Hematologic Malignancies
Series Editor: Martin Dreyling

Rüdiger Hehlmann *Editor*

Chronic Myeloid Leukemia

Second Edition

Hematologic Malignancies

Series Editor

Martin Dreyling
München, Germany

This series of professional books provides in-depth information on all aspects of the diagnosis and treatment of different hematologic cancers, including clinical evaluation, imaging diagnosis, staging, current treatment strategies, novel targeted approaches, and evaluation of treatment response. Readers will also find coverage of methodological and research issues and factors that influence treatment outcome. Each volume is designed to serve both as a quick reference and as a comprehensive source of knowledge that will be invaluable in improving management of the malignancy under consideration. The volume editors and authors have been selected for their international reputations and acknowledged expertise. The series will appeal to hematologists and oncologists in hospitals or private practices, residents, and others with an interest in the field.

More information about this series at http://www.springer.com/series/5416

Rüdiger Hehlmann
Editor

Chronic Myeloid Leukemia

Second Edition

Editor
Rüdiger Hehlmann
ELN Foundation
Weinheim
Germany

ISSN 2197-9766 ISSN 2197-9774 (electronic)
Hematologic Malignancies
ISBN 978-3-030-71912-8 ISBN 978-3-030-71913-5 (eBook)
https://doi.org/10.1007/978-3-030-71913-5

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
This work is subject to copyright. All rights are solely and exclusively licensed by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors, and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Contents

1 Cytogenetics of Chronic Myeloid Leukemia (CML) 1
Bettina Balk, Alice Fabarius, and Claudia Haferlach

2 The Biology and Pathogenesis of Chronic Myeloid Leukaemia 17
Naranie Shanmuganathan, Bradley Chereda, and Junia V. Melo

3 Epidemiology of Chronic Myeloid Leukaemia 37
Martin Höglund, Fredrik Sandin, Bengt Simonsson, and Leif Stenke

4 Imatinib: The First-Line CML Therapy 49
Carmen Fava, Giovanna Rege-Cambrin, and Giuseppe Saglio

5 CML Therapy: A Focus on Second- and Third-Generation Tyrosine Kinase Inhibitors 61
Alexandra Lovell, Elias Jabbour, Jorge Cortes, and Hagop Kantarjian

6 Adverse Events Associated with ATP-Competitive BCR-ABL1 Tyrosine Kinase Inhibitors in Chronic Myeloid Leukemia 77
Delphine Rea

7 Pharmacoeconomic Considerations for Tyrosine Kinase Inhibitors in the Treatment of Chronic Myeloid Leukemia 93
Benyam Muluneh, Rena M. Conti, Joehl Nguyen, Ashley Cole, Richard A. Larson, and Stacie B. Dusetzina

8 Standardization of Molecular Monitoring for Chronic Myeloid Leukemia: 2021 Update 105
Matthew Salmon, Helen E. White, Nicholas C. P. Cross, and Andreas Hochhaus

9 Prognostic Scores for Patients with Chronic Myeloid Leukemia under Particular Consideration of Disease-Specific Death 119
Markus Pfirrmann, Michael Lauseker, Verena S. Hoffmann, and Joerg Hasford

10 A Review and an Update of European LeukemiaNet Recommendations for the Management of Chronic Myeloid Leukemia 145
Gabriele Gugliotta, Fausto Castagnetti, Simona Soverini, Gianantonio Rosti, and Michele Baccarani

11 The Role of Hematopoietic Stem Cell Transplantation in CML 159
Jane F. Apperley and A. Gratwohl

12 CML End Phase and Blast Crisis: Implications and Management 179
Rüdiger Hehlmann, Susanne Saußele, Astghik Voskanyan, and Richard T. Silver

13 The Interferon-Alpha Revival in CML 197
Moshe Talpaz, Jessica Mercer, and Rüdiger Hehlmann

14 Managing Pregnancy in Chronic Myeloid Leukaemia 227
Elisabetta Abruzzese and Jane F. Apperley

15 Response-Related Predictors of Survival and of Treatment-Free Remission in CML 245
Susan Branford, Naranie Shanmuganathan, and Timothy P. Hughes

16 Discontinuation or Cessation of Tyrosine Kinase Inhibitor Treatment in Chronic Myeloid Leukemia Patients with Deep Molecular Response 265
Susanne Saußele and Francois-Xavier Mahon

Introduction to Chronic Myeloid Leukemia in 2021

156 years after the first attempt at treating CML with Fowler's solution in 1865, CML therapy has reached a stage at which discontinuation of any treatment has become a realistic perspective for a substantial minority of patients. The ELN 2020 recommendations for treating CML consider treatment discontinuation at stable deep molecular remission (DMR) and treatment-free remission (TFR) new goals of CML management. The second edition of Springer's CML book accounts for this new development.

After reviews of cytogenetics, molecular biology, and epidemiology of CML, the book covers the various modalities available for treating CML, such as tyrosine kinase inhibitors (TKI), hematopoietic cell transplantation, and interferon alpha, as well as diagnostic and monitoring procedures. Several reviews deal with first-, second-, or higher-line treatment, management of resistance, intolerance, side effects, TKI contraindications and comorbidities. The elucidation of pathogenesis of CML with detection of the Philadelphia chromosome and recognition of the BCR-ABL1 translocation underlying diagnostic tests and treatment is reviewed in detail. Standardized molecular BCR-ABL1 monitoring with derivation of the International Scale (IS) has become the posterchild for molecular monitoring of other leukemias and diseases and is highlighted. Prognostic scores for predicting outcome and management of pregnancy in CML are also covered. Costs as important public health aspect are addressed by analyzing cost-effectiveness of TKI treatment under consideration of unreasonably high TKI prices and the now general availability of much cheaper generic imatinib. A review on CML end phase and blast crisis points to the current limits of treatment. 5–7% of patients still progress to blast crisis.

The positive outlook of current CML management is presented in the last two reviews which report on response-related predictors of survival and successful TFR, and on the prospects of TKI cessation in patients in sustained DMR. The current expectation is that patients in sustained DMR for 2–3 years have a 50% chance to stay in TFR at 2 years after cessation, and the hope is that the rate will increase by treatment optimization and better drugs. Currently, the majority of CML patients, however, will likely need life-long TKI treatment.

Figure 1 shows updated molecular response rates of imatinib-treated patients from CML study IV. The majority of patients reached DMR (MR^4 or deeper) by 3 years.

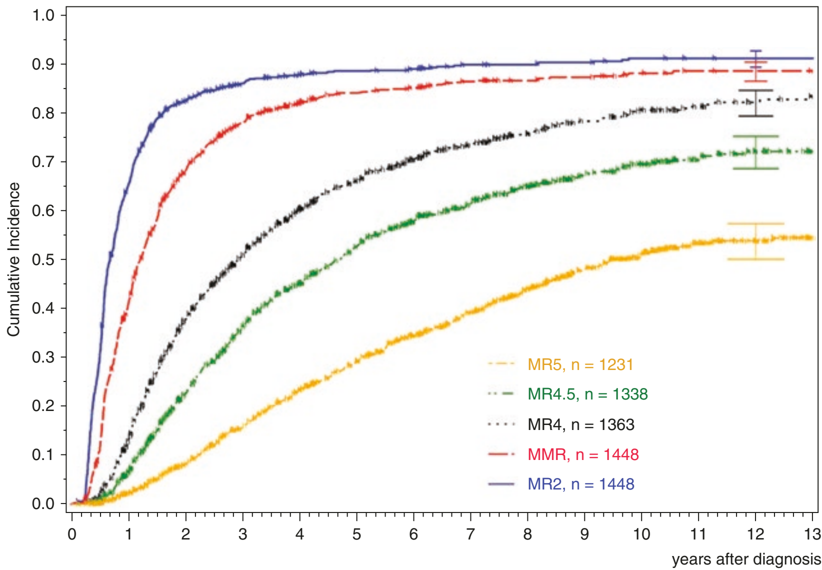


Fig. 1 Molecular responses of imatinib-treated patients from CML study IV (M. Pfirrmann, update 2020 from Kalmanti et al. Leukemia 2015; 29:1123–1132)

Table 1 5- and 10-year benchmarks for DMR (MR^4, $MR^{4.5}$)

Study		5 years (%)	10 years (%)
CML study IV[a]	Imatinib MR^4	68	81
	Imatinib $MR^{4.5}$	53	72
ENESTnd[b]	Nilotinib MR^4	66	73
	Nilotinib $MR^{4.5}$	54	64
Dasision[c]	Dasatinib $MR^{4.5}$	42	NA
Bfore[d,e]	Bosutinib MR^4	58	NA
	Bosutinib $MR^{4.5}$	47	NA

Updated from the European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Hochhaus et al., Leukemia. 2020; 34(4):966–984.
NA not available
[a]imatinib 400(-800) mg once daily (*n* = 1442)
[b]nilotinib 300 mg twice daily (*n* = 282)
[c]dasatinib 100mg once daily (*n* = 259)
[d]bosutinib 400mg once daily (*n* = 268)
[e]Bosutinib data are from Brümmendorf et al., Blood (2020) 136 (Supplement 1):41–42. DMR rates of these trials cannot be directly compared owing to different methods of trial evaluation

Table 1 has been updated from the ELN 2020 recommendations for treating CML and now includes 5-year DMR rates also of bosutinib. Based on long-term clinical trials, the table provides 5- and 10-year benchmarks when DMR can be expected for patients treated with imatinib, nilotinib, dasatinib, and bosutinib.

The book comprises 16 original reviews on selected relevant topics of CML, one review more than the first edition. Half of the reviews are newly written. The other eight reviews have been updated from the first edition. All reviews reflect the state of the art in 2020. The authors hope that this book provides in-depth information for the successful management of CML in various situations after treatment discontinuation has become possible, and after a cure of CML appears within reach in at least a minority of patients.

Weinheim, Germany, Rüdiger Hehlmann for the authors
15 November 2020

Cytogenetics of Chronic Myeloid Leukemia (CML)

1

Bettina Balk, Alice Fabarius, and Claudia Haferlach

1.1 The Discovery of the Philadelphia Chromosome (Ph)

Telling the story of the advances in chronic myeloid leukemia (CML), seen from a historical perspective, one cannot deny the extraordinary role of cytogenetics. When John Hughes Bennett and Rudolf Virchow reported what is thought to be the first descriptions of CML in 1845, nothing was known about the mechanism and the underlying genetics. Therefore, it was a quantum leap when the Philadelphia chromosome was discovered by Peter Nowel and David Hungerford in 1960 [1, 2]. By that time, they still used very basic chromosome staining techniques. The cells were grown on slides using short-term cell cultures [3], rinsed with tap water, and stained with Giemsa [4, 5]. Investigating acute leukemia they initially did not find consistent genetic abnormalities, but eventually they identified a characteristic small chromosome in two patients with CML. Together with other scientists like Paul Moorhead they were able to improve their preparation technique and report a series of seven patients all displaying a minute chromosome. In accordance with the Committee for the Standardization of Chromosomes, Tough and colleagues called this minute chromosome Philadelphia chromosome after the city it was first detected [4]. As cytogenetic techniques improved in the 1970s, Rowley discovered that the Philadelphia chromosome is the result of a translocation t(9;22)(q34;q11) between the long arms of chromosomes 9 and 22 with the derivative chromosome 22, der(22)t(9;22), being the Philadelphia chromosome [6]. de Klein et al. were then able to demonstrate that a small segment of chromosome 9 was translocated back to chromosome 22, providing evidence for the reciprocal nature of the translocation t(9;22) [7]. Later, Bartram and co-workers could show that the tyrosine kinase gene *ABL1* (*abelson*) on chromosome 9 and the *BCR (breakpoint cluster region)* gene on chromosome 22 are fused and generate the *BCR-ABL1* fusion gene on the Philadelphia chromosome [8–10]. This was the basis for the characterization of the BCR-ABL1 fusion protein, the development of the first BCR-ABL1 tyrosine kinase inhibitor (TKI) imatinib in 1996 and the success story of CML treatment [11–13]. Currently, the life expectancy of patients with newly diagnosed CML in chronic phase (CP) is very close to that of age-matched individuals [14, 15].

B. Balk (✉) · C. Haferlach
Münchner Leukämie Labor, MLL, Munich, Germany
e-mail: bettina.balk@mll.com

A. Fabarius
III. Medizinische Klinik, Hämatologie und Internistische Onkologie, Universitätsmedizin Mannheim, Pettenkoferstraße 22, 68169 Mannheim, Germany

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
R. Hehlmann (ed.), *Chronic Myeloid Leukemia*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-71913-5_1

1.2 The Translocation t(9;22)

Prakash and Yunis located the breakpoints in CML to the sub-bands 22q11.21 and 9q34.1 [16]. A scheme and a picture of a karyogram are shown in Fig. 1.1. The rearrangement of the *BCR* and *ABL1* gene can also be visualized by fluorescence in situ hybridization (FISH) in interphase nuclei and on metaphase chromosomes using dual-color dual-fusion probes (Fig. 1.2).

The so-called standard translocation t(9;22)(q34;q11) is found in about 85–90% of all CML patients via the banding technique. In addition variant translocations or cytogenetically cryptic rearrangements occur.

Variant translocations are defined by the involvement of one or more additional chromosomes besides the chromosomes 9 and 22. They can be found in 5–10% of newly diagnosed CML cases [18–21]. Various chromosomes can be involved in a variant translocation, but there seems to be a non-random pattern. Marked breakpoint cluster to chromosome bands 1p36, 3p21, 5q13, 6p21, 9q22, 11q13, 12p13, 17p13, 17q21, 17q25, 19q13, 21q22, 22q12, and 22q13 [22]. Most cases can be explained by a one-step or two-step mechanism. For the one-step mechanism chromosome breakage occurs simultaneously on three or even more chromosomes and leads to a three-way or more-way translocation, respectively. The two-step mechanism consists of sequential translocations including a standard translocation t(9;22) followed by a second translocation with another chromosome [20]. However, there are also rare variant translocations that are more complex and even include loss of small chromosomal regions. Moreover, Fisher et al. observed a significant positive correlation between breakpoint locations and CG composition [23], suggesting that repetitive elements or chromatin structure might cause genomic instability that promotes these areas for being involved in the variant translocations.

The prognostic impact of variant translocations has been frequently discussed. However, variant translocations are not frequent, and the numbers of patients included in these studies are small. In the pre-imatinib era it was suggested that patients with variant translocations do have adverse outcomes [24], whereas other studies with patients treated with chemotherapy and interferon-alpha revealed no differences compared to the standard translocation t(9;22) [25–27]. Studies with patients treated with imatinib showed no significant prognostic difference between patients with standard and variant translocations [20, 22, 28, 29]. The latter is also supported by a large systematic study performed within prospective trials that showed that time to complete cytogenetic response (no Ph-positive cell in 20 metaphases, CCyR) and time to major molecular response (MMR, $\leq$ 0.1% *BCR-ABL1/ABL1* using quantitative polymerase chain reaction (PCR)) [30] do not differ significantly. Furthermore, event-free survival (EFS), failure-free survival, progression-free survival (PFS), and overall survival (OS) display no significant variation [20]. Besides that, no striking difference regarding the response rates and the survival rates between one-step or two-step variant translocations or the number of involved chromosomes was discovered [20]. Thus, it is generally agreed that variant translocations no longer have any prognostic significance.

Another aspect that has provoked controversy is that 10–15% of all patients with CML have a deletion of a sizable portion on the derivative chromosome 9 [28, 31–35]. These small deletions cannot be detected by classical cytogenetics, but only by FISH or molecular genetic methods. Huntly et al. and Reid et al. suggested for these patients, treated with hydroxyurea and interferon-alpha, respectively, a poor prognosis and inferior survival [36]. Sinclair et al. and Huntly et al. reported that these deletions are more frequent in patients with variant translocations compared to patients with standard translocation t(9;22). However, studies particularly in the imatinib era demonstrated that there is only a trend that variant translocations more frequently show deletions at the derivative chromosome 9 [20, 28, 37, 38] and that these deletions do not influence response or outcome of CML patients in CP treated with imatinib [20, 28, 39–41]. In summary, deletions in the breakpoint region of *BCR* and *ABL1* do not have any prognostic significance in the era of TKI treatment [38].

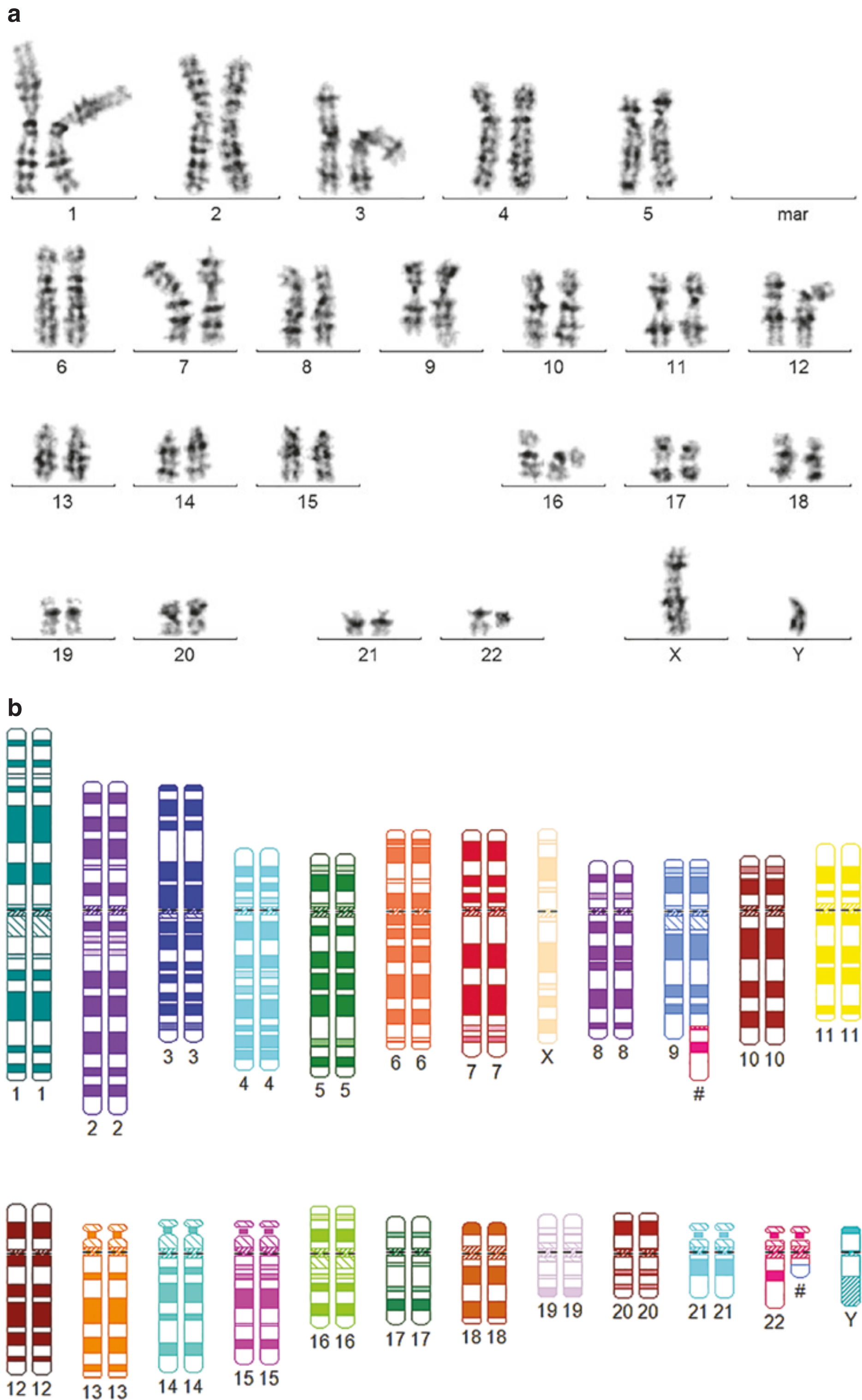


Fig. 1.1 (**a**) Karyogram and (**b**) scheme of the translocation t(9;22)(q34;q11) generated with CyDAS [17]

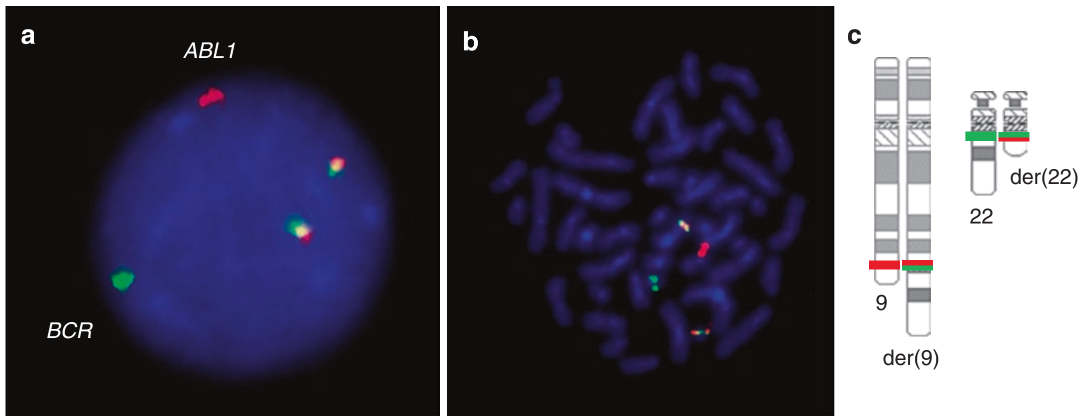


Fig. 1.2 (**a**) Interphase FISH using dual-color dual-fusion probes, with *BCR* labelled in green and *ABL1* in red to detect the *BCR-ABL1* rearrangement that leads to two yellow fusion signals. (**b**) Metaphase FISH with the *BCR-ABL1* probes. (**c**) Scheme of the *BCR* and *ABL1* probes binding to the chromosomes

Cryptic *BCR-ABL1*-rearrangements, which can only be detected by FISH or molecular genetic techniques such as RT-PCR, are found in about 1–5% of patients [42, 43]. The *BCR-ABL1* fusion signal can be found either on chromosome 9, 22, or even another chromosome. Two different mechanisms have been postulated, which are either the insertion of *ABL1* into the *BCR* region (or less frequent vice versa) or by a multi-step mechanism that starts with the standard translocation t(9;22) followed by additional translocations [44]. Considering that the *BCR-ABL1* rearrangement in these cases cannot be identified by chromosome banding analysis, as the cytogenetic correlate, the Philadelphia chromosome, is missing, either FISH or a PCR-based technique is necessary to establish the diagnosis of *BCR-ABL1*-positive CML. Cryptic *BCR-ABL1*-rearrangements seem to have no prognostic relevance compared to the standard *BCR-ABL1*-rearrangement [42].

1.3 Relevance of Additional Cytogenetic Aberrations in a Ph-Positive Clone

At diagnosis, 80–90% of patients with CML in CP show the standard or a variant translocation as a sole cytogenetic change. The remaining patients display additional cytogenetic aberrations (ACAs) [45]. The percentage of patients with ACAs is relatively low in CP, but increases during the course of disease to 30% in the accelerated phase (AP) [46] and 60–80% in blast crisis (BC) [22, 47, 48]. These secondary changes accompany or precede the transformation into a more malignant form for a few months [45, 48–50].

ACAs clearly follow a non-random pattern and according to their frequency, they were separated into major and minor route aberrations referring to the major and minor route of clonal cytogenetic evolution. However, the major or minor route only relates to the frequency of these aberrations. Most frequently observed are trisomy 8 (+8), which is depicted in Fig. 1.3a, an additional Philadelphia chromosome (+der(22)t(9;22)) or an isochromosome of the long arm of the Philadelphia chromosome (ider(22)(q10)t(9;22)), an isochromosome of the long arm of chromosome 17 (i(17)(q10)) as shown in Fig. 1.3b and trisomy 19 (+19). These aberrations are considered as major route aberrations [51]. The frequencies are quite similar for patients with standard and variant translocations. If both groups are combined the following frequencies were found: +8 (34%), +der(22) t(9;22) (31%), i(17)(q10) (20%), and + 19 (13%) [22]. It should be mentioned that according to Fioretos et al. the majority of i(17)(q10) are dicentric [52] and should rather be designated idic(17)(p11). All other ACAs occur in less than 10% of cases, the most frequent being

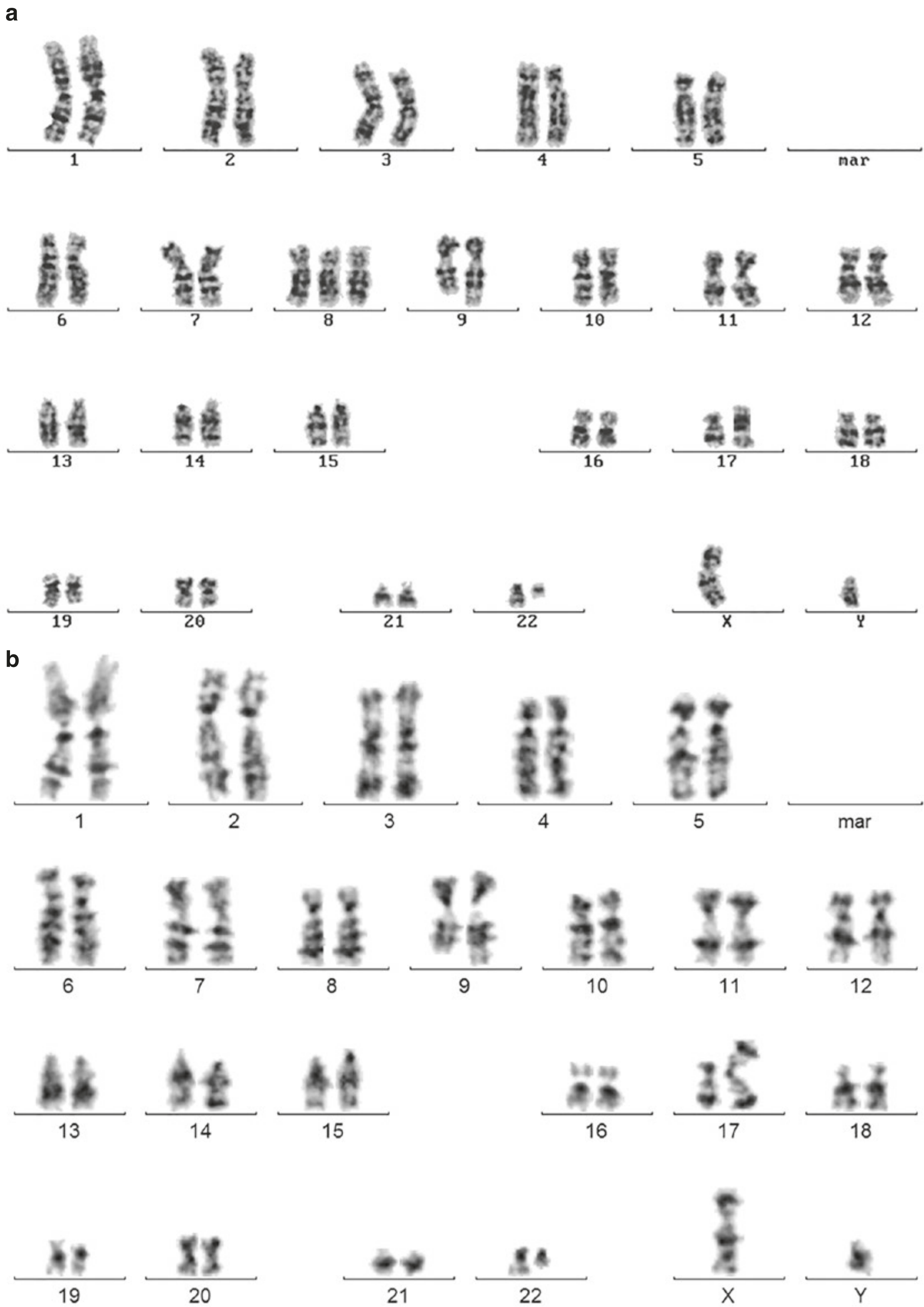


Fig. 1.3 (**a**) Karyogram of the translocation t(9;22)(q34;q11) with trisomy 8 and (**b**) i(17)(q10) as additional chromosomal aberrations (ACAs), respectively

loss of Y-chromosome, trisomy 21 (+21), trisomy 17 (+17), monosomy 7 (−7), and monosomy 17 (−17), which were then regarded as minor route aberrations [51]. It was suggested to expand the major evolutionary route to all aberration with a frequency higher than 5 % [22] and therefore include –Y, −7, +17 and +21 as major route aberrations.

Besides these unbalanced aberrations, also certain balanced ACAs occur, which are rather typical for acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). About 1% of patients gain a translocation t(3;21)(q26;q22) in addition to t(9;22) and this is usually a sign of transformation into BC although the t(3;21) can also be found in CML prior to the onset of BC [48, 49, 53–55]. Likewise, the AML-typical aberrations like t(15;17)(q24;q21) with *PML-RARA* fusion transcript, inv(3)(q21q26)/t(3;3)(q21;q26) involving the *MECOM* locus, t(7;11)(p15;p15) with *NUP98-HOXA9,* t(8;21)(q22;q22) with *RUNX1-RUNX1T1*, rearrangements involving the *KMT2A* gene at 11q23, and inv(16)(p13q22) with *CBFB-MYH11* can occur during disease progression [22, 54, 56–62]. These AML-specific aberrations can be seen as a warning sign and they have been related to quite specific phenotypic features. For example, the blasts of a CML patient in BC with additional t(15;17)(q24;q21) had morphological features of promyelocytes. Upon treatment with all-*trans* retinoic acid and arsenic trioxide this patient achieved CCyR with no evidence of *PML-RARA* by FISH and low levels of *BCR-ABL1* by RT-PCR [63].

It is hard to tell whether these cytogenetic changes appear just due to the increasing genomic instability during the transformation from CP to BC or whether these ACAs are drivers of this process. It also needs to be mentioned that some clones already undetectable in remission under treatment can reappear within the course of disease and that new clones with additional mutations can overgrow the original clones [22, 50, 64]. The impact of unbalanced ACAs on the pathobiology of CML is hardly understood. Trisomy cannot be reduced to an increased copy number of a certain gene, as global expression studies of hematologic malignancies have demonstrated that +8 and trisomy of other chromosomes lead to a general up-regulation of a large fraction of genes located on such additional chromosomes [65–67]. Furthermore, genes on a chromosome can be differentially silenced. For example, the effects of +8 cannot be cut down to an increased copy number of the *MYC* transcription factor gene at 8q24 because many other genes are also up-regulated as shown for +8 in AML [68]. It is also assumed that the effects of i(17)(q10) are due to reduced copy number of tumor suppressor gene *TP53* ([69, 70]; [22, 52]). So far it is still unclear whether the expression of *BCR-ABL1* is increased for patients with an additional Philadelphia chromosome [45]. It was reported that a patient in lymphoid BC displayed three Philadelphia chromosomes and strongly increased *BCR-ABL1* levels in blasts compared to granulocytes [71]. A case with multiple copies of *BCR-ABL1* fusion gene on two isodicentric Philadelphia chromosomes was described in an imatinib-resistant patient [72]. Moreover, Gaiger et al. report increased *BCR-ABL1* mRNA expression during disease progression [73], whereas Andrews et al. report rather heterogeneous expression levels in BC [74]. Therefore, the genomic imbalance as such demonstrated by an additional Philadelphia chromosome might be more important and more relevant for the pathological mechanism than the additional fusion gene. Besides that, overexpression of *EVI1* frequently occurs in CML in BC, but also in cases without cytogenetically detectable involvement of the *MECOM* locus at 3q26 [75]. Intriguingly, it was demonstrated in 14 CML patients with +21 that six of them had mutations within the DNA-binding domain of *RUNX1* at 21q22, of which two also displayed a translocation t(1;21)(p36;q22) leading to biallelic *RUNX1* aberrations [76]. This phenomenon has also been reported by Preudhomme and colleagues. They showed that myeloid leukemia patients with +21 had a high frequency of biallelic point mutations in *RUNX1*, and they even found patients with no functional *RUNX1* allele left [77]. Thus, a + 21 is not necessarily associated with an increased copy number of functional *RUNX1* alleles. Hence, transformation to BC involves many different genetic changes not only cytogenetically detectable ones [78, 79].

As mentioned above, the pattern of ACAs is not random and follows certain patterns without therapy [22, 50]. It was described that this pattern of secondary aberrations during AP or BC can be influenced by the type of treatment during CP. Trisomy 8 was more common after busulfan treatment as compared to hydroxyurea treatment [22]. As most patients are now treated with TKI these differences seem to be less important. Cytogenetic changes that appear in Ph-positive clones under TKI treatment seem to follow the same cytogenetic evolution pattern as before [45, 80].

The question was raised whether certain aberrations are more frequently found in myeloid BC compared to lymphoid BC. Trisomy 8, 3q26 rearrangements, i(17)(q10), and + 19 were more common in myeloid BC, and − 7 more common in lymphoid BC. +Ph and + 21 were equally distributed in myeloid and lymphoid BC [22, 56]. Most common ACAs like +8, +Ph, −7, i(17)(q10), +19, or + 21 were associated with other chromosomal alterations and rarely detected as sole ACA in BC.

So what is the prognostic impact of these ACAs? The type of cytogenetic aberration needs to be considered. Although the differentiation between major and minor route ACAs is just based on the frequency of these ACAs, studies used this differentiation as the numbers of individual ACAs at that time were too low for distinct analyses. Whereas major route ACAs (+8, i(17) (q10), +19 and +der(22)t(9;22)) seemed to have a significant prognostic impact, minor route aberrations (all other ACAs) seemed to be less concerning [29, 81]. It is difficult to predict the prognostic impact of certain individual ACAs as the total numbers are still small and as these ACAs can occur in various combinations. However, more data is emerging about the prognostic impact of individual ACAs. A study on 2015 CML patients treated with TKIs was trying to shed light on the impact of +8 [82]. Patients with +8 as a sole ACA showed a significantly worse OS than patients without ACAs but a significantly better OS than patients with other ACAs in addition to +8 (most commonly +der(22)t(9;22) and i(17)(q10)). Another study revealed that 5.8% of patients showed abnormalities at chromosome 3 and about 50% of these aberrations were at the *MECOM* locus (3q26), which is a rather AML typical aberration. These patients with 3q26 rearrangements displayed a marginal response to TKI treatment, and no long-term remission on a cytogenetic or molecular level was achieved. Moreover, the OS of patients with chromosome 3 abnormalities was significantly poorer compared to patients with no ACAs and other ACAs not involving chromosome 3 [55]. Patients with -Y were also specifically investigated as -Y frequently occurs in healthy elderly men [83]. Comparing the OS of patients without ACAs at diagnosis to patients with -Y as a sole ACA, it was found that -Y alone had no significant impact [29, 82]. It was also depicted that in some patients the aberrant Ph-positive clone with -Y disappeared during therapy, whereas a clone with just -Y was still detectable despite CCyR.

Wang et al. proposed risk stratification of ACAs in CML for patients treated with TKI by focusing on the six most common ACAs that appeared as single ACAs in their study cohort. According to the OS of patients after ACA emerge, these ACAs were divided into two groups. -Y, +8, and + Ph were combined in group 1 with relatively good prognosis whereas i(17)(q10), −7, deletion in the long arm of chromosome 7 (7q-), and 3q26 rearrangements comprised group 2 with relatively poor prognosis. Patients with two or more ACAs (complex aberrant karyotype) were categorized into the poor prognostic group 2 as they showed inferior survival, which was consistent with previous data [84]. Group 2 patients showed worse treatment response and OS irrelevant of the time and phase they emerged. Conversely, ACAs in group 1 had no adverse impact on survival if they were detected in CP or at the time of CML diagnosis [85]. Using the same cohort of patients Gong et al. worked out that the time interval from ACA emerge to onset of BC is dependent on the type of ACA. Based on the risk of developing BC associated with each ACA, patients were stratified into four risk groups. Patients without ACAs formed the standard-risk group and patients with 3q26.2 rearrangement, −7/7q- or i(17q) as isolated ACAs or in a complex setting formed the high-risk (HR) group. Patients with +8, +Ph, or other

single ACAs formed the intermediate-1 (Int-1) risk group. Patients with complex aberrant karyotype and no HR ACA formed the intermediate-2 (Int-2)-risk group. For patients of Int-1, Int-2, and HR groups the time interval from ACA emerge to onset of BC the median duration was unreached, 19.2 months, and 1.9 months, respectively. The overall 5-year cumulative probability of BC was 9.8%, 28.0%, 41.7%, and 67.4% for these four groups, respectively. Loss of Y-chromosome was not regarded as an ACA. It was concluded that ACAs play a crucial role in determining the risk of transformation into BC and that patients with HR ACAs may benefit from timely alternative treatment to prevent progression to BC [49]. Furthermore, Hehlmann et al. monitored 1536 CML patients in CP and analyzed ACAs and transformation to BC. ACAs were grouped according to their impact on survival. 3q26 rearrangements, −7, +8, 11q23 rearrangements, i(17)(q10), +17, +19, +21, +Ph, and complex aberrant karyotypes were classified as "high-risk" ACAs and all others as "low-risk." Prognosis of +8 alone was better than +8 accompanied with other ACA but was worse than low-risk ACA. They concluded that "high-risk" ACAs herald death by BC already at low blast levels, and more intensive therapy might be indicated at the emerge of "high-risk" ACAs [48].

All these data was taken into account in the latest version of the European LeukemiaNet (ELN) 2020 recommendations for CML. 3q26 aberrations, −7/7q-, +8, 11q23 aberrations, i(17)(q10), +19, +Ph, and complex aberrant karyotypes were classified as "high-risk" ACAs, which predict poorer response to TKI and a higher risk of progression. The panel recommends classifying ACAs and treating patients with "high-risk" ACAs as high-risk patients [15].

What is known about the time point ACAs appear? Most ACAs are detected during the course of disease, but there are also cases that show ACAs at the initial diagnosis. Although the type of ACA present at diagnosis and acquired during the course of disease are similar [80], "high-risk" ACAs at diagnosis are regarded as a warning sign according to the ELN recommendations [15]. Though, "high-risk" ACAs arising at any other time point are seen as a sign of disease progression. They define therapy failure and indicate a change in therapy [15]. CML patients with emergence of "high-risk" ACAs should be observed closely and an intensification of treatment should be considered.

1.4 Ph-Negative Clones

So far, the appearance of ACAs in Ph-positive clones has been addressed. However, new clones with cytogenetic aberrations, which do not show a translocation t(9;22), have also been found in patients in partial or complete cytogenetic remission during treatment. These clones are termed Ph-negative clones. They have to be clearly distinguished from clonal evolution with ACAs in the Ph-positive clone, which is a sign of disease progression.

Ph-negative clones carrying cytogenetic aberrations have already been described during CML treatment with interferon-alpha [45, 86, 87], but could be more carefully investigated in patients treated with imatinib, as these clones become obvious only in complete or at least partial cytogenetic remission of the Ph-positive clone, which was basically only achieved by the introduction of TKI. The incidence of Ph-negative clones is in the range of 2–10% and is mostly similar across TKI groups [88–95]. The median interval from the start of therapy to the first observation of a Ph-negative clone was 5–24 months and these clones might also just appear transiently [88, 91, 92, 96, 97]. The most common aberrations found are +8, −7, deletions in the long arm of chromosome 20, monosomy 5, 7q-, and loss of Y-chromosome, whereby +8 can also be found in addition to other aberrations [88, 89, 91, 92]. Unsurprisingly, loss of Y-chromosome occurred mainly in older patients. Besides that, various other rare aberrations in single cases [89] and even cases with complex aberrant karyotype (≥ 3 chromosomal abnormalities) were reported [91]. Most patients show CCyR or major cytogenetic response regarding the Ph-positive clone [98], but few

cases with minor cytogenetic remission were described [88]. Interestingly, cases were published with clonal evolution in the Ph-positive clone and emergence of a Ph-negative clone as well [88, 91].

How do Ph-negative clones arise? One argument is the general genomic instability in CML patients. Likewise, it is discussed that TKIs cause aberrant Ph-negatives clones more frequently. However, this might be due to the limited number of studied patients with interferon-alpha treatment and the far better cytogenetic response rates with TKI treatment. It is probably not dependent on the type of treatment, but rather a process of selection. By suppressing CML-cells, a small pre-existing clone could gain a growth advantage and become detectable or new arising clones are not suppressed by the Ph-positive clone. This is supported by findings of Terre et al., who found Ph-independent clonal aberrations in 4 out of 15 patients before the start of the imatinib therapy [95]. Noteworthy, it was demonstrated that 38% of patients with Ph-negative clones had at least one mutation in a panel of genes known to be mutated in other myeloid malignancies, whereas for randomly selected patients in MMR it was only 4% [99, 100]. The most frequently mutated genes were *ASXL1, DNMT3A, RUNX1, NRAS*, and *TET2*. In comprehensible cases, a few of these mutations were already detectable at a low level at CML diagnosis, but in most cases, these mutations were first detected during treatment with TKIs. Mutation levels were inversely related to *BCR-ABL1* expression. It is proposed that these chromosomal aberrations in Ph-negative clones are an indicator for genomic instability, also at the molecular level [99, 100]. However, it cannot be ruled out that the constant inhibition of the ABL1 kinase and its down-stream partners, which are also involved in DNA repair, does not have an impact on genomic stability [101].

How about the prognostic impact of these Ph-negative clones? Generally, the arising aberrations are similar to those observed in MDS and AML and some patients developing MDS or even AML were described [88, 93, 98]. Thus, it was proposed that these patients might have a worse outcome and might tend to develop therapy-associated MDS or AML. As the number of patients with Ph-negative clones is low and the number of patients developing MDS or AML is even lower, it is hard to find statistically significant correlations. It was found that in 17 cases with MDS or AML reported in the literature, eight had a − 7 in the Ph-negative clone whereas nine cases showed other aberrations [98]. Groves et al. performed a more systematically and detailed literature search and were able to study 53 patients of which 29 displayed −7 as a sole aberration, 14 showed −7 and + 8, and 10 patients had a 7q-. Out of these, 32% developed MDS or AML, but among them none with 7q-. They also revealed that if −7 is found later than 15 months after start of TKI treatment, patients had a higher risk of developing MDS or AML [102]. Furthermore, they detected that the transformation most likely takes place during the first 6 months after detection of −7. The authors do not recommend preventive therapeutic strategies as more than half of the patients do not develop MDS or AML although the outcome of patients, particularly for the patients that develop AML is very poor [45, 102]. More recently, Bidet et al. monitored 102 patients with Ph-negative clones and reported 26 patients with −7 or 7q-. These patients had a lower EFS and PFS after 3 years of starting TKI treatment compared to patients with Ph-negative clones other than −7 or 7q- [103]. Issa et al. examined 598 patients with CML in CP and described that -Y as a sole aberration had no significant influence on survival. On the other hand, patients with Philadelphia-negative clones with aberrations other than just -Y had worse EFS and OS compared to patients with no Philadelphia-negative clones. Two patients in this study developed AML or MDS and deceased, both with −7, confirming the risk of this occurrence. They concluded that Ph-negative clones other than just -Y are associated with decreased survival when emerging in patients with CP CML across various TKIs [91]. Regarding the appearance of clonal chromosome aberrations in a Ph-negative clone, constant monitoring is not recommended, just if there is any hint of myelodysplasia or a Ph-negative clone with involvement of chromosome 7 [30].

1.5 Significance of Cytogenetics for Current and Future Diagnosis and Monitoring of CML

Although chromosome banding analysis (CBA) is a comparably old technique that needs cultivation of viable proliferating bone marrow (BM) or peripheral blood (PB) cells, it is still very important and recommended by the ELN for CML diagnosis together with quantitative PCR and FISH in case of Philadelphia chromosome negativity [15]. Cytogenetics provide evidence of a *BCR-ABL1*-fusion by detection of the Philadelphia chromosome. This is of particular importance if the patient was initially not suspected to have CML. CBA can provide a warning sign or reveal disease progression by emerge of ACAs or even a complex aberrant karyotype. According to the ELN, cytogenetics should be part of the diagnostic work-up at CML diagnosis. Additionally, cytogenetics should be performed in patients with atypical translocations and rare or atypical *BCR-ABL1* transcripts, which cannot be measured by quantitative PCR. Cytogenetics are also recommended to exclude ACAs in patients with treatment failure, resistance, or disease progression to AP or BC. FISH might be needed for monitoring patients with atypical transcripts [15]. However, cytogenetics became less important for monitoring TKI response rate. CCyR is defined as no Ph-positive cell in at least 20 metaphases of BM or PB. Equivalently, $<1\%$ *BCR-ABL1*-positive nuclei of at least 200 nuclei evaluated by FISH using a double-color double-fusion probe can be used for the assessment of CCyR as a substitute if chromosome banding analysis cannot be performed [30, 104]. CCyR correlates to a molecular remission of $\leq 1\%$ *BCR-ABL1/ABL1* by quantitative PCR [105]. Therefore, the ELN recommends monitoring TKI response by quantitative PCR whenever possible due to a higher sensitivity of this diagnostic method [15]. How about the future of cytogenetics? As the molecular methods improve, *BCR-ABL1*-positive cells can also be diagnosed and monitored using quantitative PCR, which provides more accurate and much more sensitive data. Deep molecular responses are defined as MR 4 ($\leq 0.01\%$ *BCR-ABL1/ABL1*), MR4.5 ($\leq 0.0032\%$ *BCR-ABL1/ABL1*), and MR5 ($\leq 0.001\%$ *BCR-ABL1/ABL1*) [106, 107] and are important for the decision of treatment discontinuation.

Although quantitative PCR is a very useful tool for CML monitoring and for the decision about therapy discontinuation [108, 109], it cannot completely replace cytogenetics. CBA will still be necessary for CML diagnosis to assess "high-risk" ACAs and complex aberrant karyotypes. Furthermore, it should be performed if there is any risk of disease progression into AP or BC and if there is any suspicion of myelodysplasia that indicates the appearance of a Ph-negative clone.

At some point in the future, whole-genome sequencing (WGS), but not whole-exome sequencing, will be able to replace conventional cytogenetics. For the detection of translocations, the intronic regions are also of importance. At the moment, however, WGS is still by far more expensive and more labor-intensive compared to conventional cytogenetics and, therefore, not applicable in routine diagnostics.

References

1. Deininger MW. Milestones and monitoring in patients with CML treated with imatinib. Hematology Am Soc Hematol Educ Program. 2008:419–26.
2. Nowell PC, Hungerford DA. A minute chromosome in human granulocytic leukemia. Science. 1960;132:1497.
3. Osgood EE, Kripphahne ML. The gradient tissue culture method. Exp Cell Res. 1955;9(1):116–27.
4. Nowell PC. Discovery of the Philadelphia chromosome: a personal perspective. J Clin Invest. 2007;117(8):2033–5.
5. Tough IM, WM CB, Baikie AG, Buckton KE, Harnden DG, Jacobs PA, King MJ, McBride JA. Cytogenetic studies in chronic myeloid leukaemia and acute leukaemia associated with monogolism. Lancet. 1961;1(7174):411–7.
6. Rowley JD. Letter: a new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature. 1973;243(5405):290–3.
7. de Klein A, van Kessel AG, Grosveld G, Bartram CR, Hagemeijer A, Bootsma D, Spurr NK,

Heisterkamp N, Groffen J, Stephenson JR. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature. 1982;300(5894):765–7.
8. Balabanov S, Braig M, Brummendorf TH. Current aspects in resistance against tyrosine kinase inhibitors in chronic myelogenous leukemia. Drug Discov Today Technol. 2014;11:89–99.
9. Bartram CR, de KA, Hagemeijer A, van AT, Geurts van KA, Bootsma D, Grosveld G, Ferguson-Smith MA, Davies T, Stone M. Translocation of c-ab1 oncogene correlates with the presence of a Philadelphia chromosome in chronic myelocytic leukaemia. Nature. 1983;306(5940):277–80.
10. Groffen J, Stephenson JR, Heisterkamp N, de KA, Bartram CR, Grosveld G. Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. Cell. 1984;36(1):93–9.
11. Druker BJ. Translation of the Philadelphia chromosome into therapy for CML. Blood. 2008;112(13):4808–17.
12. Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian HM, Gattermann N, Deininger MW, Silver RT, Goldman JM, Stone RM, Cervantes F, Hochhaus A, Powell BL, Gabrilove JL, Rousselot P, Reiffers J, Cornelissen JJ, Hughes T, Agis H, Fischer T, Verhoef G, Shepherd J, Saglio G, Gratwohl A, Nielsen JL, Radich JP, Simonsson B, Taylor K, Baccarani M, So C, Letvak L, Larson RA. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med. 2006;355(23):2408–17.
13. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, Zimmermann J, Lydon NB. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med. 1996;2(5):561–6.
14. Bower H, Bjorkholm M, Dickman PW, Hoglund M, Lambert PC, Andersson TM. Life expectancy of patients with chronic myeloid Leukemia approaches the life expectancy of the general population. J Clin Oncol. 2016;34(24):2851–7.
15. Hochhaus A, Baccarani M, Silver RT, Schiffer C, Apperley JF, Cervantes F, Clark RE, Cortes JE, Deininger MW, Guilhot F, Hjorth-Hansen H, Hughes TP, Janssen J, Kantarjian HM, Kim DW, Larson RA, Lipton JH, Mahon FX, Mayer J, Nicolini F, Niederwieser D, Pane F, Radich JP, Rea D, Richter J, Rosti G, Rousselot P, Saglio G, Saussele S, Soverini S, Steegmann JL, Turkina A, Zaritskey A, Hehlmann R. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020;34(4):966–84. https://doi.org/10.1038/s41375-020-0776-2.
16. Prakash O, Yunis JJ. High resolution chromosomes of the t(9;22) positive leukemias. Cancer Genet Cytogenet. 1984;11(4):361–7.
17. Hiller B, Bradtke J, Balz H, Rieder H. CyDAS: a cytogenetic data analysis system. Bioinformatics. 2005;21(7):1282–3.
18. Gorusu M, Benn P, Li Z, Fang M. On the genesis and prognosis of variant translocations in chronic myeloid leukemia. Cancer Genet Cytogenet. 2007;173(2):97–106.
19. Huret JL. Complex translocations, simple variant translocations and Ph-negative cases in chronic myelogenous leukaemia. Hum Genet. 1990;85(6):565–8.
20. Marzocchi G, Castagnetti F, Luatti S, Baldazzi C, Stacchini M, Gugliotta G, Amabile M, Specchia G, Sessarego M, Giussani U, Valori L, Discepoli G, Montaldi A, Santoro A, Bonaldi L, Giudici G, Cianciulli AM, Giacobbi F, Palandri F, Pane F, Saglio G, Martinelli G, Baccarani M, Rosti G, Testoni N. Variant Philadelphia translocations: molecular-cytogenetic characterization and prognostic influence on frontline imatinib therapy, a GIMEMA working party on CML analysis. Blood. 2011;117(25):6793–800.
21. O'Brien S, Thall PF, Siciliano MJ. Cytogenetics of chronic myelogenous leukaemia. Baillieres Clin Haematol. 1997;10(2):259–76.
22. Johansson B, Fioretos T, Mitelman F. Cytogenetic and molecular genetic evolution of chronic myeloid leukemia. Acta Haematol. 2002;107(2):76–94.
23. Fisher AM, Strike P, Scott C, Moorman AV. Breakpoints of variant 9;22 translocations in chronic myeloid leukemia locate preferentially in the CG-richest regions of the genome. Genes Chromosomes Cancer. 2005;43(4):383–9.
24. Potter AM, Watmore AE, Cooke P, Lilleyman JS, Sokol RJ. Significance of non-standard Philadelphia chromosomes in chronic granulocytic leukaemia. Br J Cancer. 1981;44(1):51–4.
25. De Braekeleer M. Variant Philadelphia translocations in chronic myeloid leukemia. Cytogenet Cell Genet. 1987;44(4):215–22.
26. Verma RS, Macera MJ. Genomic diversity of Philadelphia-positive chronic myelogenous leukemia. Leuk Res. 1987;11(9):833–42.
27. Yehuda O, Abeliovich D, Ben-Neriah S, Sverdlin I, Cohen R, Varadi G, Orr R, Ashkenazi YJ, Heyd J, Lugassy G, Ben YD. Clinical implications of fluorescence in situ hybridization analysis in 13 chronic myeloid leukemia cases: Ph-negative and variant Ph-positive. Cancer Genet Cytogenet. 1999;114(2):100–7.
28. El-Zimaity MM, Kantarjian HM, Talpaz M, O'Brien S, Giles F, Garcia-Manero G, Verstovsek S, Thomas D, Ferrajoli A, Hayes K, Nebiyou BB, Zhou X, Rios MB, Glassman AB, Cortes JE. Results of imatinib mesylate therapy in chronic myelogenous leukaemia with variant Philadelphia chromosome. Br J Haematol. 2004;125(2):187–95.
29. Fabarius A, Leitner A, Hochhaus A, Muller MC, Hanfstein B, Haferlach C, Gohring G, Schlegelberger B, Jotterand M, Reiter A, Jung-Munkwitz S, Proetel U, Schwaab J, Hofmann WK, Schubert J, Einsele H, Ho AD, Falge C, Kanz L, Neubauer A, Kneba M, Stegelmann F, Pfreundschuh M, Waller CF,

Spiekermann K, Baerlocher GM, Lauseker M, Pfirrmann M, Hasford J, Saussele S, Hehlmann R. Impact of additional cytogenetic aberrations at diagnosis on prognosis of CML: long-term observation of 1151 patients from the randomized CML study IV. Blood. 2011;118(26):6760–8.
30. Baccarani M, Deininger MW, Rosti G, Hochhaus A, Soverini S, Apperley JF, Cervantes F, Clark RE, Cortes JE, Guilhot F, Hjorth-Hansen H, Hughes TP, Kantarjian HM, Kim DW, Larson RA, Lipton JH, Mahon FX, Martinelli G, Mayer J, Muller MC, Niederwieser D, Pane F, Radich JP, Rousselot P, Saglio G, Saussele S, Schiffer C, Silver R, Simonsson B, Steegmann JL, Goldman JM, Hehlmann R. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood. 2013;122(6):872–84.
31. Cohen N, Rozenfeld-Granot G, Hardan I, Brok-Simoni F, Amariglio N, Rechavi G, Trakhtenbrot L. Subgroup of patients with Philadelphia-positive chronic myelogenous leukemia characterized by a deletion of 9q proximal to ABL gene: expression profiling, resistance to interferon therapy, and poor prognosis. Cancer Genet Cytogenet. 2001;128(2):114–9.
32. Herens C, Tassin F, Lemaire V, Beguin Y, Collard E, Lampertz S, Croisiau C, Lecomte M, De PB, Longree L, Koulischer L. Deletion of the 5'-ABL region: a recurrent anomaly detected by fluorescence in situ hybridization in about 10% of Philadelphia-positive chronic myeloid leukaemia patients. Br J Haematol. 2000;110(1):214–6.
33. Huntly BJ, Reid AG, Bench AJ, Campbell LJ, Telford N, Shepherd P, Szer J, Prince HM, Turner P, Grace C, Nacheva EP, Green AR. Deletions of the derivative chromosome 9 occur at the time of the Philadelphia translocation and provide a powerful and independent prognostic indicator in chronic myeloid leukemia. Blood. 2001;98(6):1732–8.
34. Kolomietz E, Al-Maghrabi J, Brennan S, Karaskova J, Minkin S, Lipton J, Squire JA. Primary chromosomal rearrangements of leukemia are frequently accompanied by extensive submikroskopic deletions and may lead to altered prognosis. Blood. 2001;97(11):3581–8.
35. Sinclair PB, Nacheva EP, Leversha M, Telford N, Chang J, Reid A, Bench A, Champion K, Huntly B, Green AR. Large deletions at the t(9;22) breakpoint are common and may identify a poor-prognosis subgroup of patients with chronic myeloid leukemia. Blood. 2000;95(3):738–43.
36. Reid AG, Huntly BJ, Grace C, Green AR, Nacheva EP. Survival implications of molecular heterogeneity in variant Philadelphia-positive chronic myeloid leukaemia. Br J Haematol. 2003;121(3):419–27.
37. Huh J, Jung CW, Kim JW, Kim HJ, Kim SH, Shin MG, Kim YK, Kim HJ, Suh JS, Moon JH, Sohn SK, Nam GH, Lee JE, Kim DH. Genome-wide high density single-nucleotide polymorphism array-based karyotyping improves detection of clonal aberrations including der(9) deletion, but does not predict treatment outcomes after imatinib therapy in chronic myeloid leukemia. Ann Hematol. 2011;90(11):1255–64.
38. Kreil S, Pfirrmann M, Haferlach C, Waghorn K, Chase A, Hehlmann R, Reiter A, Hochhaus A, Cross NC. Heterogeneous prognostic impact of derivative chromosome 9 deletions in chronic myelogenous leukemia. Blood. 2007;110(4):1283–90.
39. Castagnetti F, Testoni N, Luatti S, Marzocchi G, Mancini M, Kerim S, Giugliano E, Albano F, Cuneo A, Abruzzese E, Martino B, Palandri F, Amabile M, Iacobucci I, Alimena G, Pane F, Martinelli G, Saglio G, Baccarani M, Rosti G. Deletions of the derivative chromosome 9 do not influence the response and the outcome of chronic myeloid leukemia in early chronic phase treated with imatinib mesylate: GIMEMA CML working party analysis. J Clin Oncol. 2010;28(16):2748–54.
40. Huntly BJP, Guilhot F, Reid AG, Vassiliou G, Hennig E, Franke C, Byrne J, Brizard A, Niederwieser D, Freeman-Edward J, Cuthbert G, Bown N, Clark RE, Nacheva EP, Green AR, Deininger MW. Imatinib improves but may not fully reverse the poor prognosis of patients with CML with derivative chromosome 9 deletions. Blood. 2003;102(6):2205–12.
41. Quintas-Cardama A, Kantarjian H, Shan J, Jabbour E, Abruzzo LV, Verstovsek S, Garcia-Manero G, O'Brien S, Cortes J. Prognostic impact of deletions of derivative chromosome 9 in patients with chronic myelogenous leukemia treated with nilotinib or dasatinib. Cancer. 2011;117(22):5085–93.
42. Luatti S, Baldazzi C, Marzocchi G, Ameli G, Bochicchio MT, Soverini S, Castagnetti F, Tiribelli M, Gugliotta G, Martinelli G, Baccarani M, Cavo M, Rosti G, Testoni N. Cryptic BCR-ABL fusion gene as variant rearrangement in chronic myeloid leukemia: molecular cytogenetic characterization and influence on TKIs therapy. Oncotarget. 2017;8(18):29906–13. https://doi.org/10.18632/oncotarget.15369.
43. Pelz AF, Kroning H, Franke A, Wieacker P, Stumm M. High reliability and sensitivity of the BCR/ABL1 D-FISH test for the detection of BCR/ABL rearrangements. Ann Hematol. 2002;81(3):147–53.
44. Virgili A, Brazma D, Reid AG, Howard-Reeves J, Valganon M, Chanalaris A, De MV, Marin D, Apperley JF, Grace C, Nacheva EP. FISH mapping of Philadelphia negative BCR/ABL1 positive CML. Mol Cytogenet. 2008;1:14.
45. Heim S, Mitelman F. Cancer cytogenetics: chromosomal and molecular genetic aberrations of tumor cells. 4th ed. Chichester: Wiley Blackwell; 2015.
46. Cortes JE, Talpaz M, Giles F, O'Brien S, Rios MB, Shan J, Garcia-Manero G, Faderl S, Thomas DA, Wierda W, Ferrajoli A, Jeha S, Kantarjian HM. Prognostic significance of cytogenetic clonal evolution in patients with chronic myelogenous leukemia on imatinib mesylate therapy. Blood. 2003;101(10):3794–800.
47. Anastasi J, Feng J, Le Beau MM, Larson RA, Rowley JD, Vardiman JW. The relationship between

secondary chromosomal abnormalities and blast transformation in chronic myelogenous leukemia. Leukemia. 1995;9(4):628–33.

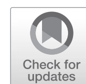
48. Hehlmann R, Voskanyan A, Lauseker M, Pfirrmann M, Kalmanti L, Rinaldetti S, Kohlbrenner K, Haferlach C, Schlegelberger B, Fabarius A, Seifarth W, Spieß B, Wuchter P, Krause S, Kolb H-J, Neubauer A, Hossfeld DK, Nerl C, Gratwohl A, Baerlocher GM, Burchert A, Brümmendorf TH, Hasford J, Hochhaus A, Saußele S, Baccarani M, von Weikersthal LF, Hahn M, Schlimok G, Reichert D, Janssen J, Martens U, Majunke P, Reichert P, Neben K, Korsten S, Scholz C, Oldenkott B, Heßling J, Kingreen D, Sperling C, Schelenz C, Blau I, Urmersbach A, Ludwig W, Le Coutre P, Arnold R, de Wit M, Pezzutto A, Schäfer E, Schroers R, Lochter A, Behringer D, Ko Y, Weidenhöfer S, Verbeek W, Brossart P, Trenn G, Pommerien W, Krauter J, Doering G, Munzinger H, Diekmann C, Hertenstein B, Stier S, Möller-Faßbender F, Hänel M, Zöller T, Lamberti C, Koch B, Henzel A, Wagner S, Schmalenbach A, Hoffknecht M, Ehninger G, Kiani A, Illmer T, Aul C, Flaßhove M, Henneke F, Simon M, Müller L, Becker H, Janz R, Eckart MJ, Fuchs R, Schlegel F, Wattad M, Rudolph R, Beelen DW, Lindemann A, Linck D, Wassman JE, Al-Batran S, Reiber T, Waller CF, Hoeffkes H, Schulz L, Tajrobehkar K, Mittermüller J, Pralle H, Runde V, Hoyer A, Tessen H, Trümper L, Schmidt C, Sieber M, Eschenburg H, Depenbusch R, Rösel S, Lindemann HW, Wolf H, Spohn C, Moeller R, Hossfeld D, Zander A, Schafhausen P, Köster H, Hollburg W, Schmitz N, Dürk H, Hemeier M, Grote-Metke A, Weischer H, Bechtel B, Balleisen L, Sosada M, Ho A, Petersen V, Dengler J, Bildat S, Hahn L, Dietzfelbinger H, Gröschel W, Bartholomäus A, Freier W, Sievers B, Pfreundschuh IM, Herrmann T, Fauser A, Menzel J, Kemmerling M, Hansen R, Link H, Schatz M, Bentz M, Prümmer O, Kneba M, Heymanns J, Schmitz S, Scheid C, Lollert A, Neise M, Planker M, Stauch M, Schröder M, Kempf B, Vehling-Kaiser U, Kremers S, Köchling G, Müller L, Hartmann F, Neuhaus T, Fetscher S, Kämpfe D, Heil G, Uppenkamp M, Goldmann B, Huber TF, Hieber U, Plöger C, Griesshammer M, Lange C, Göttler B, Lunscken C, Schiel X, Scheidegger C, Stötzer O, Hitz H, Schick H, Völkl S, Spiekermann K, Berdel W, Hebart H, Ladda E, Schmidt P, Burkhardt U, Hentschke S, Falge C, Reschke D, Köhne CA, Müller-Naendrup C, Sauer M, Frühauf S, Ranft K, Dencausse Y, Sandritter B, Baake G, Hofknecht M, Dengler R, Edinger M, Schenk M, Wehmeier A, Weidelich HP, Pihusch R, Stahlhut K, Baldus M, Matzdorff A, Geer T, Schanz S, Käfer G, Gassmann W, Priebe-Richter C, Demandt M, Springer G, Fiechtner H, Denzlinger C, Schleicher J, Assman D, Gaeckler R, Adam G, Waladkhani A, Rendenbach B, Forstbauer H, Kanz L, Jacki S, Stegelmann F, Kalhori N, Nusch A, Langer W, Müller F, Brettner S, Uebelmesser B, Kamp T, Schadeck-Gressel C, Josten K, Klein O, Schwerdtfeger R, Baurmann H, Strotkötter H, Fett W, Raghavachar A, Maintz C, Goebler MC, Schlag R, Elsel W, Wernli M, Heim D, Wuillemin W, Hess U, Gmür J, Mayer J, for the S, the German CMLSG. High-risk additional chromosomal abnormalities at low blast counts herald death by CML. Leukemia. 2020; https://doi.org/10.1038/s41375-020-0826-9.
49. Gong Z, Medeiros LJ, Cortes JE, Chen Z, Zheng L, Li Y, Bai S, Lin P, Miranda RN, Jorgensen JL, McDonnell TJ, Wang W, Kantarjian HM, Hu S. Cytogenetics-based risk prediction of blastic transformation of chronic myeloid leukemia in the era of TKI therapy. Blood Adv. 2017;1(26):2541–52.
50. Hagemeijer A, Stenfert Kroeze WF, Abels J. Cytogenetic follow-up of patients with nonlymphocytic leukemia I. Philadelphia chromosome-positive chronic myeloid leukemia. Cancer Genet Cytogenet. 1980;2(4):317–26.
51. Mitelman F, Levan G, Nilsson PG, Brandt L. Non-random karyotypic evolution in chronic myeloid leukemia. Int J Cancer. 1976;18(1):24–30.
52. Fioretos T, Strombeck B, Sandberg T, Johansson B, Billstrom R, Borg A, Nilsson PG, van den Berghe H, Hagemeijer A, Mitelman F, Höglund M. Isochromosome 17q in blast crisis of chronic myeloid leukemia and in other hematologic malignancies is the result of clustered breakpoints in 17p11 and is not associated with coding TP53 mutations. Blood. 1999;94(1):225–32.
53. Coyle T, Najfeld V. Translocation (3;21) in Philadelphia chromosome-positive chronic myelogenous leukemia prior to the onset of blast crisis. Am J Hematol. 1988;27(1):56–9.
54. Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer (2021). Mitelman F, Johansson B and Mertens F (Eds.), https://mitelman-database.isb-cgc.org.
55. Wang W, Cortes JE, Lin P, Beaty MW, Ai D, Amin HM, McDonnell TJ, Ok CY, Kantarjian HM, Medeiros LJ, Hu S. Clinical and prognostic significance of 3q26.2 and other chromosome 3 abnormalities in CML in the era of tyrosine kinase inhibitors. Blood. 2015a;126(14):1699–706.
56. Chen Z, Shao C, Wang W, Zuo Z, Mou X, Hu SJ, DiGiuseppe JA, Zu Y, Medeiros LJ, Hu S. Cytogenetic landscape and impact in blast phase of chronic myeloid leukemia in the era of tyrosine kinase inhibitor therapy. Leukemia. 2017;31(3):585–92. https://doi.org/10.1038/leu.2016.231.
57. Ferro MT, Steegman JL, Escribano L, Heiurichs B, Parada L, Garcia-Sagredo JM, Resino M, Cabello P, San RC. Ph-positive chronic myeloid leukemia with t(8;21)(q22;q22) in blastic crisis. Cancer Genet Cytogenet. 1992;58(1):96–9.
58. Heim S, Christensen BE, Fioretos T, Sorensen AG, Pedersen NT. Acute myelomonocytic leukemia with inv(16)(p13q22) complicating Philadelphia chromosome positive chronic myeloid leukemia. Cancer Genet Cytogenet. 1992;59(1):35–8.
59. Pintado T, Ferro MT, San RC, Mayayo M, Larana JG. Clinical correlations of the 3q21;q26 cytogenetic

anomaly. A leukemic or myelodysplastic syndrome with preserved or increased platelet production and lack of response to cytotoxic drug therapy. Cancer. 1985;55(3):535–41.
60. Rubin CM, Larson RA, Bitter MA, Carrino JJ, Le Beau MM, Diaz MO, Rowley JD. Association of a chromosomal 3;21 translocation with the blast phase of chronic myelogenous leukemia. Blood. 1987;70(5):1338–42.
61. Wang W, Tang G, Cortes JE, Liu H, Ai D, Yin CC, Li S, Khoury JD, Bueso-Ramos C, Medeiros LJ, Hu S. Chromosomal rearrangement involving 11q23 locus in chronic myelogenous leukemia: a rare phenomenon frequently associated with disease progression and poor prognosis. J Hematol Oncol. 2015c;8:32.
62. Yin CC, Medeiros LJ, Glassman AB, Lin P. T(8;21)(q22;q22) in blast phase of chronic myelogenous leukemia. Am J Clin Pathol. 2004;121(6):836–42.
63. Hoehn D, Lu G, Konoplev S, Zhou Y, Bueso-Ramos CE, Zuo Z, Hsu B, Medeiros LJ, Yin CC. t(15;17)(q24.1;q21.2)/PML-RARA in blast phase of chronic myelogenous leukemia: a rare form of clonal evolution. J Hematop. 2013;6(4):187–93.
64. Bernstein R, Morcom G, Pinto MR, Mendelow B, Dukes I, Penfold G, Bezwoda W. Cytogenetic findings in chronic myeloid leukemia (CML); evaluation of karyotype, blast morphology, and survival in the acute phase. Cancer Genet Cytogenet. 1980;2(1):23–37.
65. Andersson A, Olofsson T, Lindgren D, Nilsson B, Ritz C, Eden P, Lassen C, Rade J, Fontes M, Morse H, Heldrup J, Behrendtz M, Mitelman F, Hoglund M, Johansson B, Fioretos T. Molecular signatures in childhood acute leukemia and their correlations to expression patterns in normal hematopoietic subpopulations. Proc Natl Acad Sci U S A. 2005;102(52):19069–74.
66. Ross ME, Zhou X, Song G, Shurtleff SA, Girtman K, Williams WK, Liu HC, Mahfouz R, Raimondi SC, Lenny N, Patel A, Downing JR. Classification of pediatric acute lymphoblastic leukemia by gene expression profiling. Blood. 2003;102(8):2951–9.
67. Virtaneva K, Wright FA, Tanner SM, Yuan B, Lemon WJ, Caligiuri MA, Bloomfield CD, de la Chapelle A, Krahe R. Expression profiling reveals fundamental biological differences in acute myeloid leukemia with isolated trisomy 8 and normal cytogenetics. Proc Natl Acad Sci U S A. 2001;98(3):1124–9.
68. Schoch C, Kern W, Kohlmann A, Hiddemann W, Schnittger S, Haferlach T. Acute myeloid leukemia with a complex aberrant karyotype is a distinct biological entity characterized by genomic imbalances and a specific gene expression profile. Genes Chromosomes Cancer. 2005;43(3):227–38.
69. Schütte J, Opalka B, Becher R, Bardenheuer W, Szymanski S, Lux A, Seeber S. Analysis of the p53 gene in patients with isochromosome 17q and Ph1-positive or -negative myeloid leukemia. Leuk Res. 1993;17(6):533–539.
70. Rege-Cambrin G, Gaidano G, Serra A, Scaravaglio P, Guglielmelli T, Guerrasio A, Giovinazzo B, Saglio G. Analysis of the p53 gene in myeloid malignancies associated with chromosomal abnormalities involving the short arm of chromosome 17. Leukemia. 1994;8 Suppl 1:S23–6.
71. Collins SJ, Groudine MT. Chronic myelogenous leukemia: amplification of a rearranged c-abl oncogene in both chronic phase and blast crisis. Blood. 1987;69(3):893–8.
72. Al-Achkar W, Wafa A, Ikhtiar A, Liehr T. Three-way Philadelphia translocation t(9;10;22)(q34;p11.2;q11.2) as a secondary abnormality in an imatinib mesylate-resistant chronic myeloid leukemia patient. Oncol Lett. 2013;5(5):1656–8.
73. Gaiger A, Henn T, Horth E, Geissler K, Mitterbauer G, Maier-Dobersberger T, Greinix H, Mannhalter C, Haas OA, Lechner K, Lion T. Increase of bcr-abl chimeric mRNA expression in tumor cells of patients with chronic myeloid leukemia precedes disease progression. Blood. 1995;86(6):2371–8.
74. Andrews DF III, Collins SJ. Heterogeneity in expression of the bcr-abl fusion transcript in CML blast crisis. Leukemia. 1987;1(10):718–24.
75. Ogawa S, Kurokawa M, Tanaka T, Tanaka K, Hangaishi A, Mitani K, Kamada N, Yazaki Y, Hirai H. Increased Evi-1 expression is frequently observed in blastic crisis of chronic myelocytic leukemia. Leukemia. 1996;10(5):788–94.
76. Roche-Lestienne C, Deluche L, Corm S, Tigaud I, Joha S, Philippe N, Geffroy S, Lai JL, Nicolini FE, Preudhomme C. RUNX1 DNA-binding mutations and RUNX1-PRDM16 cryptic fusions in BCR-ABL+ leukemias are frequently associated with secondary trisomy 21 and may contribute to clonal evolution and imatinib resistance. Blood. 2008;111(7):3735–41.
77. Preudhomme C, Warot-Loze D, Roumier C, Grardel-Duflos N, Garand R, Lai JL, Dastugue N, MacIntyre E, Denis C, Bauters F, Kerckaert JP, Cosson A, Fenaux P. High incidence of biallelic point mutations in the runt domain of the AML1/PEBP2 alpha B gene in Mo acute myeloid leukemia and in myeloid malignancies with acquired trisomy 21. Blood. 2000;96(8):2862–9.
78. Branford S, Kim DDH, Apperley JF, Eide CA, Mustjoki S, Ong ST, Nteliopoulos G, Ernst T, Chuah C, Gambacorti-Passerini C, Mauro MJ, Druker BJ, Kim DW, Mahon FX, Cortes J, Radich JP, Hochhaus A, Hughes TP. Laying the foundation for genomically-based risk assessment in chronic myeloid leukemia. Leukemia. 2019;33(8):1835–50. https://doi.org/10.1038/s41375-019-0512-y.
79. Grossmann V, Kohlmann A, Zenger M, Schindela S, Eder C, Weissmann S, Schnittger S, Kern W, Muller MC, Hochhaus A, Haferlach T, Haferlach C. A deep-sequencing study of chronic myeloid leukemia patients in blast crisis (BC-CML) detects mutations in 76.9% of cases. Leukemia. 2011;25(3):557–60.

80. Haferlach C, Bacher U, Schnittger S, Weiss T, Kern W, Haferlach T. Similar patterns of chromosome abnormalities in CML occur in addition to the Philadelphia chromosome with or without tyrosine kinase inhibitor treatment. Leukemia. 2010;24(3):638–40.
81. Fabarius A, Kalmanti L, Dietz CT, Lauseker M, Rinaldetti S, Haferlach C, Gohring G, Schlegelberger B, Jotterand M, Hanfstein B, Seifarth W, Hanel M, Kohne CH, Lindemann HW, Berdel WE, Staib P, Muller MC, Proetel U, Balleisen L, Goebeler ME, Dengler J, Falge C, Kanz L, Burchert A, Kneba M, Stegelmann F, Pfreundschuh M, Waller CF, Spiekermann K, Brummendorf TH, Edinger M, Hofmann WK, Pfirrmann M, Hasford J, Krause S, Hochhaus A, Saussele S, Hehlmann R. Impact of unbalanced minor route versus major route karyotypes at diagnosis on prognosis of CML. Ann Hematol. 2015;94(12):2015–24.
82. Wang W, Cortes JE, Lin P, Khoury JD, Ai D, Tang Z, Tang G, Jorgensen JL, Medeiros LJ, Hu S. Impact of trisomy 8 on treatment response and survival of patients with chronic myelogenous leukemia in the era of tyrosine kinase inhibitors. Leukemia. 2015b;29(11):2263–6.
83. Guttenbach M, Koschorz B, Bernthaler U, Grimm T, Schmid M. Sex chromosome loss and aging: in situ hybridization studies on human interphase nuclei. Am J Hum Genet. 1995;57(5):1143–50.
84. Verma D, Kantarjian H, Shan J, O'Brien S, Estrov Z, Garcia-Manero G, Koller C, Borthakur G, Cortes J. Survival outcomes for clonal evolution in chronic myeloid leukemia patients on second generation tyrosine kinase inhibitor therapy. Cancer. 2010;116(11):2673–81.
85. Wang W, Cortes JE, Tang G, Khoury JD, Wang S, Bueso-Ramos CE, DiGiuseppe JA, Chen Z, Kantarjian HM, Medeiros LJ, Hu S. Risk stratification of chromosomal abnormalities in chronic myelogenous leukemia in the era of tyrosine kinase inhibitor therapy. Blood. 2016;127(22):2742–50. https://doi.org/10.1182/blood-2016-01-690230.
86. Ariyama T, Inazawa J, Uemura Y, Kakazu N, Maekawa T, Urase F, Irimajiri K, Horiuchi A, Nakamura Y, Abe T. Clonal origin of Philadelphia chromosome negative cells with trisomy 8 appearing during the course of alpha-interferon therapy for Ph positive chronic myelocytic leukemia. Cancer Genet Cytogenet. 1995;81(1):20–3.
87. Fayad L, Kantarjian H, O'Brien S, Seong D, Albitar M, Keating M, Talpaz M. Emergence of new clonal abnormalities following interferon-alpha induced complete cytogenetic response in patients with chronic myeloid leukemia: report of three cases. Leukemia. 1997;11(5):767–71.
88. Bacher U, Hochhaus A, Berger U, Hiddemann W, Hehlmann R, Haferlach T, Schoch C. Clonal aberrations in Philadelphia chromosome negative hematopoiesis in patients with chronic myeloid leukemia treated with imatinib or interferon alpha. Leukemia. 2005;19(3):460–3.
89. Bumm T, Muller C, Al-Ali HK, Krohn K, Shepherd P, Schmidt E, Leiblein S, Franke C, Hennig E, Friedrich T, Krahl R, Niederwieser D, Deininger MW. Emergence of clonal cytogenetic abnormalities in Ph- cells in some CML patients in cytogenetic remission to imatinib but restoration of polyclonal hematopoiesis in the majority. Blood. 2003;101(5):1941–9.
90. Corbin AS, La Rosee P, Stoffregen EP, Druker BJ, Deininger MW. Several Bcr-Abl kinase domain mutants associated with imatinib mesylate resistance remain sensitive to imatinib. Blood. 2003;101(11):4611–4.
91. Issa GC, Kantarjian HM, Gonzalez GN, Borthakur G, Tang G, Wierda W, Sasaki K, Short NJ, Ravandi F, Kadia T, Patel K, Luthra R, Ferrajoli A, Garcia-Manero G, Rios MB, Dellasala S, Jabbour E, Cortes JE. Clonal chromosomal abnormalities appearing in Philadelphia chromosome-negative metaphases during CML treatment. Blood. 2017;130(19):2084–91. https://doi.org/10.1182/blood-2017-07-792143.
92. Medina J, Kantarjian HM, Talpaz M, O'Brien S, Garcia-Manero G, Giles F, Rios MB, Hayes K, Cortes J. Chromosomal abnormalities in Philadelphia chromosome-negative metaphases appearing during imatinib mesylate therapy in patients with Philadelphia chromosome-positive chronic myelogenous leukemia in chronic phase. Cancer. 2003;98(9):1905–11.
93. Meeus P, Demuynck H, Martiat P, Michaux L, Wouters E, Hagemeijer A. Sustained, clonal karyotype abnormalities in the Philadelphia chromosome negative cells of CML patients successfully treated with Imatinib. Leukemia. 2003;17(2):465–7.
94. Schoch C, Haferlach T, Kern W, Schnittger S, Berger U, Hehlmann R, Hiddemann W, Hochhaus A. Occurrence of additional chromosome aberrations in chronic myeloid leukemia patients treated with imatinib mesylate. Leukemia. 2003;17(2):461–3.
95. Terre C, Eclache V, Rousselot P, Imbert M, Charrin C, Gervais C, Mozziconacci MJ, Maarek O, Mossafa H, Auger N, Dastugue N, Talmant P, Van Den AJ, Leonard C, N'Guyen KF, Mugneret F, Viguie F, Lafage-Pochitaloff M, Bastie JN, Roux GL, Nicolini F, Maloisel F, Vey N, Laurent G, Recher C, Vigier M, Yacouben Y, Giraudier S, Vernant JP, Salles B, Roussi J, Castaigne S, Leymarie V, Flandrin G, Lessard M. Report of 34 patients with clonal chromosomal abnormalities in Philadelphia-negative cells during imatinib treatment of Philadelphia-positive chronic myeloid leukemia. Leukemia. 2004;18(8):1340–6.
96. Kantarjian HM, Sawyers C, Hochhaus A, Guilhot F, Schiffer C, Gambacorti-Passerini C, Niederwieser D, Resta D, Capdeville R, Zoellner U, Talpaz M, Druker BJ, Goldman J, O'Brien SG, Russell N, Fischer T, Ottmann O, Cony-Makhoul P, Facon T, Stone R, Miller C, Tallman M, Brown R, Loughran TP, Gratwohl A, Mandelli F, Saglio G, Lazzarino

M, Russo D, Baccarani M, Morra E. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. N Engl J Med. 2002;346(9):645–52.

97. O'Dwyer ME, Gatter KM, Loriaux M, Druker BJ, Olson SB, Magenis RE, Lawce H, Mauro MJ, Maziarz RT, Braziel RM. Demonstration of Philadelphia chromosome negative abnormal clones in patients with chronic myelogenous leukemia during major cytogenetic responses induced by imatinib mesylate. Leukemia. 2003;17(3):481–7.
98. Deininger MW, Cortes J, Paquette R, Park B, Hochhaus A, Baccarani M, Stone R, Fischer T, Kantarjian HM, Niederwieser D, Gambacorti-Passerini C, So C, Gathmann I, Goldman JM, Smith D, Druker BJ, Guilhot F. The prognosis for patients with chronic myeloid leukemia who have clonal cytogenetic abnormalities in Philadelphia chromosome-negative cells. Cancer. 2007;110(7):1509–19.
99. Schnittger S, Kuznia S, Meggendorfer M, Nadarajah N, Jeromin S, Alpermann T, Roller A, Albuquerque A, Weissmann S, Ernst T, Eder C, Dicker F, Kern W, Kohlmann A, Haferlach T, Hochhaus A, Haferlach C. Tyrosine kinase inhibitor treated CML patients Harboring Philadelphia-negative cytogenetically aberrant clones show molecular mutations in 31% of cases not present at diagnosis: a high-throughput amplicon sequencing study of 29 genes. Blood. 2013;122(21):611a.
100. Schnittger S, Meggendorfer M, Nadarajah N, Alpermann T, Kern W, Haferlach T, Haferlach C. In CML patients with good response to TKIs other gene mutations are frequently (37%) present in addition to Philadelphia negative, cytogenetically aberrant clones but are rare (4%) in cases with MMR and normal karyotype. Blood. 2014;124(21):3126a.
101. Wang JY. Regulation of cell death by the Abl tyrosine kinase. Oncogene. 2000;19(49):5643–50.
102. Groves MJ, Sales M, Baker L, Griffiths M, Pratt N, Tauro S. Factors influencing a second myeloid malignancy in patients with Philadelphia-negative −7 or del(7q) clones during tyrosine kinase inhibitor therapy for chronic myeloid leukemia. Cancer Genet. 2011;204(1):39–44.
103. Bidet A, Dulucq S, Smol T, Marceau-Renaut A, Morisset S, Coiteux V, Noel-Walter MP, Nicolini FE, Tigaud I, Luquet I, Struski S, Gaillard B, Penther D, Tondeur S, Nadal N, Hermet E, Veronese L, Rea D, Gervais C, Theisen O, Terre C, Cony-Makhoul P, Lefebvre C, Gaillard JB, Radford I, Vervaeke AL, Barin C, Chapiro E, Nguyen-Khac F, Etienne G, Preudhomme C, Mahon FX, Roche-Lestienne C. Poor prognosis of chromosome 7 clonal aberrations in Philadelphia-negative metaphases and relevance of potential underlying myelodysplastic features in chronic myeloid leukemia. Haematologica. 2019;104(6):1150–5. https://doi.org/10.3324/haematol.2018.208801.
104. Testoni N, Marzocchi G, Luatti S, Amabile M, Baldazzi C, Stacchini M, Nanni M, Rege-Cambrin G, Giugliano E, Giussani U, Abruzzese E, Kerim S, Grimoldi MG, Gozzetti A, Crescenzi B, Carcassi C, Bernasconi P, Cuneo A, Albano F, Fugazza G, Zaccaria A, Martinelli G, Pane F, Rosti G, Baccarani M. Chronic myeloid leukemia: a prospective comparison of interphase fluorescence in situ hybridization and chromosome banding analysis for the definition of complete cytogenetic response: a study of the GIMEMA CML WP. Blood. 2009;114(24):4939–43.
105. Lauseker M, Hanfstein B, Haferlach C, Schnittger S, Pfirrmann M, Fabarius A, Schlegelberger B, Saussele S, Dietz CT, Erben P, Hehlmann R, Hasford J, Hochhaus A, Muller MC. Equivalence of BCR-ABL transcript levels with complete cytogenetic remission in patients with chronic myeloid leukemia in chronic phase. J Cancer Res Clin Oncol. 2014;140(11):1965–9.
106. Cross NC, White HE, Colomer D, Ehrencrona H, Foroni L, Gottardi E, Lange T, Lion T, Machova PK, Dulucq S, Martinelli G, Oppliger LE, Pallisgaard N, Barbany G, Sacha T, Talmaci R, Izzo B, Saglio G, Pane F, Muller MC, Hochhaus A. Laboratory recommendations for scoring deep molecular responses following treatment for chronic myeloid leukemia. Leukemia. 2015;29(5):999–1003.
107. Cross NC, White HE, Muller MC, Saglio G, Hochhaus A. Standardized definitions of molecular response in chronic myeloid leukemia. Leukemia. 2012;26(10):2172–5.
108. Mahon FX, Rea D, Guilhot J, Guilhot F, Huguet F, Nicolini F, Legros L, Charbonnier A, Guerci A, Varet B, Etienne G, Reiffers J, Rousselot P. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre stop Imatinib (STIM) trial. Lancet Oncol. 2010;11(11):1029–35.
109. Rousselot P, Charbonnier A, Cony-Makhoul P, Agape P, Nicolini FE, Varet B, Gardembas M, Etienne G, Rea D, Roy L, Escoffre-Barbe M, Guerci-Bresler A, Tulliez M, Prost S, Spentchian M, Cayuela JM, Reiffers J, Chomel JC, Turhan A, Guilhot J, Guilhot F, Mahon FX. Loss of major molecular response as a trigger for restarting tyrosine kinase inhibitor therapy in patients with chronic-phase chronic myelogenous leukemia who have stopped imatinib after durable undetectable disease. J Clin Oncol. 2014;32(5):424–30.



2 The Biology and Pathogenesis of Chronic Myeloid Leukaemia

Naranie Shanmuganathan, Bradley Chereda, and Junia V. Melo

2.1 The Molecular Biology of CML

2.1.1 The t(9;22) Translocation and the BCR-ABL1 Gene

The Philadelphia (Ph) chromosome is formed by a reciprocal t(9;22)(q34;q11) translocation between the long arms of chromosomes 9 and 22, causing the juxtaposition of the BCR (breakpoint cluster region) and ABL1 (Abelson) genes. The *BCR-ABL1* fusion gene consists of the 5′ end of the BCR gene and the 3′ end of the ABL1 gene (Fig. 2.1a). The location of the BCR and ABL1 genomic breakpoints is highly variable, but the recombination usually involves fusion of intron 13 or 14 of BCR with a 140-kilobase (kb) region of ABL1 surrounding exons 1b and 2 (Fig. 2.1a) [1, 2]. Regardless of the breakpoint location on the ABL1 gene, mRNA splicing gives rise to major *BCR-ABL1* transcripts with e13a2 (BCR exon 13 and ABL1 exon 2) or e14a2 junctions, originally referred to as b2a2 and b3a2, respectively. Both transcripts result in the expression of a 210 kDa *BCR-ABL1* protein with a 75-amino acid difference. In <2% of chronic phase (CP)-CML, 'atypical' transcripts can form when the breakpoint occurs between exons 1 and 2 (e1a2 transcript) or exons 19 and 20 (e19a2) of BCR. Alternative atypical transcripts have also been described although even less frequently [3, 4].

There has been much debate regarding the consequence of a patient expressing either the e13a2 or e14a2 transcripts [2]. Before the tyrosine kinase inhibitor (TKI) era, most reports on large series refuted the importance of the BCR breakpoint [5–8]. However, a recent revival of this debate has found consistent evidence that patients with either the e14a2 transcript or both the e14a2 and e13a2 transcripts exhibit a higher platelet count, approximating 1.5 times higher than that in the e13a2 group [3, 9, 10]. Several laboratories have also found that patients with e14a2 transcripts achieve optimal ELN-defined responses more rapidly, including the deep

N. Shanmuganathan
Department of Haematology, Royal Adelaide Hospital and SA Pathology, Adelaide, Australia

Centre for Cancer Biology, University of South Australia, Adelaide, Australia

B. Chereda
Centre for Cancer Biology, University of South Australia, Adelaide, Australia

J. V. Melo (✉)
Faculty of Health and Medical Sciences, University of Adelaide, Adelaide, Australia

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
R. Hehlmann (ed.), *Chronic Myeloid Leukemia*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-71913-5_2

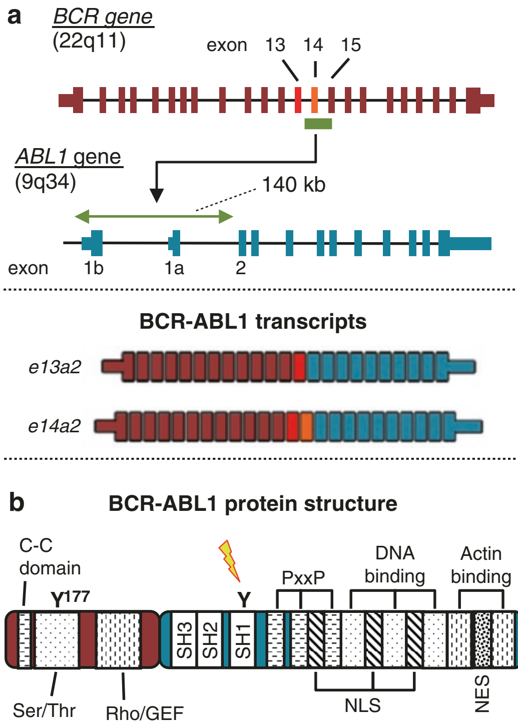


Fig. 2.1 The gene and protein structure of *BCR-ABL1*. (**a**) The *BCR-ABL1* fusion gene consists of the 5′ end of the *BCR* gene and the 3'end of *ABL1*. The location of the translocation usually involves fusion of intron 13 or 14 of *BCR* with a 140-kilobase (kb) region of *ABL1* surrounding exons 1b and 2. Examples of the two *BCR-ABL1* major mRNA isoforms are shown to highlight the *BCR* breakpoint variants. Depending on the breakpoint on the *ABL1* gene, exons 1a and/or 1b may be included in the primary transcript but are always excluded from the mRNA because they lack a splice acceptor sequence. (**b**) The BCR-ABL1 protein contains the dimerisation or coiled-coil (C-C), the Ser-Thr kinase and the Rho/GEF domains of BCR, as well as the SH-domains, Proline-rich (PxxP) nuclear localisation signal (NLS), DNA-binding nuclear export signal (NES) and Actin-binding domains from ABL. The ATP-binding site in the SH1 domain is indicated, highlighting the site of traditional tyrosine kinase inhibitor binding. The tyrosine residues in the Ser/Thr and SH1 kinase domains have been highlighted with a Y. The diagrams in A and B are not to scale

molecular response which is mandated for consideration of a treatment-free remission attempt [3, 11–13]. Patients expressing both transcripts tend to track with the e14a2 group [3, 10]. More recently, the transcript type has been demonstrated to influence long-term treatment-free remission outcomes with e14a2 expression correlating with higher treatment-free remission success [13, 14]. Furthermore, despite the rarity, atypical *BCR-ABL1* transcripts are generally associated with inferior outcomes [15, 16].

BCR-ABL1 has also been detected in healthy individuals with neither clinical nor laboratory evidence of CML [17–21]. With limited follow-up, these patients do not develop CML, likely due to these events being detected in terminally differentiated leukocytes as opposed to the leukaemic stem cell [19]. The absence of *BCR-ABL1* in the pluripotent stem cell explains the lack of proliferative potential, corroborating that this genetic event must develop in the leukaemic stem cell for CML to develop.

2.1.2 Protein Structure

The 210 kDa *BCR-ABL1* protein observed in CML contains more than ten protein domains (Fig. 2.1b). The SH1 tyrosine kinase region is the most studied due to its inherent role in CML pathogenesis and, consequently, the target for TKIs [22]. However other important regions include the SH2, SH3 and the N-terminal cap [23]. Myristoyl modification of the N-terminal cap permits the regulation of the kinase domain by SH2 and SH3 [24]. The fusion of BCR to ABL1 eventuates in loss of the N-terminal cap which results in constitutive activation of the SH1 kinase domain, inducing uncontrolled signal transduction and abnormal cellular proliferation [22]. TKIs, such as imatinib, compete with ATP for binding at the catalytic domain, inhibiting the phosphorylation of the tyrosine residues on substrates and impeding the downstream signalling effects of the oncogenic protein [22, 24]. In contrast, asciminib mimics the actions of the myristoyl site of the N-terminal cap, leading to the allosteric inhibition of *BCR-ABL1* [25].

2.1.3 The Consequence of BCR-ABL1

The *BCR-ABL1* protein gives rise to aberrant activation of cell signalling pathways and a shift to a micro-environment that is optimal for the development of leukaemia. For example, CML cells exhibit changes in growth-factor dependence, apoptosis, proliferation and cell adhesion [24]. These changes result in excessive proliferation of granulocytes, leading to the clinical features observed in CP-CML [26, 27]. The importance of *BCR-ABL1* signalling (particularly via the tyrosine kinase domain) is ultimately corroborated by the efficacy of TKI therapy.

BCR-ABL1 is a multi-faceted fusion gene with a marked effect on downstream signalling pathways, all of which promote the leukaemic phenotype observed in CML. Early work involving the transplantation of murine bone marrow transfected with *BCR-ABL1* induced a CML-like disease in transplanted mice [26, 28, 29]. Additional experiments confirmed the oncogenic potential of *BCR-ABL1* through gradual disruption of cellular differentiation, dysregulated proliferation, growth factor independence and interference of apoptosis through downstream signalling pathways [30–32]. Moreover, studies targeting *BCR-ABL1* by antisense oligonucleotides [33–36] demonstrated that *BCR-ABL1* was crucial for maintenance of the leukaemic process. These early observations underpinned the function of *BCR-ABL1* and provided affirmation that this fusion oncogene is the sole driver of CP-CML. However evolution to the more aggressive stages of CML is likely dependent on the cooperation of *BCR-ABL1* with other genetic events implicated in malignancy [37].

2.2 Important Pathways Affected by *BCR-ABL1* Activity

2.2.1 JAK/STAT

The JAK/STAT signalling pathway has been heavily implicated in leukaemogenesis, including the pathogenesis of CML [38]. *BCR-ABL1* augments activation of JAK2 through enhanced efficiency of JAK2 phosphorylation, promoting cell growth/survival while numerous STAT proteins are activated by the JAK receptor [39, 40]. Furthermore, murine models have illustrated the pivotal role of STAT5 signalling in the development and maintenance of CML. One experiment utilizing retroviral transduction of *BCR-ABL1* in STAT5-knockout bone marrow failed to induce CML in recipient mice after both primary and secondary transplantation [41]. In a second model, STAT5 deletion resulted in marked depletion of *BCR-ABL1*-expressing leukaemic cells, demonstrating the importance of STAT5 in the maintenance of CML [42]. Furthermore, enhanced STAT5 expression reduced imatinib-mediated cytotoxicity in *BCR-ABL1*-positive cells, potentially linked to marked anti-apoptotic activity mediated by increased STAT5 downstream signalling [43]. Increased STAT3 levels have also correlated with imatinib resistance [44]. Regardless of the mechanism, JAK inhibitors have exhibited efficacy against *BCR-ABL1*-positive cells, overcoming TKI resistance [45]. Furthermore, the combination of the JAK-inhibitor, ruxolitinib, and nilotinib has been demonstrated to induce undetectable *BCR-ABL1* levels in patients with low-level disease [46].

2.2.2 PI3K/AKT and Autophagy

PI3K proteins communicate extra-cellular signals to modulate transcription factor activation and programming that favour cell growth/survival and inhibition of cell death. AKT is a downstream effector of PI3K and plays a major role in its signalling [47]. *BCR-ABL1* can stimulate PI3K signalling through the adapter proteins Grb2/Gab2 [48] and CBL [49] but also through loss of function of the tumour suppressor gene PTEN, which is frequently silenced in malignancy [50]. Several reports indicate that the PI3K/AKT pathway is critical for *BCR-ABL1*-induced leukaemogenesis and for CML maintenance [51] and that its interruption can circumvent *BCR-ABL1* oncogenesis [52, 53]. Another

consequence of PI3K activation is stimulation of the mTOR pathway [54], which is responsible for controlling protein synthesis, cell growth/size and autophagy.

Autophagy can occur following cell stress (i.e. loss of *BCR-ABL1* signalling) to promote cell hibernation as opposed to apoptosis, and can be reversed with restoration of the optimal environment. Recent studies have observed that whilst *BCR-ABL1* inhibits autophagy, TKI treatment restores this pathway and may contribute to molecular relapse in failed treatment-free remission attempts, despite undetectable *BCR-ABL1* levels prior to TKI discontinuation [55]. Moreover, *BCR-ABL1*-positive stem cells with knockdown of genes vital for the autophagy process failed to proliferate in an optimized environment [56]. Therefore, autophagy may be an appropriate mechanism to target for the future.

2.2.3 Ras/MEK Pathway

Activation of Ras GTPases/MEK kinases stimulates cell growth via a membrane receptor-binding cascade to activate transcription of a number of growth factor genes and is a key pathway deregulated in cancer [57]. *BCR-ABL1* activates Ras via Grb2/Gab2 phosphorylation to promote cell growth [58, 59], and persistence of Ras activity has been demonstrated in TKI-resistant CML cells [60]. Disruption of Ras signalling impairs development of *BCR-ABL1*-induced CML-like disease in mice [49, 61]. In addition, MEK inhibitors can induce apoptosis in blast crisis (BC)- and drug-resistant CML cells with targeting of CML progenitors [60, 62]. Further work is required to investigate the true potential of inhibition of this pathway in CML.

2.2.4 Src Kinases

The Src-family kinases (SFKs) are another group of widely studied downstream targets of *BCR-ABL1*. Their role is to coordinate cell growth, differentiation and motility in response to extracellular signals [63]. Initial CML cell line models showed that *BCR-ABL1* expression significantly activated the Hck and Lyn SFKs [64]. Subsequent studies demonstrated that Hck, Lyn and Fyn were required for *BCR-ABL1* cell line transformation as well as functionally phosphorylating several *BCR-ABL1* tyrosines [65, 66]. One mechanism by which SFKs contribute to disease is in assisting *BCR-ABL1* in its activation of STAT5 and AKT [67, 68]. In addition, knockdown of Lyn exhibited impressive killing of BC cells, and its upregulation in BC-CML suggested a potential role for promoting disease progression [69, 70]. However, the importance of SFKs in CML remains unclear because mouse models show that SFKs are not required for initiation of CML but, rather, support the generation of acute lymphoid leukaemia [71, 72]. The second generation TKIs, dasatinib and bosutinib, are dual Src/Abl1 inhibitors, so defining the role of SFKs in CML could have an impact on both understanding its biology and treatment [73].

2.2.5 Crkl

The adaptor protein Crkl is constitutively activated by *BCR-ABL1* [74]. Protein networks involving *BCR-ABL1* and Crkl include Cbl, STAT, PI3K, paxillin and Ras [75]. Indeed, loss of the interaction between Ckrl and *BCR-ABL1* impaired *BCR-ABL1*-induced transformation in mice [76]. The potent phosphorylation of Crkl by *BCR-ABL1* allows the measurement of the percentage of phospho-Crkl as a surrogate to *BCR-ABL1* phosphorylation levels (which are more difficult to measure) in order to experimentally examine the patient's response to TKI therapy and to predict outcome [77].

2.2.6 Long Non-coding (lnc) RNA

LncRNAs are heavily involved in normal haematopoiesis and have increasingly been implicated in haematological malignancies [78]. In CML cell line models, lncRNA-BGL3 sensitizes *BCR-ABL1*-positive cells to imatinib-induced apoptosis [79]. It also acts as a decoy for several

microRNAs that target the tumour suppressor gene PTEN, leading to its stabilisation and associated inhibition of leukaemogenesis [79]. In contrast, lncRNA-H19 facilitates leukaemogenesis in CML through upregulation of MYC, and its knockdown perturbs the pathogenicity of *BCR-ABL1* in CML cell lines [80]. Further work is required to understand the full mechanisms and impact of these lncRNAs in CML.

2.2.7 Apoptosis Deregulation

In addition to promoting cell proliferation, *BCR-ABL1* can disrupt cell death. An example of this involves a *BCR-ABL1*, Bad, BCL2 and BCL-X_L circuit (Fig. 2.2). Expression of *BCR-ABL1* can inhibit apoptosis by increasing expression of the anti-apoptotic proteins BCL2 and BCL-X_L [81]. Both STAT5 and PI3K signalling are important mediators of *BCR-ABL1*'s anti-apoptotic function. STAT5 activation by *BCR-ABL1* causes increased BCL-X_L expression [82, 83]. Furthermore, phosphorylation of the pro-apoptotic protein Bad by PI3K/Akt facilitates the interaction between the chaperone protein 14–3-3 and Bad, which restricts Bad to the cytoplasm [84]. This prevents Bad opposing BCL2 and BCL-X_L inhibition of apoptosis in the mitochondrion.

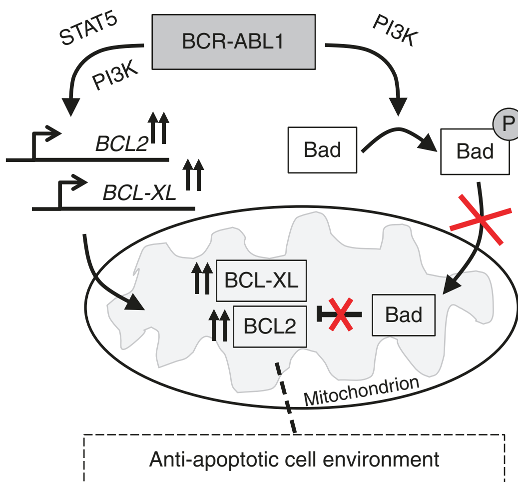


Fig. 2.2 An example of apoptotic circuitry controlled by *BCR-ABL1*. *BCR-ABL1* promotes the expression of anti-apoptotic genes BCL2 and BCL-XL and inhibits the function of pro-apoptotic protein Bad via phosphorylation (grey circle) and cytoplasmic sequestration

2.3 CML Stem Cells

2.3.1 Leukaemic Stem Cells (LSCs) Are Refractory to TKIs

A seminal paper from the Holyoake laboratory showed that *BCR-ABL1* inhibition reduced LSC proliferation but failed to deplete quiescent LSCs [85]. Furthermore, LSCs have also been shown to be insensitive to more potent second-generation TKIs, despite complete silencing of *BCR-ABL1* activity [86, 87]. These studies raised the possibility of early relapse despite TKI therapy, but long-term TKI usage has rebuffed this theory [88]. Subsequent studies have strengthened the notion that survival of the LSC is independent of *BCR-ABL1* activity [89, 90]. It has also been reported that therapy-refractory LSCs exhibit a bias for *low BCR-ABL1* expression [91, 92]. Persistence of the LSCs have been postulated to be the primary causes of molecular relapse following a treatment-free remission attempt despite long-term *BCR-ABL1* negativity [88]. Several pathways have been shown to play key roles in stem cell biology (Fig. 2.3), and targeting them could lead to a promising strategy to eliminate the LSC in CML.

2.3.2 Wnt/β-Catenin Pathway

The Wnt signalling pathway has been demonstrated to be crucial for LSC self-renewal [93], and β-catenin is one of its components [94, 95]. Binding of Wnt to its receptor, Frizzled, causes disruption of ubiquitin-mediated degradation of β-catenin, freeing the molecule for nuclear translocation to activate the transcription of target genes such as the *cyclin D1* and *MYC* oncogenes [96]. *BCR-ABL1* induces aberrant PI3K/AKT signalling, resulting in upregulated β-catenin activity [53], which has also been implicated in risk of progression to BC [97]. Enhanced

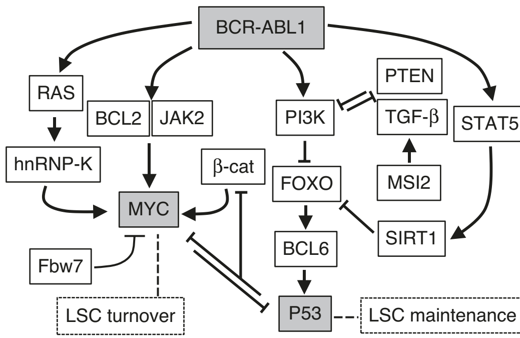


Fig. 2.3 LSC circuitry of genes discussed in this chapter. STAT5, JAK2 and PI3K all feature to control LSC-effector genes. However, quiescent stem cells have intrinsic counters to prevent potent *BCR-ABL1* signalling depleting the LSC population, such as MSI2/TGF-β, PTEN, FOXO transcription factors and Fbw7. In the context of BC-CML, the reliance on countering *BCR-ABL1* is not as important due to the incapacity of leukaemic progenitor cells to differentiate. This may explain how enhanced pathway activation (JAK2 / β-catenin) is compatible with expansion of the stem/progenitor compartment in BC. Hes1 activity enhances that of PI3K while the Hh pathway via the transmembrane receptors of PTCH and Smo regulate Gli signalling, also important for LSC maintenance

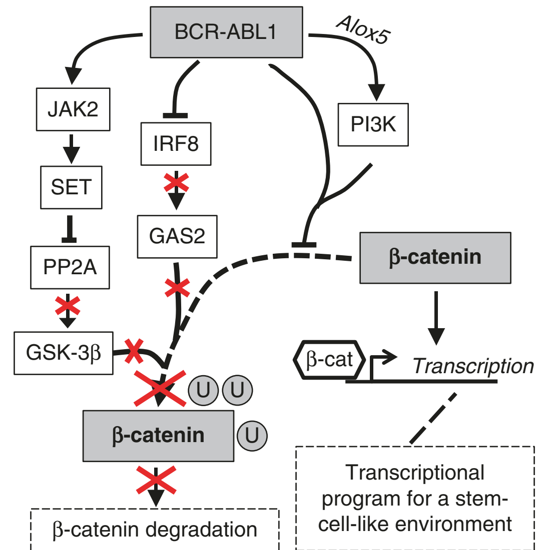


Fig. 2.4 Complex control of β-catenin in CML. *BCR-ABL1* stabilises β-catenin signalling via PI3K, JAK2 and inhibition of IRF8. Canonical stability of β-catenin is controlled by protein ubiquitination (grey circles). Thus, in CML, this pathway is activated to promote a stem-cell like environment. However, inhibition of, e.g. PP2A activation, can reverse pathogenic β-catenin signalling and synergise with *BCR-ABL1* inhibition to enhance treatment efficacy

β-catenin signalling in BC-CML confers stem-cell-like characteristics to progenitor cells leading to cellular expansion [98]. Future strategies may be to target both β-catenin and *BCR-ABL1*, as murine models have demonstrated that this approach is synergistic, delaying disease progression while depleting CML-LSCs [99] (Fig. 2.4).

2.3.3 Hedgehog (Hh) Pathway

Signalling in the Hh pathway is critical for LSC self-renewal and contributes to tissue homeostasis, regeneration and healing [100]. In *BCR-ABL1*-positive progenitor cells, increased Hh signalling is observed with marked upregulation in BC-CML [101, 102]. It also induces malignant expansions of LSCs in murine models [103]. Upregulation of Smoothened (Smo), a membrane receptor for the hedgehog ligand, has been found to augment LSCs and to drive disease progression [104]. Activation of Smo, in turn, activates Gli transcription factors, which drive expression of their downstream transcriptional targets [105]. Studies on primary CML cells found that Smo/Gli2 promoted LSC dormancy via cell cycle arrest, and an enhanced hedgehog pathway signature is observed in BC patients. Inhibition of Gli2 was able to restore LSC cycling and sensitise LSCs to TKI eradication [106]. Dual targeting with Smo inhibitors and TKIs may be a future therapeutic strategy to target both stem and progenitor cells as in vitro data suggest a reduced rate of leukaemic progression [100].

2.3.4 Notch Pathway

The Notch pathway has been demonstrated to be vital for cellular signalling and is dysregulated in multiple malignancies, including haematological cancers [107]. A member of the Notch family, Hairy enhancer of split 1 (Hes1), cooperates with *BCR-ABL1* to induce BC-CML in murine models [108]. Furthermore, over-expression of Hes1 has

been shown in BC but not CP, while dominant-negative Hes1 deterred growth of Hes1-expressing cell lines [108]. Interfering with the cross-talk between Notch signalling and *BCR-ABL1* may be achievable with combined targeting of both pathways and may be a treatment option for future exploration [109].

2.3.5 FoxO Family

BCR-ABL1 promotes deregulation of several transcription factors, including forkhead box class O (FoxO), through activation of the PI3K/Akt pathway [94]. Members of the FoxO family, in particular FoxO3a, are vital to the maintenance of LSCs [110]. *BCR-ABL1* promotes nuclear export and deactivation of these transcription factors via PI3K/Akt [111]. In mature cells, Akt signalling is strong and assists propagation of *BCR-ABL1*'s proliferative advantage. However, in LSCs, Akt signalling is inhibited by PTEN [112] and TGF-β [113]. This reverses *BCR-ABL1* inactivation of FoxO3a and allows for BCL6 transcription, which favours quiescence and self-renewal [112]. Targeting this mechanism with BCL6 or TGF-β inhibitors together with TKIs perturbed CML development and induced cell death/turnover of primitive CML cells [112, 113].

2.3.6 BCL2 Family

The proteins in the BCL2 family are key regulators of apoptosis and crucial for LSC survival [114]. BCL2 anti-apoptotic protein expression is increased in CML and is further increased in CML-BC. *BCR-ABL1* signalling also promotes CML cell survival by upregulation of anti-apoptotic BCL2 proteins, including BCL-X_L [115]. Furthermore, BCL2 acts synergistically with *BCR-ABL1* to induce BC-CML [116]. Another member of the BCL2 family, the BH3-only pro-apoptotic protein (BIM), is also down-regulated in CML, supporting LSC survival [116]. TKI therapy leads to upregulation of pro-apoptotic proteins, including BIM [117]. The presence of a common synonymous variant in the BH3 functional domain of BIM has been associated with imatinib resistance and inferior molecular target achievement [118]. Selective inhibition of BCL-2 through combined therapy with venetoclax (a novel BCL2 inhibitor primarily utilized in chronic lymphocytic leukaemia) and TKI has been demonstrated to target the LSC in a *BCR-ABL1* transgenic mouse model, potentially offering a long-term cure in CML [114].

2.3.7 PP2A-JAK2-SET

BCR-ABL1 was reported to circumvent the requirement for JAK2 in its activation of STAT5 [119], but there are data demonstrating a role for JAK2 within the LSC compartment. A network involving PP2A/JAK2/Set/GSK-3β was shown to play a critical role in LSC survival [120]. Central to this pathway is PP2A, a tyrosine phosphatase whose activity is impaired in CML. 'Active' PP2A has the ability to silence key pathways which are activated by *BCR-ABL1*, including *BCR-ABL1* itself [121]. In CML-LSCs, *BCR-ABL1*/JAK2 signalling overcomes PP2A activity by enhancing the activity of SET, a PP2A-inhibitor. Blocking the PP2A inhibitory role of SET restores PP2A function and impairs the self-renewal and survival of CML-LSCs but not normal haematopoietic stem cells (HSCs) [120]. A major mechanism by which PP2A activation affects LSC maintenance is thought to be the loss of β-catenin signalling via GSK-3β mediated ubiquitination. This is coupled with PP2A silencing of *BCR-ABL1* to allow for LSC turnover and reduced leukaemic potential.

2.3.8 Bone Marrow Microenvironment

HSCs reside in the bone marrow, which provides an environment that controls haemopoiesis by coordinating HSC renewal and differentiation into functional blood cells. The bone marrow supportive environment comprises the osteoblast and vascular niches [122, 123]. The former

promotes self-renewal and quiescence, while the vascular niche is permissive of differentiation into progenitor and subsequent functional cells. In CML, it is thought that the osteoblast niche nurtures LSCs, which may explain why LSCs do not require *BCR-ABL1* kinase activity to survive TKI exposure [124, 125]. This may also contribute to BC. Since progenitor cells attain stem cell like properties, a progenitor-contingent may retreat towards the osteoblast niche for protection against TKIs, whilst retaining cycling properties that allow for faster accumulation of mutations (compared to LSCs) required for transformation.

2.4 Biology of Blast Crisis

The mechanism of disease evolution to BC-CML is still incompletely understood. This stage of the disease is characterised by the expansion of haemopoietic progenitors that fail to differentiate and interfere with normal haematopoiesis. These progenitor cells gain self-renewal capacity, differentiation arrest and survival properties that lead to uncontrolled proliferation, [98] exhibiting more stem-cell like characteristics compared to CP-progenitors. This is partially attributed to increased β-catenin activity [98] but also marked genomic and genetic instability [126, 127]. Extra chromosomal abnormalities are observed in approximately 80% of BC patients (e.g. Ph duplication, trisomy 8 or 19, loss of 17p) [128]. Pathogenic mutations in tumour suppressor and oncogenes have also been detected in BC-CML [129], and it is hypothesised that these additional hits contribute to the transition into BC [127, 129]. The rapid recent technological advances in next-generation sequencing has not only enabled attempts at unmasking the genomic landscape involved in BC-CML but has further highlighted the vast gaps of knowledge which yet remain.

2.4.1 *BCR-ABL1* and *CML-BC*

Inhibition of *BCR-ABL1* kinase activity effectively delays the onset of BC but does not eliminate the primitive population that establishes advanced disease. One interpretation is that *BCR-ABL1* signalling is required for transition to BC, especially since progression is rare in TKI responsive patients. A number of studies have found increased expression of *BCR-ABL1* in BC compared to CP. This was observed when comparing matched CP and BC samples (from the same patient) at both the mRNA [130–133] and protein levels [121, 130, 134]. Additionally, it has been shown that cells expressing higher amounts of *BCR-ABL1* have an increase in genomic instability as well as perturbed differentiation, which are intrinsic properties of BC-CML [127, 135]. These findings imply more than a passenger role for *BCR-ABL1* in BC-transformation.

2.4.2 DNA Damage/Repair

BCR-ABL1 has been shown to facilitate genomic instability via disrupting DNA repair pathways, generating reactive oxygen species and inhibiting DNA-damage-induced apoptosis, all of which may lead to retention of genomic mutations [136–140]. These events are in part tied to the level of *BCR-ABL1* expression [141]. CML CD34+ cells express high levels of *BCR-ABL1* as compared to mature cells [132], and they are highly susceptible to genomic instability compared to their healthy counterparts [89]. Although not formally shown, it is reasonable to suggest that *BCR-ABL1* provides progenitor cells with the genomic plasticity required for malignant transformation [127, 142, 143].

2.4.3 *C/EBPα* and *hnRNP-E2*

Required for myeloid differentiation [144], C/EBPα expression is reduced in cell lines expressing *BCR-ABL1* [145]. These lines responded poorly to growth-factor-induced differentiation [135], but ectopic expression of C/EBPα and *BCR-ABL1* kinase inhibition were able to reverse this differentiation block [145]. Further experiments revealed that *BCR-ABL1* negatively regulates the expression of C/EBPα via upregulation of hnRNP-E2, an RNA-binding protein which

inhibits C/EBPα expression [135]. Interestingly, analysis of CML-patient cells found that loss of C/EBPα and expression of hnRNP-E2 was restricted to BC [135]. In addition, hnRNP-E2 upregulation and C/EBPα downregulation were directly proportional to increasing levels of *BCR-ABL1* [135]. To add extra complexity to this pathway, it was recently shown that the microRNA miR-328 acts in a non-canonical way to block hnRNP-E2 regulation of C/EBPα and promotes myeloid differentiation [146]. The expression of miR-328 negatively correlates with *BCR-ABL1* expression levels and is thus downregulated in BC [146]. These experiments provide evidence of a sophisticated circuit by which enhanced *BCR-ABL1* expression can facilitate a switch to BC by disrupting myeloid differentiation.

2.4.4 Important Pathways Involved in BC-CML

2.4.4.1 MYC

The MYC proto-oncogene was one of the first genes implicated in CML disease progression. MYC is a transcription factor which governs the expression of genes enabling cell growth and proliferation and, thus, commonly activated in cancer [147]. It was originally observed that patients with BC exhibited higher levels of MYC compared to CP patients [148]. This was followed by reports that ABL1 expression enhances MYC expression and that MYC is required for *BCR-ABL1*-induced transformation [149, 150]. Although excess MYC can induce apoptosis [151], early cell line models show that *BCR-ABL1* activation of BCL2 can inhibit MYC apoptotic activity whilst retaining its proliferative advantage [152]. This is one of many examples by which *BCR-ABL1* creates 'a perfect storm' to promote leukaemogenesis.

BCR-ABL1 can control MYC expression via PI3K, JAK2 and the transcription factor E2F1 [51, 153–155], while maintaining protein stability via MEK and hnRNP-K [156]. A CML mouse model demonstrated that MYC expression is required for CML maintenance and progression. It also showed that high levels of MYC are harmful for LSCs, and ubiquitination (degradation) of MYC by ubiquitin ligase Fbw7 keeps MYC levels in check in LSCs [157]. This provides a rationale for the constrained *BCR-ABL1* kinase activity observed in quiescent LSCs [120] and selection of low *BCR-ABL1* expression in TKI-refractive LSCs [91, 92] (suggesting that enhanced *BCR-ABL1* signalling is toxic for quiescent cells). These findings, coupled with MYC's established role in myeloid differentiation [158], present MYC deregulation as a strong candidate for driving BC-transformation in CML.

2.4.4.2 p53

The normal function of p53 is to respond to cell stress events, where it becomes activated and drives transcription of genes that decide cell fate (apoptosis, DNA repair, cell cycle arrest or senescence) [159]. Early genetic studies observed inactivating mutations of p53 in approximately 20% of CML patients who progressed to BC [160, 161]. Regulation of p53 by *BCR-ABL1* is complex and unclear, with both p53 activation [162, 163] and inactivation [164, 165] being reported. However, loss or inhibition of p53 promotes BC-like disease in mice [165–167], and stabilisation of p53 in BC cells induces apoptosis [167, 168]. It has also been shown that MYC over-expression is only toxic to LSCs if p53 is present [157].

2.4.4.3 XPO1

The nuclear export protein, XPO1, is another novel candidate for the regulation of BC. Its expression is enhanced in BC patients, and pharmacological blockade of its function was shown as sufficient to kill both CP and BC-primary CD34+ cells [169]. Inhibition of XPO1 in *BCR-ABL1*-positive cell lines demonstrated that impaired nuclear transport could explain XPO1-inhibition lethality. For example, both SET and p53 were abnormally enriched in the nucleus leading to their inactivation [169]. Additional experiments revealed that long-term XPO1 inhibition caused *BCR-ABL1* degradation (via loss of SET control of PP2A activity) whereas short-term inhibition shutdown STAT5, AKT and MEK signalling prior to affecting *BCR-ABL1* activity

[169]. This suggests that both *BCR-ABL1*-dependent and -independent cell death results through XPO1 inhibition. Remarkably, an XPO1 inhibitor reversed CML symptoms (WBC count/splenomegaly) in a patient with disease progression and who was resistant to TKI therapy, highlighting a potential strategy to treat advanced disease [169].

2.4.4.4 SIRT1

Expression of SIRT1 is enhanced in CML and is, in part, regulated by *BCR-ABL1*/STAT5 [170]. This protein-deacetylase has been linked to CML BC due to its disruption of LSC turnover and DNA repair. SIRT1 suppression of p53/FoxO-controlled LSC maintenance is believed to prolong the survival of CML LSCs [170, 171]. In contrast, knockout or inhibition of SIRT1 impairs CML development and disease progression in mice by reducing proliferative and self-renewal capacity of LSCs [170, 171]. SIRT1 regulation of the DNA repair protein Ku70 in CML cell lines causes enhancement of less faithful non-homologous end joining DNA repair, which enhances mutations [172]. The knowledge that SIRT1 provides a route for LSC survival and genomic instability—the key drivers of BC-CML—offers strong evidence that SIRT1 has a major role in BC development.

2.4.4.5 ADAR1

ADAR1 is an RNA editor whose enzymatic activity converts adenosine to inosine in RNA, resulting in these nucleotides being interpreted as guanine in the ribosome, thus altering RNA behaviour and protein amino acid composition. Analysis of ADAR1 expression in CML patients showed a marked increase in expression from CP to BC, and was correlated with *BCR-ABL1* levels [173]. The BC samples also had enhanced A to I editing and altered expression of RNA-edited genes, providing evidence that the increased expression of ADAR1 in BC had a functional effect on its downstream targets [173]. Two mouse models have been developed which successfully demonstrate the important role that ADAR1 plays in CML stem cells. Following disruption of ADAR1 expression in CML mouse models, leukaemia development, maintenance and BC onset were all impaired due to the loss of primitive leukaemic cells [174]. In contrast, ADAR1 over-expression caused myeloid progenitor expansion [173]. Moreover, specific deletion of the ADAR1's RNA-editing moiety demonstrated that RNA editing is vital for CML progenitor self-renewal [174]. It is known that the RNA-editing activity of ADAR1 is required for HSC survival [175], so it is speculated that the enhanced activity of ADAR1 in BC locks the LSCs in a primitive state.

2.4.4.6 Polycomb Repressive Complexes (PRCs) and Epigenetic Regulation

Dysregulation of PRCs have been implicated in a number of haematological malignancies, including CML [176]. Early data indicated that overexpression of BMI1, a member of PRC1, correlated with inferior survival and higher risk of BC transformation [177]. Enhanced EZH2 activity, a catalytic subunit of PRC2, has also been demonstrated as necessary for the propagation of CML [178]. More recent exploration of the BC genome indicates substantial enrichment for mutations affecting the PRCs: transcriptomic interrogation of BC progenitors demonstrated both upregulation and depletion of PRC1- and PRC2-related gene sets, respectively [179].

The impact of epigenetic reprogramming is still an emerging area of research in CML. The PRCs are heavily involved with epigenetic reprogramming in BC-CML with PRC2-driven DNA hypermethylation being responsible for arrested myeloid differentiation and loss of tumour suppressor function [179]. However, DNA methylation inhibitors have failed to produce durable responses in BC-CML [179]. Gene expression analysis of BC-cells treated with hypomethylating agents revealed failure to normalize the majority of the gene expression changes associated with DNA-methylation, indicating additional layers of unidentified epigenetic regulation [179]. However, in vitro combinatorial therapy with directed inhibition of BMI1 and hypomethylating agents reduced colony formation in BC-CML cell lines by approximately 90% [179].

2.4.4.7 Mutational Landscape

While *BCR-ABL1* alone is sufficient to induce CP-CML, it is unlikely to be the sole event in more advanced stages of the disease. *BCR-ABL1* has been associated with substantial genetic instability [180], assisting in the acquisition of additional mutational events that could trigger progression to BC. In order to identify putative BC driver genes, Giotopoulos et al. utilised an impressive mouse model. The experiment centred on a transposable cassette array in the presence or absence of *BCR-ABL1* [181]. Transposition of the cassettes can either activate or deactivate the genes in proximity to the genomic insertion site. Gene activation is achieved by a transposition event within the 5′ region of the gene due to enhancer/promoter sequences in the cassette [181]. Conversely, intragenic transposition can disrupt genes causing loss-of-function. Mice with a *BCR-ABL1* only genetic background succumbed to a CML-CP phenotype, whilst 85% of the *BCR-ABL1*/transposon mice exhibited CML-BC, 5% CP and 10% AP-like disease [181]. Microarray gene expression analysis of the mice showed clustering within disease type and inter-type separation, identifying several genes known to be involved in in the development of BC. Transposition events within the BC sample cohort included STAT5, XPO1, PTEN, MYC-target genes and JAK1 [181].

The current era has been characterized by dramatic technological advances in next-generation sequencing which have enabled the identification of somatic mutational profiles that characterize various haematological malignancies, influencing diagnosis, treatment and prognosis [182–184]. In BC-CML, most patients have been identified to harbour additional mutational events in known cancer genes [185, 186] seen in up to 95% of patients in one study [185]. Mutations in RUNX1, ASXL1 and IKZF1 exon deletions are the most frequently observed events [187] while single nucleotides, insertions, deletions, fusions and aberrant splicing in multiple different cancer-related genes have all been described in BC-CML. Aberrant RAG-mediated recombination has also been demonstrated to contribute to structural rearrangements in lymphoid BC [188]. A novel class of variant, termed 'Ph-associated rearrangements', involving gene rearrangements and novel fusions on the chromosome arms involved with the inciting Ph-translocation, has also been observed in poor outcome patients at the time of diagnosis, including those progressing to BC-CML [185]. Moreover, the Ph-associated rearrangements were more frequently identified in patients progressing to lymphoid BC [185]. While there are minimal data regarding this novel group of mutations, their presence may highlight a cohort of patients with increased genetic instability and, therefore, increased propensity for adverse outcomes. Kinase domain mutations can be identified in approximately 50% of patients in BC-CML [185], more frequently in lymphoid BC [185]. However, these are rarely the sole event [185], frequently co-occurring with IKZF1 variants. Additionally, cancer-gene variants often pre-date the development of kinase domain mutations in approximately 60% of patients, emphasizing the genomic instability associated with the acquisition of cancer-gene mutations [185].

2.5 Concluding Remarks

The biology of CML is centred on *BCR-ABL1*'s constitutive kinase activity, which is sufficient to cause the clinical features of CP. The ability to readily model CML in both cell lines and mice has allowed for a large accumulation of knowledge regarding the molecular network of CML. These studies have shown that *BCR-ABL1* is implicated in altering almost every process within the cell to drive CML pathogenesis. This extends to dampening its own excessive signalling in LSCs, which would be otherwise unfavourable. Current literature has shown that STAT5 stands out as a vital component of *BCR-ABL1*'s induction of CML as demonstrated by two conditional knockout models [41, 189]. The investigation of primitive CML-cell biology has benefitted from the utilisation of new and powerful techniques to identify a number of important genes within this compartment. The best studied

are p53, MYC and β-catenin, which have prominent roles in both stem cell biology and BC transformation.

The link between LSCs and BC and treatment response has put the LSC and progenitor populations at the forefront of CML biology. Of particular interest is the finding that LSCs do not rely on *BCR-ABL1* kinase activity for survival. It is unknown if another protein domain of *BCR-ABL1* confers LSC survival properties. Another possibility is that *BCR-ABL1* can program LSCs in such a way that its kinase activity is no longer required. It is unknown whether the HSC or progenitor compartment gives rise to the clones responsible for BC-CML. Pinpointing the latter is important because each of these compartments has discrete biological properties and, thus, requires alternative therapeutic strategies.

Next-generation sequencing and powerful experimental modelling tools will no doubt provide a flood of information regarding CML biology as well as highlight the potential drivers of disease progression. These advances are likely to generate evidence of recurrent mutations and epigenetic marks that favour or hinder CML pathogenesis or response to treatment.

In the proteomics field, improved methods to study proteins and more powerful mass spectrometers have the potential to uncover post-translational modifications and protein interactomes. The study of proteome networks is relatively untapped in CML (although elegant examples do exist [190, 191]), making this an attractive area of interest to improve the knowledge of CML biology. The same can be said of non-coding RNA (ncRNA). It is known that deregulation of these molecules occurs in CML, for example in CP versus BC, and in primitive cells versus granulocytes [192, 193]. However, most functional work is limited to a single microRNA and target. Further work is required to understand the global ncRNA circuitry in key areas within this disease. These fields of interest are bolstered with the emerging accessibility to high-powered fluorescence microscopy, which can monitor the spatiotemporal behaviour of proteins and RNA.

Finally, availability of pathway inhibitors and genome editing (including crispR) systems [194] are powerful options to functionally validate pathways identified by genomic and proteomic studies in both cell lines and mouse models. These technologies will make for an exciting time to uncover novel mechanisms behind CML pathogenesis and the potential for translation to other diseases.

Conflict of Interest The authors declare that they have no conflict of interest for the writing of this chapter.

References

1. Score J, Calasanz MJ, Ottman O, Pane F, Yeh RF, Sobrinho-Simoes MA, et al. Analysis of genomic breakpoints in p190 and p210 BCR-ABL indicate distinct mechanisms of formation. Leukemia. 2010;24(10):1742–50. https://doi.org/10.1038/leu.2010.174.
2. Melo JV. The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. Blood. 1996;88(7):2375–84.
3. Jain P, Kantarjian H, Patel KP, Gonzalez GN, Luthra R, Shamanna RK, et al. Impact of BCR-ABL transcript type on outcome in patients with chronic-phase CML treated with tyrosine kinase inhibitors. Blood. 2016;127(10):1269–75. https://doi.org/10.1182/blood-2015-10-674242.
4. Baccarani M, Castagnetti F, Gugliotta G, Rosti G, Soverini S, Albeer A, et al. The proportion of different BCR-ABL1 transcript types in chronic myeloid leukemia. An international overview. Leukemia. 2019;33(5):1173–83. https://doi.org/10.1038/s41375-018-0341-4.
5. Dowding C, Guo AP, Maisin D, Gordon MY, Goldman JM. The effects of interferon-alpha on the proliferation of CML progenitor cells in vitro are not related to the precise position of the M-BCR breakpoint. Br J Haematol. 1991;77(2):165–71.
6. Fioretos T, Nilsson PG, Aman P, Heim S, Kristoffersson U, Malm C, et al. Clinical impact of breakpoint position within M-bcr in chronic myeloid leukemia. Leukemia. 1993;7(8):1225–31.
7. Rozman C, Urbano-Ispizua A, Cervantes F, Rozman M, Colomer D, Feliz P, et al. Analysis of the clinical relevance of the breakpoint location within M-BCR and the type of chimeric mRNA in chronic myelogenous leukemia. Leukemia. 1995;9(6):1104–7.
8. Shepherd P, Suffolk R, Halsey J, Allan N. Analysis of molecular breakpoint and m-RNA transcripts in a prospective randomized trial of interferon in chronic myeloid leukaemia: no correlation with clinical features, cytogenetic response, duration

of chronic phase, or survival. Br J Haematol. 1995;89(3):546–54.

9. Balatzenko G, Vundinti BR, Margarita G. Correlation between the type of bcr-abl transcripts and blood cell counts in chronic myeloid leukemia - a possible influence of mdr1 gene expression. Hematol Rep. 2011;3(1):e3. https://doi.org/10.4081/hr.2011.e3. hr.2011.e3 [pii]
10. Hanfstein B, Lauseker M, Hehlmann R, Saussele S, Erben P, Dietz C, et al. Distinct characteristics of e13a2 versus e14a2 BCR-ABL1 driven chronic myeloid leukemia under first-line therapy with imatinib. Haematologica. 2014;99(9):1441–7. https://doi.org/10.3324/haematol.2013.096537.
11. Castagnetti F, Gugliotta G, Breccia M, Iurlo A, Levato L, Albano F, et al. The BCR-ABL1 transcript type influences response and outcome in Philadelphia chromosome-positive chronic myeloid leukemia patients treated frontline with imatinib. Am J Hematol. 2017;92(8):797–805. https://doi.org/10.1002/ajh.24774.
12. Genthon A, Nicolini FE, Huguet F, Colin-Gil C, Berger M, Saugues S, et al. Influence of major BCR-ABL1 transcript subtype on outcome in patients with chronic myeloid leukemia in chronic phase treated frontline with nilotinib. Oncotarget. 2020;11:26.
13. D'Adda M, Farina M, Schieppati F, Borlenghi E, Bottelli C, Cerqui E, et al. The e13a2 BCR-ABL transcript negatively affects sustained deep molecular response and the achievement of treatment-free remission in patients with chronic myeloid leukemia who receive tyrosine kinase inhibitors. Cancer. 2019;125(10):1674–82. https://doi.org/10.1002/cncr.31977.
14. Claudiani S, Apperley JF, Gale RP, Clark R, Szydlo R, Deplano S, et al. e14a2 BCR-ABL1 transcript is associated with a higher rate of treatment-free remission in individuals with chronic myeloid leukemia after stopping tyrosine kinase inhibitor therapy. Haematologica. 2017;102(8):e297–e9. https://doi.org/10.3324/haematol.2017.168740.
15. Gong Z, Medeiros LJ, Cortes JE, Zheng L, Khoury JD, Wang W, et al. Clinical and prognostic significance of e1a2 BCR-ABL1 transcript subtype in chronic myeloid leukemia. Blood Cancer J. 2017;7(7):e583-e. https://doi.org/10.1038/bcj.2017.62.
16. Qin Y-Z, Jiang Q, Jiang H, Lai Y-Y, Shi H-X, Chen W-M, et al. Prevalence and outcomes of uncommon BCR-ABL1 fusion transcripts in patients with chronic myeloid leukaemia: data from a single centre. Br J Haematol. 2018;182(5):693–700. https://doi.org/10.1111/bjh.15453.
17. Biernaux C, Loos M, Sels A, Huez G, Stryckmans P. Detection of major bcr-abl gene expression at a very low level in blood cells of some healthy individuals. Blood. 1995;86(8):3118–22.
18. Bose S, Deininger M, Gora-Tybor J, Goldman JM, Melo JV. The presence of typical and atypical BCR-ABL fusion genes in leukocytes of normal individuals: biologic significance and implications for the assessment of minimal residual disease. Blood. 1998;92(9):3362–7.
19. Basecke J, Griesinger F, Trumper L, Brittinger G. Leukemia- and lymphoma-associated genetic aberrations in healthy individuals. Ann Hematol. 2002;81(2):64–75. https://doi.org/10.1007/s00277-002-0427-x.
20. Boquett JA, Alves JR, de Oliveira CE. Analysis of BCR/ABL transcripts in healthy individuals. Genet Mol Res. 2013;12(4):4967–71. https://doi.org/10.4238/2013.October.24.8.
21. Ismail SI, Naffa RG, Yousef AM, Ghanim MT. Incidence of bcr-abl fusion transcripts in healthy individuals. Mol Med Rep. 2014;9(4):1271–6. https://doi.org/10.3892/mmr.2014.1951.
22. Goldman JM, Melo JV. Chronic myeloid Leukemia—advances in biology and new approaches to treatment. N Engl J Med. 2003;349(15):1451–64. https://doi.org/10.1056/NEJMra020777.
23. Hantschel O. Structure, regulation, signaling, and targeting of abl kinases in cancer. Genes Cancer. 2012;3(5–6):436–46. https://doi.org/10.1177/1947601912458584.
24. Quintás-Cardama A, Cortes J. Molecular biology of bcr-abl1–positive chronic myeloid leukemia. Blood. 2009;113(8):1619–30. https://doi.org/10.1182/blood-2008-03-144790.
25. Wylie AA, Schoepfer J, Jahnke W, Cowan-Jacob SW, Loo A, Furet P, et al. The allosteric inhibitor ABL001 enables dual targeting of BCR–ABL1. Nature. 2017;543:733. https://doi.org/10.1038/nature21702. https://www.nature.com/articles/nature21702#supplementary-information
26. Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science (New York, NY). 1990;247(4944):824–30.
27. Deininger MW, Goldman JM, Melo JV. The molecular biology of chronic myeloid leukemia. Blood. 2000;96(10):3343–56.
28. Heisterkamp N, Jenster G, ten Hoeve J, Zovich D, Pattengale PK, Groffen J. Acute leukaemia in bcr/abl transgenic mice. Nature. 1990;344(6263):251–3. https://doi.org/10.1038/344251a0.
29. Kelliher MA, McLaughlin J, Witte ON, Rosenberg N. Induction of a chronic myelogenous leukemia-like syndrome in mice with v-abl and BCR/ABL. Proc Natl Acad Sci U S A. 1990;87(17):6649–53.
30. Bedi A, Zehnbauer BA, Barber JP, Sharkis SJ, Jones RJ. Inhibition of apoptosis by BCR-ABL in chronic myeloid leukemia. Blood. 1994;83(8):2038–44.
31. Daley GQ, Baltimore D. Transformation of an interleukin 3-dependent hematopoietic cell line by the chronic myelogenous leukemia-specific P210bcr/abl protein. Proc Natl Acad Sci U S A. 1988;85(23):9312–6.
32. Hariharan IK, Adams JM, Cory S. bcr-abl oncogene renders myeloid cell line factor independent:

potential autocrine mechanism in chronic myeloid leukemia. Oncogene Res. 1988;3(4):387–99.
33. Ratajczak MZ, Kant JA, Luger SM, Hijiya N, Zhang J, Zon G, et al. In vivo treatment of human leukemia in a scid mouse model with c-myb antisense oligodeoxynucleotides. Proc Natl Acad Sci U S A. 1992;89(24):11823–7.
34. Skorski T, Szczylik C, Malaguarnera L, Calabretta B. Gene-targeted specific inhibition of chronic myeloid leukemia cell growth by BCR-ABL antisense oligodeoxynucleotides. Folia Histochem Cytobiol. 1991;29(3):85–9.
35. Szczylik C, Skorski T, Nicolaides NC, Manzella L, Malaguarnera L, Venturelli D, et al. Selective inhibition of leukemia cell proliferation by BCR-ABL antisense oligodeoxynucleotides. Science (New York, NY). 1991;253(5019):562–5.
36. Engelman A, Rosenberg N. Temperature-sensitive mutants of Abelson murine leukemia virus deficient in protein tyrosine kinase activity. J Virol. 1990;64(9):4242–51.
37. Calabretta B, Perrotti D. The biology of CML blast crisis. Blood. 2004;103(11):4010–22. https://doi.org/10.1182/blood-2003-12-4111.
38. Lin TS, Mahajan S, Frank DA. STAT signaling in the pathogenesis and treatment of leukemias. Oncogene. 2000;19(21):2496–504. https://doi.org/10.1038/sj.onc.1203486.
39. Chai SK, Nichols GL, Rothman P. Constitutive activation of JAKs and STATs in BCR-Abl-expressing cell lines and peripheral blood cells derived from leukemic patients. J Immunol. 1997;159(10):4720–8.
40. Warsch W, Walz C, Sexl V. JAK of all trades: JAK2-STAT5 as novel therapeutic targets in BCR-ABL1+ chronic myeloid leukemia. Blood. 2013;122(13):2167–75. https://doi.org/10.1182/blood-2013-02-485573.
41. Walz C, Ahmed W, Lazarides K, Betancur M, Patel N, Hennighausen L, et al. Essential role for Stat5a/b in myeloproliferative neoplasms induced by BCR-ABL1 and JAK2(V617F) in mice. Blood. 2012;119(15):3550–60. https://doi.org/10.1182/blood-2011-12-397554.
42. Hoelbl A, Schuster C, Kovacic B, Zhu B, Wickre M, Hoelzl MA, et al. Stat5 is indispensable for the maintenance of bcr/abl-positive leukaemia. EMBO Mol Med. 2010;2(3):98–110. https://doi.org/10.1002/emmm.201000062.
43. Warsch W, Kollmann K, Eckelhart E, Fajmann S, Cerny-Reiterer S, Hölbl A, et al. High STAT5 levels mediate imatinib resistance and indicate disease progression in chronic myeloid leukemia. Blood. 2011;117(12):3409–20. https://doi.org/10.1182/blood-2009-10-248211.
44. Bewry NN, Nair RR, Emmons MF, Boulware D, Pinilla-Ibarz J, Hazlehurst LA. Stat3 contributes to resistance toward BCR-ABL inhibitors in a bone marrow microenvironment model of drug resistance. Mol Cancer Ther. 2008;7(10):3169–75. https://doi.org/10.1158/1535-7163.MCT-08-0314.
45. Samanta AK, Chakraborty SN, Wang Y, Kantarjian H, Sun X, Hood J, et al. Jak2 inhibition deactivates Lyn kinase through the SET-PP2A-SHP1 pathway, causing apoptosis in drug-resistant cells from chronic myelogenous leukemia patients. Oncogene. 2009;28(14):1669–81. https://doi.org/10.1038/onc.2009.7.
46. Sweet K, Hazlehurst L, Sahakian E, Powers J, Nodzon L, Kayali F, et al. A phase I clinical trial of ruxolitinib in combination with nilotinib in chronic myeloid leukemia patients with molecular evidence of disease. Leuk Res. 2018;74:89–96. https://doi.org/10.1016/j.leukres.2018.10.002.
47. Zhao JJ, Cheng H, Jia S, Wang L, Gjoerup OV, Mikami A, et al. The p110alpha isoform of PI3K is essential for proper growth factor signaling and oncogenic transformation. Proc Natl Acad Sci U S A. 2006;103(44):16296–300. https://doi.org/10.1073/pnas.0607899103.
48. Sattler M, Salgia R, Okuda K, Uemura N, Durstin MA, Pisick E, et al. The proto-oncogene product p120CBL and the adaptor proteins CRKL and c-CRK link c-ABL, p190BCR/ABL and p210BCR/ABL to the phosphatidylinositol-3′ kinase pathway. Oncogene. 1996;12(4):839–46.
49. Sattler M, Mohi MG, Pride YB, Quinnan LR, Malouf NA, Podar K, et al. Critical role for Gab2 in transformation by BCR/ABL. Cancer Cell. 2002;1(5):479–92. S1535610802000740 [pii]
50. Morotti A, Panuzzo C, Crivellaro S, Pergolizzi B, Familiari U, Berger AH, et al. BCR-ABL disrupts PTEN nuclear-cytoplasmic shuttling through phosphorylation-dependent activation of HAUSP. Leukemia. 2014;28(6):1326–33. https://doi.org/10.1038/leu.2013.370.
51. Skorski T, Bellacosa A, Nieborowska-Skorska M, Majewski M, Martinez R, Choi JK, et al. Transformation of hematopoietic cells by BCR/ABL requires activation of a PI-3k/Akt-dependent pathway. EMBO J. 1997;16(20):6151–61. https://doi.org/10.1093/emboj/16.20.6151.
52. Klejman A, Rushen L, Morrione A, Slupianek A, Skorski T. Phosphatidylinositol-3 kinase inhibitors enhance the anti-leukemia effect of STI571. Oncogene. 2002;21(38):5868–76. https://doi.org/10.1038/sj.onc.1205724.
53. Hu J, Feng M, Liu Z-L, Liu Y, Huang Z-L, Li H, et al. Potential role of Wnt/β-catenin signaling in blastic transformation of chronic myeloid leukemia: cross talk between β-catenin and BCR-ABL. Tumor Biol. 2016;37(12):15859–72. https://doi.org/10.1007/s13277-016-5413-3.
54. Mayerhofer M, Valent P, Sperr WR, Griffin JD, Sillaber C. BCR/ABL induces expression of vascular endothelial growth factor and its transcriptional activator, hypoxia inducible factor-1alpha, through a pathway involving phosphoinositide 3-kinase and the mammalian target of rapamycin. Blood. 2002;100(10):3767–75. https://doi.org/10.1182/blood-2002-01-0109. 2002-01-0109 [pii]

55. Sheng Z, Ma L, Sun JE, Zhu LJ, Green MR. BCR-ABL suppresses autophagy through ATF5-mediated regulation of mTOR transcription. Blood. 2011;118(10):2840–8. https://doi.org/10.1182/blood-2010-12-322537.
56. Ianniciello A, Dumas P-Y, Drullion C, Guitart A, Villacreces A, Peytour Y, et al. Chronic myeloid leukemia progenitor cells require autophagy when leaving hypoxia-induced quiescence. Oncotarget. 2017;8(57):96984–92. https://doi.org/10.18632/oncotarget.18904.
57. Steelman LS, Franklin RA, Abrams SL, Chappell W, Kempf CR, Basecke J, et al. Roles of the Ras/Raf/MEK/ERK pathway in leukemia therapy. Leukemia. 2011;25(7):1080–94. https://doi.org/10.1038/leu.2011.66.
58. Chu S, Li L, Singh H, Bhatia R. BCR-tyrosine 177 plays an essential role in Ras and Akt activation and in human hematopoietic progenitor transformation in chronic myelogenous leukemia. Cancer Res. 2007;67(14):7045–53. https://doi.org/10.1158/0008-5472.CAN-06-4312.
59. Puil L, Liu J, Gish G, Mbamalu G, Bowtell D, Pelicci PG, et al. Bcr-Abl oncoproteins bind directly to activators of the Ras signalling pathway. EMBO J. 1994;13(4):764–73.
60. Packer LM, Rana S, Hayward R, O'Hare T, Eide CA, Rebocho A, et al. Nilotinib and MEK inhibitors induce synthetic lethality through paradoxical activation of RAF in drug-resistant chronic myeloid leukemia. Cancer Cell. 2011;20(6):715–27. https://doi.org/10.1016/j.ccr.2011.11.004.
61. Baum KJ, Ren R. Effect of Ras inhibition in hematopoiesis and BCR/ABL leukemogenesis. J Hematol Oncol. 2008;1:5. https://doi.org/10.1186/1756-8722-1-5.
62. Pellicano F, Simara P, Sinclair A, Helgason GV, Copland M, Grant S, et al. The MEK inhibitor PD184352 enhances BMS-214662-induced apoptosis in CD34+ CML stem/progenitor cells. Leukemia. 2011;25(7):1159–67. https://doi.org/10.1038/leu.2011.67.
63. Kim LC, Song L, Haura EB. Src kinases as therapeutic targets for cancer. Nat Rev Clin Oncol. 2009;6(10):587–95. https://doi.org/10.1038/nrclinonc.2009.129.
64. Danhauser-Riedl S, Warmuth M, Druker BJ, Emmerich B, Hallek M. Activation of Src kinases p53/56lyn and p59hck by p210bcr/abl in myeloid cells. Cancer Res. 1996;56(15):3589–96.
65. Lionberger JM, Wilson MB, Smithgall TE. Transformation of myeloid leukemia cells to cytokine independence by Bcr-Abl is suppressed by kinase-defective Hck. J Biol Chem. 2000;275(24):18581–5. https://doi.org/10.1074/jbc.C000126200. C000126200 [pii]
66. Wilson MB, Schreiner SJ, Choi HJ, Kamens J, Smithgall TE. Selective pyrrolo-pyrimidine inhibitors reveal a necessary role for Src family kinases in Bcr-Abl signal transduction and oncogenesis. Oncogene. 2002;21(53):8075–88. https://doi.org/10.1038/sj.onc.1206008.
67. Klejman A, Schreiner SJ, Nieborowska-Skorska M, Slupianek A, Wilson M, Smithgall TE, et al. The Src family kinase Hck couples BCR/ABL to STAT5 activation in myeloid leukemia cells. EMBO J. 2002;21(21):5766–74.
68. Warmuth M, Simon N, Mitina O, Mathes R, Fabbro D, Manley PW, et al. Dual-specific Src and Abl kinase inhibitors, PP1 and CGP76030, inhibit growth and survival of cells expressing imatinib mesylate-resistant Bcr-Abl kinases. Blood. 2003;101(2):664–72. https://doi.org/10.1182/blood-2002-01-0288. 2002-01-0288 [pii]
69. Ban K, Gao Y, Amin HM, Howard A, Miller C, Lin Q, et al. BCR-ABL1 mediates up-regulation of Fyn in chronic myelogenous leukemia. Blood. 2008;111(5):2904–8. https://doi.org/10.1182/blood-2007-05-091769.
70. Ptasznik A, Nakata Y, Kalota A, Emerson SG, Gewirtz AM. Short interfering RNA (siRNA) targeting the Lyn kinase induces apoptosis in primary, and drug-resistant, BCR-ABL1(+) leukemia cells. Nat Med. 2004;10(11):1187–9. https://doi.org/10.1038/nm1127.
71. Engelman A, Rosenberg N. bcr/abl and src but not myc and ras replace v-abl in lymphoid transformation. Mol Cell Biol. 1990;10(8):4365–9.
72. Hu Y, Liu Y, Pelletier S, Buchdunger E, Warmuth M, Fabbro D, et al. Requirement of Src kinases Lyn, Hck and Fgr for BCR-ABL1-induced B-lymphoblastic leukemia but not chronic myeloid leukemia. Nat Genet. 2004;36(5):453–61. https://doi.org/10.1038/ng1343. ng1343 [pii]
73. Rusconi F, Piazza R, Vagge E, Gambacorti-Passerini C. Bosutinib : a review of preclinical and clinical studies in chronic myelogenous leukemia. Expert Opin Pharmacother. 2014;15(5):701–10. https://doi.org/10.1517/14656566.2014.882898.
74. ten Hoeve J, Arlinghaus RB, Guo JQ, Heisterkamp N, Groffen J. Tyrosine phosphorylation of CRKL in Philadelphia+ leukemia. Blood. 1994;84(6):1731–6.
75. Birge RB, Kalodimos C, Inagaki F, Tanaka S. Crk and CrkL adaptor proteins: networks for physiological and pathological signaling. Cell Commun Signal. 2009;7:13. https://doi.org/10.1186/1478-811X-7-13.
76. Seo J-H, Wood LJ, Agarwal A, O'Hare T, Elsea CR, Griswold IJ, et al. A specific need for CRKL in p210BCR-ABL-induced transformation of mouse hematopoietic progenitors. Cancer Res. 2010;70(18):7325–35. https://doi.org/10.1158/0008-5472.can-10-0607.
77. White D, Saunders V, Lyons AB, Branford S, Grigg A, To LB, et al. In vitro sensitivity to imatinib-induced inhibition of ABL kinase activity is predictive of molecular response in patients with de novo CML. Blood. 2005;106(7):2520–6. https://doi.org/10.1182/blood-2005-03-1103.

78. Li W, Ren Y, Si Y, Wang F, Yu J. Long non-coding RNAs in hematopoietic regulation. Cell Regen. 2018;7(2):27–32. https://doi.org/10.1016/j.cr.2018.08.001.
79. Guo G, Kang Q, Zhu X, Chen Q, Wang X, Chen Y, et al. A long noncoding RNA critically regulates Bcr-Abl-mediated cellular transformation by acting as a competitive endogenous RNA. Oncogene. 2015;34(14):1768–79. https://doi.org/10.1038/onc.2014.131.
80. Guo G, Kang Q, Chen Q, Chen Z, Wang J, Tan L, et al. High expression of long non-coding RNA H19 is required for efficient tumorigenesis induced by Bcr-Abl oncogene. FEBS Lett. 2014;588(9):1780–6. https://doi.org/10.1016/j.febslet.2014.03.038.
81. Salomoni P, Condorelli F, Sweeney SM, Calabretta B. Versatility of BCR/ABL-expressing leukemic cells in circumventing proapoptotic BAD effects. Blood. 2000;96(2):676–84.
82. de Groot RP, Raaijmakers JA, Lammers JW, Koenderman L. STAT5-dependent CyclinD1 and Bcl-xL expression in Bcr-Abl-transformed cells. Mol Cell Biol Res Commun. 2000;3(5):299–305. https://doi.org/10.1006/mcbr.2000.0231. S1522472400902319 [pii]
83. Horita M, Andreu EJ, Benito A, Arbona C, Sanz C, Benet I, et al. Blockade of the Bcr-Abl kinase activity induces apoptosis of chronic myelogenous leukemia cells by suppressing signal transducer and activator of transcription 5-dependent expression of Bcl-xL. J Exp Med. 2000;191(6):977–84.
84. Neshat MS, Raitano AB, Wang HG, Reed JC, Sawyers CL. The survival function of the Bcr-Abl oncogene is mediated by bad-dependent and -independent pathways: roles for phosphatidylinositol 3-kinase and Raf. Mol Cell Biol. 2000;20(4):1179–86.
85. Graham SM, Jorgensen HG, Allan E, Pearson C, Alcorn MJ, Richmond L, et al. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. Blood. 2002;99(1):319–25.
86. Copland M, Hamilton A, Elrick LJ, Baird JW, Allan EK, Jordanides N, et al. Dasatinib (BMS-354825) targets an earlier progenitor population than imatinib in primary CML but does not eliminate the quiescent fraction. Blood. 2006;107(11):4532–9. https://doi.org/10.1182/blood-2005-07-2947.
87. Jorgensen HG, Allan EK, Jordanides NE, Mountford JC, Holyoake TL. Nilotinib exerts equipotent antiproliferative effects to imatinib and does not induce apoptosis in CD34+ CML cells. Blood. 2007;109(9):4016–9. https://doi.org/10.1182/blood-2006-11-057521.
88. Vetrie D, Helgason GV, Copland M. The leukaemia stem cell: similarities, differences and clinical prospects in CML and AML. Nat Rev Cancer. 2020;20(3):158–73. https://doi.org/10.1038/s41568-019-0230-9.
89. Chakraborty S, Stark JM, Sun CL, Modi H, Chen W, O'Connor TR, et al. Chronic myelogenous leukemia stem and progenitor cells demonstrate chromosomal instability related to repeated breakage-fusion-bridge cycles mediated by increased nonhomologous end joining. Blood. 2012;119(26):6187–97. https://doi.org/10.1182/blood-2011-05-352252.
90. Corbin AS, Agarwal A, Loriaux M, Cortes J, Deininger MW, Druker BJ. Human chronic myeloid leukemia stem cells are insensitive to imatinib despite inhibition of BCR-ABL activity. J Clin Invest. 2011;121(1):396–409. https://doi.org/10.1172/JCI35721.
91. Chomel JC, Sorel N, Guilhot J, Guilhot F, Turhan AG. BCR-ABL expression in leukemic progenitors and primitive stem cells of patients with chronic myeloid leukemia. Blood. 2012;119(12):2964–5.; author reply 5-6. https://doi.org/10.1182/blood-2011-12-396226.
92. Kumari A, Brendel C, Hochhaus A, Neubauer A, Burchert A. Low BCR-ABL expression levels in hematopoietic precursor cells enable persistence of chronic myeloid leukemia under imatinib. Blood. 2012;119(2):530–9. https://doi.org/10.1182/blood-2010-08-303495.
93. Zhao C, Blum J, Chen A, Kwon HY, Jung SH, Cook JM, et al. Loss of beta-catenin impairs the renewal of normal and CML stem cells in vivo. Cancer Cell. 2007;12(6):528–41. https://doi.org/10.1016/j.ccr.2007.11.003.
94. Arrigoni E, Del Re M, Galimberti S, Restante G, Rofi E, Crucitta S, et al. Concise review: chronic myeloid Leukemia: stem cell niche and response to pharmacologic treatment. Stem Cells Transl Med. 2018;7(3):305–14. https://doi.org/10.1002/sctm.17-0175.
95. Grassi S, Palumbo S, Mariotti V, Liberati D, Guerrini F, Ciabatti E, et al. The WNT pathway is relevant for the BCR-ABL1-independent resistance in chronic myeloid leukemia. Front Oncol. 2019;9:532. https://doi.org/10.3389/fonc.2019.00532.
96. Moon RT, Kohn AD, Ferrari GVD, Kaykas A. WNT and [beta]-catenin signalling: diseases and therapies. Nat Rev Genet. 2004;5(9):691–701.
97. Coluccia AM, Vacca A, Dunach M, Mologni L, Redaelli S, Bustos VH, et al. Bcr-Abl stabilizes beta-catenin in chronic myeloid leukemia through its tyrosine phosphorylation. EMBO J. 2007;26(5):1456–66. https://doi.org/10.1038/sj.emboj.7601485.
98. Jamieson CH, Ailles LE, Dylla SJ, Muijtjens M, Jones C, Zehnder JL, et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. N Engl J Med. 2004;351(7):657–67. https://doi.org/10.1056/NEJMoa040258. 351/7/657 [pii]
99. Heidel FH, Bullinger L, Feng Z, Wang Z, Neff TA, Stein L, et al. Genetic and pharmacologic inhibition of beta-catenin targets imatinib-resistant leukemia stem cells in CML. Cell Stem Cell. 2012;10(4):412–24. https://doi.org/10.1016/j.stem.2012.02.017.
100. Irvine DA, Zhang B, Kinstrie R, Tarafdar A, Morrison H, Campbell VL, et al. Deregulated hedgehog path-

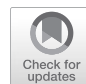
way signaling is inhibited by the smoothened antagonist LDE225 (Sonidegib) in chronic phase chronic myeloid leukaemia. Sci Rep. 2016;6(1):25476. https://doi.org/10.1038/srep25476.

101. Sengupta A, Banerjee D, Chandra S, Banerji SK, Ghosh R, Roy R, et al. Deregulation and cross talk among Sonic hedgehog, Wnt, Hox and notch signaling in chronic myeloid leukemia progression. Leukemia. 2007;21(5):949–55. https://doi.org/10.1038/sj.leu.2404657.
102. Long B, Zhu H, Zhu C, Liu T, Meng W. Activation of the Hedgehog pathway in chronic myelogeneous leukemia patients. J Exp Clin Cancer Res. 2011;30(1):8. https://doi.org/10.1186/1756-9966-30-8.
103. Taipale J, Beachy PA. The Hedgehog and Wnt signalling pathways in cancer. Nature. 2001;411(6835):349–54. https://doi.org/10.1038/35077219.
104. Zhao C, Chen A, Jamieson CH, Fereshteh M, Abrahamsson A, Blum J, et al. Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. Nature. 2009;458(7239):776–9. https://doi.org/10.1038/nature07737.
105. Briscoe J, Therond PP. The mechanisms of hedgehog signalling and its roles in development and disease. Nat Rev Mol Cell Biol. 2013;14(7):416–29. https://doi.org/10.1038/nrm3598.
106. Sadarangani A, Pineda G, Lennon KM, Chun HJ, Shih A, Schairer AE, et al. GLI2 inhibition abrogates human leukemia stem cell dormancy. J Transl Med. 2015;13:98. https://doi.org/10.1186/s12967-015-0453-9.
107. Bray SJ. Notch signalling in context. Nat Rev Mol Cell Biol. 2016;17(11):722–35. https://doi.org/10.1038/nrm.2016.94.
108. Nakahara F, Sakata-Yanagimoto M, Komeno Y, Kato N, Uchida T, Haraguchi K, et al. Hes1 immortalizes committed progenitors and plays a role in blast crisis transition in chronic myelogenous leukemia. Blood. 2010;115(14):2872–81. https://doi.org/10.1182/blood-2009-05-222836.
109. Aljedai A, Buckle A-M, Hiwarkar P, Syed F. Potential role of notch signalling in CD34+ chronic myeloid leukaemia cells: cross-talk between notch and BCR-ABL. PLoS One. 2015;10(4):e0123016-e. https://doi.org/10.1371/journal.pone.0123016.
110. Miyamoto K, Araki KY, Naka K, Arai F, Takubo K, Yamazaki S, et al. Foxo3a is essential for maintenance of the hematopoietic stem cell pool. Cell Stem Cell. 2007;1(1):101–12. https://doi.org/10.1016/j.stem.2007.02.001.
111. Atfi A, Abecassis L, Bourgeade MF. Bcr-Abl activates the AKT/fox O3 signalling pathway to restrict transforming growth factor-beta-mediated cytostatic signals. EMBO Rep. 2005;6(10):985–91. https://doi.org/10.1038/sj.embor.7400501.
112. Hurtz C, Hatzi K, Cerchietti L, Braig M, Park E, Kim YM, et al. BCL6-mediated repression of p53 is critical for leukemia stem cell survival in chronic myeloid leukemia. J Exp Med. 2011;208(11):2163–74. https://doi.org/10.1084/jem.20110304.
113. Naka K, Hoshii T, Muraguchi T, Tadokoro Y, Ooshio T, Kondo Y, et al. TGF-beta-FOXO signalling maintains leukaemia-initiating cells in chronic myeloid leukaemia. Nature. 2010;463(7281):676–80. https://doi.org/10.1038/nature08734.
114. Carter BZ, Mak PY, Mu H, Zhou H, Mak DH, Schober W, et al. Combined targeting of BCL-2 and BCR-ABL tyrosine kinase eradicates chronic myeloid leukemia stem cells. Sci Transl Med. 2016;8(355):355ra117. https://doi.org/10.1126/scitranslmed.aag1180.
115. Horita M, Andreu EJ, Benito A, Arbona C, Sanz C, Benet I, et al. Blockade of the Bcr-Abl kinase activity induces apoptosis of chronic myelogenous leukemia cells by suppressing signal transducer and activator of transcription 5–dependent expression of Bcl-XL. J Exp Med. 2000;191(6):977–84. https://doi.org/10.1084/jem.191.6.977.
116. Tzifi F, Economopoulou C, Gourgiotis D, Ardavanis A, Papageorgiou S, Scorilas A. The role of BCL2 family of apoptosis regulator proteins in acute and chronic leukemias. Adv Hematol. 2012;2012:524308. https://doi.org/10.1155/2012/524308.
117. Kuroda J, Puthalakath H, Cragg MS, Kelly PN, Bouillet P, Huang DCS, et al. Bim and bad mediate imatinib-induced killing of Bcr/Abl$^+$ leukemic cells, and resistance due to their loss is overcome by a BH3 mimetic. Proc Natl Acad Sci. 2006;103(40):14907–12. https://doi.org/10.1073/pnas.0606176103.
118. Marum JE, Yeung DT, Purins L, Reynolds J, Parker WT, Stangl D, et al. ASXL1 and BIM germ line variants predict response and identify CML patients with the greatest risk of imatinib failure. Blood Adv. 2017;1(18):1369–81. https://doi.org/10.1182/bloodadvances.2017006825.
119. Hantschel O, Warsch W, Eckelhart E, Kaupe I, Grebien F, Wagner KU, et al. BCR-ABL uncouples canonical JAK2-STAT5 signaling in chronic myeloid leukemia. Nat Chem Biol. 2012;8(3):285–93. https://doi.org/10.1038/nchembio.775.
120. Neviani P, Harb JG, Oaks JJ, Santhanam R, Walker CJ, Ellis JJ, et al. PP2A-activating drugs selectively eradicate TKI-resistant chronic myeloid leukemic stem cells. J Clin Invest. 2013;123(10):4144–57. https://doi.org/10.1172/JCI68951.
121. Neviani P, Santhanam R, Trotta R, Notari M, Blaser BW, Liu S, et al. The tumor suppressor PP2A is functionally inactivated in blast crisis CML through the inhibitory activity of the BCR/ABL-regulated SET protein. Cancer Cell. 2005;8(5):355–68. https://doi.org/10.1016/j.ccr.2005.10.015.
122. Ellis SL, Nilsson SK. The location and cellular composition of the hemopoietic stem cell niche. Cytotherapy. 2012;14(2):135–43. https://doi.org/10.3109/14653249.2011.630729.
123. Ema H, Suda T. Two anatomically distinct niches regulate stem cell activity. Blood. 2012;120(11):2174–81. https://doi.org/10.1182/blood-2012-04-424507.
124. Hazlehurst LA, Argilagos RF, Dalton WS. Beta1 integrin mediated adhesion increases Bim protein

degradation and contributes to drug resistance in leukaemia cells. Br J Haematol. 2007;136(2):269–75. https://doi.org/10.1111/j.1365-2141.2006.06435.x.
125. Zhang B, Li M, McDonald T, Holyoake TL, Moon RT, Campana D, et al. Microenvironmental protection of CML stem and progenitor cells from tyrosine kinase inhibitors through N-cadherin and Wnt-beta-catenin signaling. Blood. 2013;121(10):1824–38. https://doi.org/10.1182/blood-2012-02-412890.
126. Perrotti D, Jamieson C, Goldman J, Skorski T. Chronic myeloid leukemia: mechanisms of blastic transformation. J Clin Invest. 2010;120(7):2254–64. https://doi.org/10.1172/JCI41246.
127. Skorski T. Genetic mechanisms of chronic myeloid leukemia blastic transformation. Curr Hematol Malig Rep. 2012;7(2):87–93. https://doi.org/10.1007/s11899-012-0114-5.
128. Johansson B, Fioretos T, Mitelman F. Cytogenetic and molecular genetic evolution of chronic myeloid leukemia. Acta Haematol. 2002;107(2):76–94. https://doi.org/10.1159/000046636.
129. Melo JV, Barnes DJ. Chronic myeloid leukaemia as a model of disease evolution in human cancer. Nat Rev Cancer. 2007;7(6):441–53. https://doi.org/10.1038/nrc2147.
130. Barnes DJ, Palaiologou D, Panousopoulou E, Schultheis B, Yong AS, Wong A, et al. Bcr-Abl expression levels determine the rate of development of resistance to imatinib mesylate in chronic myeloid leukemia. Cancer Res. 2005;65(19):8912–9. https://doi.org/10.1158/0008-5472.CAN-05-0076.
131. Gaiger A, Henn T, Horth E, Geissler K, Mitterbauer G, Maier-Dobersberger T, et al. Increase of bcr-abl chimeric mRNA expression in tumor cells of patients with chronic myeloid leukemia precedes disease progression. Blood. 1995;86(6):2371–8.
132. Jiang X, Zhao Y, Smith C, Gasparetto M, Turhan A, Eaves A, et al. Chronic myeloid leukemia stem cells possess multiple unique features of resistance to BCR-ABL targeted therapies. Leukemia. 2007;21(5):926–35. https://doi.org/10.1038/sj.leu.2404609.
133. Marega M, Piazza RG, Pirola A, Redaelli S, Mogavero A, Iacobucci I, et al. BCR and BCR-ABL regulation during myeloid differentiation in healthy donors and in chronic phase/blast crisis CML patients. Leukemia. 2010;24(8):1445–9. https://doi.org/10.1038/leu.2010.101.
134. Andrews DF 3rd, Collins SJ. Heterogeneity in expression of the bcr-abl fusion transcript in CML blast crisis. Leukemia. 1987;1(10):718–24.
135. Chang JS, Santhanam R, Trotta R, Neviani P, Eiring AM, Briercheck E, et al. High levels of the BCR/ABL oncoprotein are required for the MAPK-hnRNP-E2 dependent suppression of C/EBPalpha-driven myeloid differentiation. Blood. 2007;110(3):994–1003. https://doi.org/10.1182/blood-2007-03-078303.
136. Amos TA, Lewis JL, Grand FH, Gooding RP, Goldman JM, Gordon MY. Apoptosis in chronic myeloid leukaemia: normal responses by progenitor cells to growth factor deprivation, X-irradiation and glucocorticoids. Br J Haematol. 1995;91(2):387–93.
137. Bedi A, Barber JP, Bedi GC, el-Deiry WS, Sidransky D, Vala MS, et al. BCR-ABL-mediated inhibition of apoptosis with delay of G2/M transition after DNA damage: a mechanism of resistance to multiple anticancer agents. Blood. 1995;86(3):1148–58.
138. Dierov J, Sanchez PV, Burke BA, Padilla-Nash H, Putt ME, Ried T, et al. BCR/ABL induces chromosomal instability after genotoxic stress and alters the cell death threshold. Leukemia. 2009;23(2):279–86. https://doi.org/10.1038/leu.2008.308.
139. Koptyra M, Cramer K, Slupianek A, Richardson C, Skorski T. BCR/ABL promotes accumulation of chromosomal aberrations induced by oxidative and genotoxic stress. Leukemia. 2008;22(10):1969–72. https://doi.org/10.1038/leu.2008.78.
140. Slupianek A, Falinski R, Znojek P, Stoklosa T, Flis S, Doneddu V, et al. BCR-ABL1 kinase inhibits uracil DNA glycosylase UNG2 to enhance oxidative DNA damage and stimulate genomic instability. Leukemia. 2013;27(3):629–34. https://doi.org/10.1038/leu.2012.294.
141. Deutsch E, Dugray A, AbdulKarim B, Marangoni E, Maggiorella L, Vaganay S, et al. BCR-ABL downregulates the DNA repair protein DNA-PKcs. Blood. 2001;97(7):2084–90.
142. Skorski T. Genomic instability: the cause and effect of BCR/ABL tyrosine kinase. Curr Hematol Malig Rep. 2007;2(2):69–74. https://doi.org/10.1007/s11899-007-0010-6.
143. Skorski T. BCR/ABL, DNA damage and DNA repair: implications for new treatment concepts. Leuk Lymphoma. 2008;49(4):610–4. https://doi.org/10.1080/03093640701859089.
144. Zhang P, Iwasaki-Arai J, Iwasaki H, Fenyus ML, Dayaram T, Owens BM, et al. Enhancement of hematopoietic stem cell repopulating capacity and self-renewal in the absence of the transcription factor C/EBP alpha. Immunity. 2004;21(6):853–63. https://doi.org/10.1016/j.immuni.2004.11.006.
145. Guerzoni C, Bardini M, Mariani SA, Ferrari-Amorotti G, Neviani P, Panno ML, et al. Inducible activation of CEBPB, a gene negatively regulated by BCR/ABL, inhibits proliferation and promotes differentiation of BCR/ABL-expressing cells. Blood. 2006;107(10):4080–9. https://doi.org/10.1182/blood-2005-08-3181.
146. Eiring AM, Harb JG, Neviani P, Garton C, Oaks JJ, Spizzo R, et al. miR-328 functions as an RNA decoy to modulate hnRNP E2 regulation of mRNA translation in leukemic blasts. Cell. 2010;140(5):652–65. https://doi.org/10.1016/j.cell.2010.01.007.
147. Dang CV. MYC on the path to cancer. Cell. 2012;149(1):22–35. https://doi.org/10.1016/j.cell.2012.03.003.
148. Preisler HD, Sato H, Yang PM, Wilson M, Kaufman C, Watt R. Assessment of c-myc expres-

sion in individual leukemic cells. Leuk Res. 1988;12(6):507–16.
149. Cleveland JL, Dean M, Rosenberg N, Wang JY, Rapp UR. Tyrosine kinase oncogenes abrogate interleukin-3 dependence of murine myeloid cells through signaling pathways involving c-myc: conditional regulation of c-myc transcription by temperature-sensitive v-abl. Mol Cell Biol. 1989;9(12):5685–95.
150. Sawyers CL, Callahan W, Witte ON. Dominant negative MYC blocks transformation by ABL oncogenes. Cell. 1992;70(6):901–10. doi:0092-8674(92)90241-4 [pii]
151. Bissonnette RP, Echeverri F, Mahboubi A, Green DR. Apoptotic cell death induced by c-myc is inhibited by bcl-2. Nature. 1992;359(6395):552–4. https://doi.org/10.1038/359552a0.
152. Sanchez-Garcia I, Grutz G. Tumorigenic activity of the BCR-ABL oncogenes is mediated by BCL2. Proc Natl Acad Sci U S A. 1995;92(12):5287–91.
153. Birchenall-Roberts MC, Yoo YD, Bertolette DC 3rd, Lee KH, Turley JM, Bang OS, et al. The p120-v-Abl protein interacts with E2F-1 and regulates E2F-1 transcriptional activity. J Biol Chem. 1997;272(14):8905–11.
154. Stewart MJ, Litz-Jackson S, Burgess GS, Williamson EA, Leibowitz DS, Boswell HS. Role for E2F1 in p210 BCR-ABL downstream regulation of c-myc transcription initiation. Studies in murine myeloid cells. Leukemia. 1995;9(9):1499–507.
155. Xie S, Lin H, Sun T, Arlinghaus RB. Jak2 is involved in c-Myc induction by Bcr-Abl. Oncogene. 2002;21(47):7137–46. https://doi.org/10.1038/sj.onc.1205942.
156. Notari M, Neviani P, Santhanam R, Blaser BW, Chang JS, Galietta A, et al. A MAPK/HNRPK pathway controls BCR/ABL oncogenic potential by regulating MYC mRNA translation. Blood. 2006;107(6):2507–16. https://doi.org/10.1182/blood-2005-09-3732.
157. Reavie L, Buckley SM, Loizou E, Takeishi S, Aranda-Orgilles B, Ndiaye-Lobry D, et al. Regulation of c-Myc ubiquitination controls chronic myelogenous leukemia initiation and progression. Cancer Cell. 2013;23(3):362–75. https://doi.org/10.1016/j.ccr.2013.01.025.
158. Delgado MD, Leon J. Myc roles in hematopoiesis and leukemia. Genes Cancer. 2010;1(6):605–16. https://doi.org/10.1177/1947601910377495.
159. Pant V, Quintas-Cardama A, Lozano G. The p53 pathway in hematopoiesis: lessons from mouse models, implications for humans. Blood. 2012;120(26):5118–27. https://doi.org/10.1182/blood-2012-05-356014.
160. Guinn BA, Mills KI. p53 mutations, methylation and genomic instability in the progression of chronic myeloid leukaemia. Leuk Lymphoma. 1997;26(3–4):211–26. https://doi.org/10.3109/10428199709051771.
161. Stuppia L, Calabrese G, Peila R, Guanciali-Franchi P, Morizio E, Spadano A, et al. p53 loss and point mutations are associated with suppression of apoptosis and progression of CML into myeloid blastic crisis. Cancer Genet Cytogenet. 1997;98(1):28–35. doi:S016546089600413X [pii]
162. Sionov RV, Moallem E, Berger M, Kazaz A, Gerlitz O, Ben-Neriah Y, et al. C-Abl neutralizes the inhibitory effect of Mdm2 on p53. J Biol Chem. 1999;274(13):8371–4.
163. Stoklosa T, Slupianek A, Datta M, Nieborowska-Skorska M, Nowicki MO, Koptyra M, et al. BCR/ABL recruits p53 tumor suppressor protein to induce drug resistance. Cell Cycle. 2004;3(11):1463–72. doi:1229 [pii]
164. Trotta R, Vignudelli T, Candini O, Intine RV, Pecorari L, Guerzoni C, et al. BCR/ABL activates mdm2 mRNA translation via the La antigen. Cancer Cell. 2003;3(2):145–60. doi:S1535610803000205 [pii]
165. Wendel HG, de Stanchina E, Cepero E, Ray S, Emig M, Fridman JS, et al. Loss of p53 impedes the antileukemic response to BCR-ABL inhibition. Proc Natl Acad Sci U S A. 2006;103(19):7444–9. https://doi.org/10.1073/pnas.0602402103.
166. Honda H, Ushijima T, Wakazono K, Oda H, Tanaka Y, Aizawa S, et al. Acquired loss of p53 induces blastic transformation in p210(bcr/abl)-expressing hematopoietic cells: a transgenic study for blast crisis of human CML. Blood. 2000;95(4):1144–50.
167. Velasco-Hernandez T, Vicente-Duenas C, Sanchez-Garcia I, Martin-Zanca D. p53 restoration kills primitive leukemia cells in vivo and increases survival of leukemic mice. Cell Cycle. 2013;12(1):122–32. https://doi.org/10.4161/cc.23031.
168. Peterson LF, Mitrikeska E, Giannola D, Lui Y, Sun H, Bixby D, et al. p53 stabilization induces apoptosis in chronic myeloid leukemia blast crisis cells. Leukemia. 2011;25(5):761–9. https://doi.org/10.1038/leu.2011.7.
169. Walker CJ, Oaks JJ, Santhanam R, Neviani P, Harb JG, Ferenchak G, et al. Preclinical and clinical efficacy of XPO1/CRM1 inhibition by the karyopherin inhibitor KPT-330 in Ph+ leukemias. Blood. 2013;122(17):3034–44. https://doi.org/10.1182/blood-2013-04-495374.
170. Yuan H, Wang Z, Li L, Zhang H, Modi H, Horne D, et al. Activation of stress response gene SIRT1 by BCR-ABL promotes leukemogenesis. Blood. 2012;119(8):1904–14. https://doi.org/10.1182/blood-2011-06-361691.
171. Li L, Wang L, Wang Z, Ho Y, McDonald T, Holyoake TL, et al. Activation of p53 by SIRT1 inhibition enhances elimination of CML leukemia stem cells in combination with imatinib. Cancer Cell. 2012;21(2):266–81. https://doi.org/10.1016/j.ccr.2011.12.020.
172. Wang Z, Yuan H, Roth M, Stark JM, Bhatia R, Chen WY. SIRT1 deacetylase promotes acquisition of genetic mutations for drug resistance in CML cells. Oncogene. 2013;32(5):589–98. https://doi.org/10.1038/onc.2012.83.

173. Jiang Q, Crews LA, Barrett CL, Chun HJ, Court AC, Isquith JM, et al. ADAR1 promotes malignant progenitor reprogramming in chronic myeloid leukemia. Proc Natl Acad Sci U S A. 2013;110(3):1041–6. https://doi.org/10.1073/pnas.1213021110.
174. Steinman RA, Yang Q, Gasparetto M, Robinson LJ, Liu X, Lenzner DE, et al. Deletion of the RNA-editing enzyme ADAR1 causes regression of established chronic myelogenous leukemia in mice. Int J Cancer. 2013;132(8):1741–50. https://doi.org/10.1002/ijc.27851.
175. XuFeng R, Boyer MJ, Shen H, Li Y, Yu H, Gao Y, et al. ADAR1 is required for hematopoietic progenitor cell survival via RNA editing. Proc Natl Acad Sci U S A. 2009;106(42):17763–8. https://doi.org/10.1073/pnas.0903324106.
176. Iwama A. Polycomb repressive complexes in hematological malignancies. Blood. 2017;130(1):23–9. https://doi.org/10.1182/blood-2017-02-739490.
177. Mohty M, Yong ASM, Szydlo RM, Apperley JF, Melo JV. The polycomb group BMI1 gene is a molecular marker for predicting prognosis of chronic myeloid leukemia. Blood. 2007;110(1):380–3. https://doi.org/10.1182/blood-2006-12-065599.
178. Xie H, Peng C, Huang J, Li BE, Kim W, Smith EC, et al. Chronic myelogenous leukemia- initiating cells require Polycomb group protein EZH2. Cancer Discov. 2016;6(11):1237–47. https://doi.org/10.1158/2159-8290.CD-15-1439.
179. Ko TK, Javed A, Lee KL, Pathiraja TN, Liu X, Malik S, et al. An integrative model of pathway convergence in genetically heterogeneous blast crisis chronic myeloid leukemia. Blood. 2020;135(26):2337–53. https://doi.org/10.1182/blood.2020004834.
180. Skorski T. Oncogenic tyrosine kinases and the dna-damage response. Nat Rev Cancer. 2002;2(5):351–60. https://doi.org/10.1038/nrc799.
181. Giotopoulos G, van der Weyden L, Osaki H, Rust AG, Gallipoli P, Meduri E, et al. A novel mouse model identifies cooperating mutations and therapeutic targets critical for chronic myeloid leukemia progression. J Exp Med. 2015;212(10):1551–69. https://doi.org/10.1084/jem.20141661.
182. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification and prognosis in acute myeloid Leukemia. N Engl J Med. 2016;374(23):2209–21. https://doi.org/10.1056/NEJMoa1516192.
183. Roberts KG, Li Y, Payne-Turner D, Harvey RC, Yang Y-L, Pei D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med. 2014;371(11):1005–15. https://doi.org/10.1056/NEJMoa1403088.
184. Guglielmelli P, Lasho TL, Rotunno G, Mudireddy M, Mannarelli C, Nicolosi M, et al. MIPSS70: mutation-enhanced international prognostic Score system for transplantation-age patients with primary myelofibrosis. J Clin Oncol. 2018;36(4):310–8. https://doi.org/10.1200/jco.2017.76.4886.
185. Branford S, Wang P, Yeung DT, Thomson D, Purins A, Wadham C, et al. Integrative genomic analysis reveals cancer-associated mutations at diagnosis of CML in patients with high-risk disease. Blood. 2018;132(9):948–61. https://doi.org/10.1182/blood-2018-02-832253.
186. Grossmann V, Kohlmann A, Zenger M, Schindela S, Eder C, Weissmann S, et al. A deep-sequencing study of chronic myeloid leukemia patients in blast crisis (BC-CML) detects mutations in 76.9% of cases. Leukemia. 2011;25(3):557–60. http://www.nature.com/leu/journal/v25/n3/suppinfo/leu2010298s1.html.
187. Branford S, Kim DDH, Apperley JF, Eide CA, Mustjoki S, Ong ST, et al. Laying the foundation for genomically-based risk assessment in chronic myeloid leukemia. Leukemia. 2019;33(8):1835–50.
188. Thomson DW, Shahrin NH, Wang PPS, Wadham C, Shanmuganathan N, Scott HS, et al. Aberrant RAG-mediated recombination contributes to multiple structural rearrangements in lymphoid blast crisis of chronic myeloid leukemia. Leukemia. 2020;34:2051–63. https://doi.org/10.1038/s41375-020-0751-y.
189. Hoelbl A, Schuster C, Kovacic B, Zhu B, Wickre M, Hoelzl MA, et al. Stat5 is indispensable for the maintenance of bcr/abl-positive leukaemia. EMBO Mol Med. 2010;2(3):98–110. https://doi.org/10.1002/emmm.201000062.
190. Halbach S, Rigbolt KT, Wohrle FU, Diedrich B, Gretzmeier C, Brummer T, et al. Alterations of Gab2 signalling complexes in imatinib and dasatinib treated chronic myeloid leukaemia cells. Cell Commun Signal. 2013;11(1):30. https://doi.org/10.1186/1478-811X-11-30.
191. Winter GE, Rix U, Carlson SM, Gleixner KV, Grebien F, Gridling M, et al. Systems-pharmacology dissection of a drug synergy in imatinib-resistant CML. Nat Chem Biol. 2012;8(11):905–12. https://doi.org/10.1038/nchembio.1085.
192. Agirre X, Jimenez-Velasco A, San Jose-Eneriz E, Garate L, Bandres E, Cordeu L, et al. Down-regulation of hsa-miR-10a in chronic myeloid leukemia CD34+ cells increases USF2-mediated cell growth. Mol Cancer Res. 2008;6(12):1830–40. https://doi.org/10.1158/1541-7786.MCR-08-0167.
193. Machova Polakova K, Lopotova T, Klamova H, Burda P, Trneny M, Stopka T, et al. Expression patterns of microRNAs associated with CML phases and their disease related targets. Mol Cancer. 2011;10:41. https://doi.org/10.1186/1476-4598-10-41.
194. Gaj T, Gersbach CA, Barbas CF 3rd. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. 2013;31(7):397–405. https://doi.org/10.1016/j.tibtech.2013.04.004.



3 Epidemiology of Chronic Myeloid Leukaemia

Martin Höglund, Fredrik Sandin, Bengt Simonsson, and Leif Stenke

3.1 Population-Based Registries

Important data on cancer epidemiology (e.g. incidence, prevalence, age and sex distribution, overall and relative survival), including trends over time, may be obtained from well-established cancer registries covering either the entire population of a nation [1–3] or selected regions with well-defined populations [4–6]. In Sweden, the National Cancer Registry was formed already in 1958. All pathologists, cytologists and clinicians are obliged by law to report each occurrence of cancer that they diagnose or treat to this centralised, nationwide registry [7]. In the United States, the SEER registries collect data on all newly diagnosed cancers from a large number of hospitals, including patient demographics from 18 tumour registries, covering approximately 30% of the US population [8].

M. Höglund (✉) · B. Simonsson
Division of Hematology, Department of Medical Science, Uppsala University Hospital, Uppsala, Sweden
e-mail: martin.hoglund@medsci.uu.se

F. Sandin
Regional Cancer Centre of Middle Sweden, Akademiska Sjukhuset, Uppsala, Sweden

L. Stenke
Department of Hematology, Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden
e-mail: leif.stenke@ki.se

During the last 10–20 years, in CML and in other haematological cancers, diagnosis specific national or regional population-based registries aiming to collect more detailed data on demographics, baseline patient characteristics as well as on treatment and outcome have been established [9–14]. In particular, the British Haematological Malignancy Research Network (HMRN), established in 2004 and operating across 14 hospitals using a single haematopathology laboratory [14], the Dutch CML registry [3] and the national Swedish CML registry, founded in 2002 and covering >95% of all newly diagnosed cases of [13], have generated useful population-based data. At the international level, the European Treatment and Outcome Study (EUTOS) for CML has collected detailed population-based data from adult CML patients diagnosed in 2008–2012 in 20 European countries [15]. In addition to these kinds of population-based registries, epidemiological information on CML and other haematological malignancies may be obtained from national or regional health insurance databases [16–18] and from central laboratories receiving all diagnostic samples from a well-defined region [19].

Results from these and other relatively detailed population-based registries with full coverage of the target population are useful sources for epidemiological studies. By reducing the impact of selection on outcome, they may also provide important complementary data on treatment

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
R. Hehlmann (ed.), *Chronic Myeloid Leukemia*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-71913-5_3

outcome to those obtained from clinical trials [11, 12, 20, 21]. Using such routine care data may also be helpful in evaluating adherence to guidelines and in improving the quality of care, including routines for diagnostics and follow-up [13, 22]. Moreover, useful information could be obtained by cross-linking to other population-based regional or national health care databases [23, 24]. Thus, by linking the Swedish CML registry to National Prescribed Drug Registry and National Patient Registry (information on diagnosis from in-hospital and outpatient doctor visits), important off-target effects following treatment with TKIs, in particular the increased risk of cardiovascular events following second-generation TKIs, have been studied [25].

Obviously, reliability of data from registries claiming to be population-based presupposes complete reporting, diagnostic accuracy, correct coding classification and a well-characterised background population of the registry catchment area(s) [26, 27]. However, delayed reporting, less stringent monitoring (as compared to clinical trials) and no detailed information on treatments are obvious limitations of population-based registries.

3.2 Incidence

3.2.1 Incidence of CML in the Total Adult Population

Published data on the annual incidence of CML varies from as low as 0.4/100,000 persons in some non-Western countries to 1.75/100,000 in the United States [3, 16, 28–31]. As the incidence of CML increases by age (Fig. 3.1), some of these variations are likely due to significant differences in the age distribution of the investigated

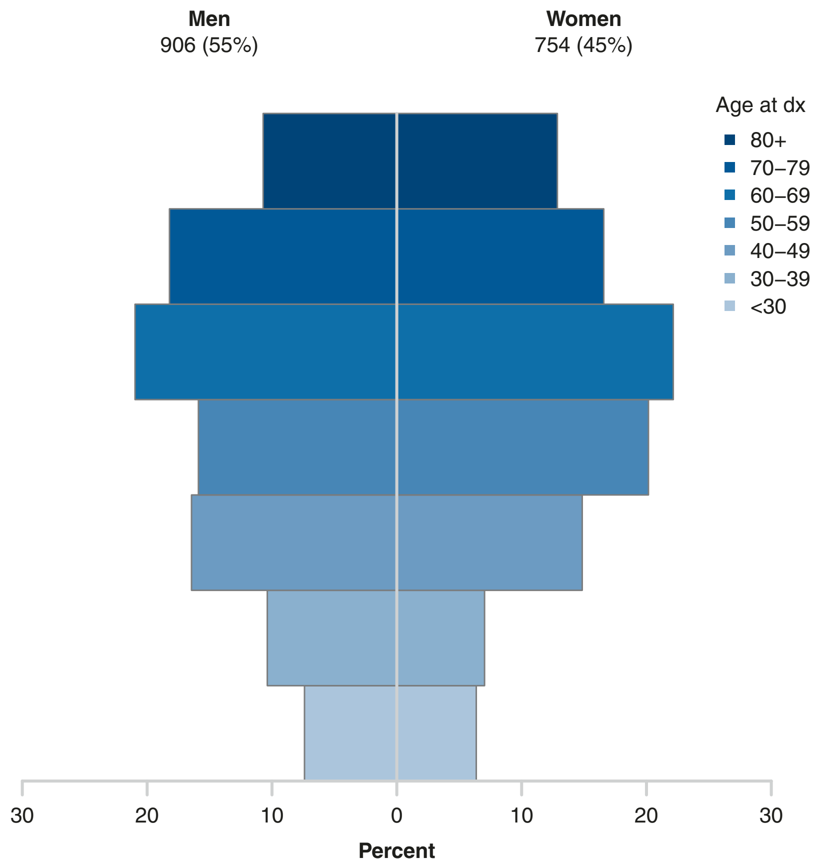


Fig. 3.1 Gender distribution in CML diagnosed in 2002–2014 (n = 1199). Data are obtained from the population-based Swedish CML registry

Table 3.1 CML incidence based on nine different population-based registries or surveys

Registry	Time of observation	No. of pts.	Median age	Raw incidence	Age-standardised incidence	Reference
United States (SEERS)	1975–2009	13,869	66	–	1.75[a]	Chen et al. [29]
NW France	1985–2006	906	56		0.8[a]	
Taiwan	1997–2007	2672	n.d.	0.7	–	Chang et al. [16]
SW Germany	1998–2000	218	57	0.62	–	Rohrbacher et al. [33]
Sweden	2002–2010	779	60	0.9	–	Hoglund et al. [13]
UK (HMRN)	2004–2011	242	59	0.97	0.7[b]	Smith et al. [31]
EUTOS	2008–2012	2887	56	0.99	0.96[c]	Hoffmann et al. [15]
Lithuania	2000–2013	601	62	1.28	0.88	Beinortas et al. [28]
The Netherlands	2001–2012	1806	59		0,8	Thielen et al. [3]

[a]American standardised population
[b]World standardised population
[c]European standardised population

population (e.g. Western vs. several non-Western countries) [32]. However, also figures on age-standardised incidence varies between different studies, although most European registries report figures in the range 0.7–1.0/100000 inhabitants (Table 3.1). Interestingly, a report from the EUTOs registry, based on population-based epidemiological data from 2287 patients aged ≥20 years and with cytogenetically confirmed CML diagnosed 2008–2012, showed that the raw incidence of CML varies from 0.69 (Poland) to 1.39 (Italy) per 100,000 persons. Correspondingly, age-standardised incidences varied from 0.70 (Poland, UK, Austria) to 1.28 (Italy) [15].

Methodological factors may explain some of these discrepancies. In particular, inclusion of patients with *BCR-ABL*-negative myeloproliferative disorders may account for the higher incidence of CML in some registries, such as SEERs reporting an incidence of 1.75/100,000, varying from 1.4 to 2.0 between different regions within the United States [29]. Moreover, incorrectly including referral patients in regional 'population-based' registries leads to an overestimation of the incidence. On the other hand, incomplete reporting of new CML cases will result in too low figs [34]. It is also possible that differences in health-care-seeking behaviours and reimbursement systems may lead to underreporting of, in particular, elderly patients in some registries. Several haematological registries have, therefore, made considerable efforts to catch all newly diagnosed cases of CML including those diagnosed at smaller hospitals [13, 31].

Although we hypothesise that the divergences in age-adjusted incidence reported so far are mainly due to methodological issues, a true difference between different geographical areas and/or ethnical subgroups cannot be excluded. Indeed, such differences have been shown in other haematological cancers such as chronic lymphocytic leukaemia and acute promyelocytic leukaemia [35, 36]. In CML, Chen et al., analysing the incidence of CML in different ethnical subgroups within the United States, showed a lower incidence in Asians as compared to Caucasians [29].

3.2.2 Age and Sex Differences

The incidence in CML increases by age, at least up to 75–80 years, with an annual incidence rising from 0.39 in young (20–29 years) to 1.52 in those 70 years or more [15, 37] (Fig. 3.1). According to the EUTOS registry report, the

median age at diagnosis of CML in Europe is 56 years, in countries such as Germany and Sweden as high as 61–62 years (Table 3.1). The latter is about 10 years above the median age typically seen in clinical trials [15, 33]. In children, CML is a very rare disease with an incidence as low as 0.6–1.2 million children/year [38].

CML is more common in males than in females with male-to-female ratio varying between 1.2 and 1.7 in different studies [3, 13, 39]. The gender difference in incidence is slightly less prominent in younger age groups (Fig. 3.2).

3.2.3 Has the Incidence of CML Increased over Time?

In several countries, cancer statistics are available since the 1970s or even earlier. Data from SEERs and the Dutch and Swedish Cancer Registries (Fig. 3.2) give no clear evidence of a change in incidence over time in CML [3, 29, 40]. However, changes in the classification system, the development of more accurate diagnostics by the centralisation of haematopathology to more specialised units and the introduction of cytogenetics make it very difficult to compare present figures on incidence with data from the mid-1980s and earlier.

3.3 Prevalence

Reliable data on the exact prevalence of CML are relatively scarce. In an epidemiological survey from northern France, Corn et al. reported prevalence for 1998, 2003 and 2007, respectively, of 5.8, 6.8 and 7.3 per 100,000 inhabitants. Due to the significant improvement in survival, following the introduction of imatinib and other TKIs [41], as well as the increasing life expectancy in the general population, the prevalence of CML is increasing [18, 42]. Thus, in a study from Sweden, the observed prevalence tripled from 1985 to 2012, from 3.9 to 11.9 per 100,000 inhabitants [43]. Assuming no further improvements in relative survival, the prevalence is projected to further increase to 15 per 100,000 in 2020 and 22.0 per 100,000 inhabitants by 2060

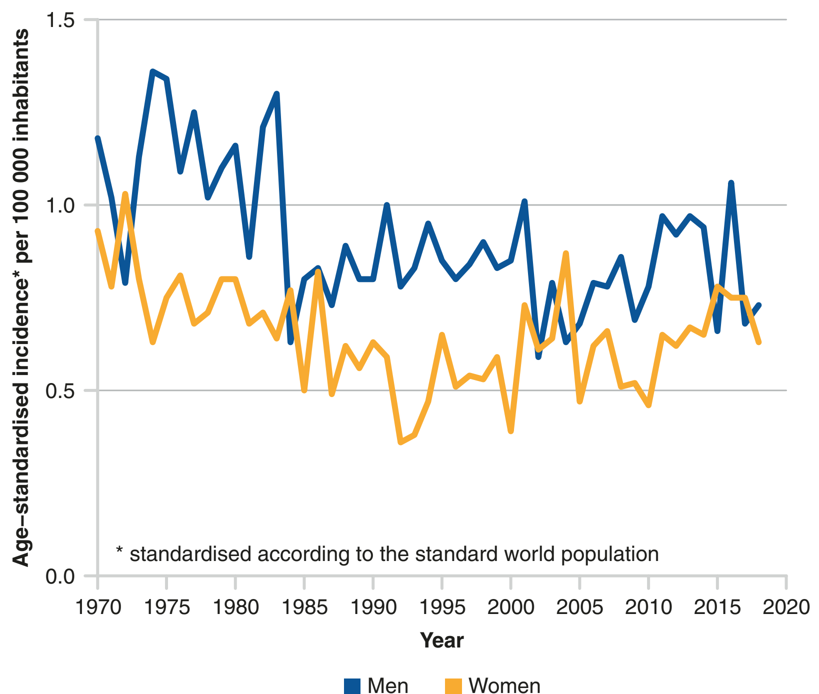


Fig. 3.2 Age-standardised annual incidence (adjusted to WSP) of CML diagnosed in 1970–2010 (n = 4393) (Data are obtained from the Swedish Cancer Registry (www.socialstyrelsen.se/register/halsodataregister/cancerregistret/inenglish). Note that data from 1970s and 1980s may be imprecise due to decentralised haematopathology and in most cases no cytogenetics

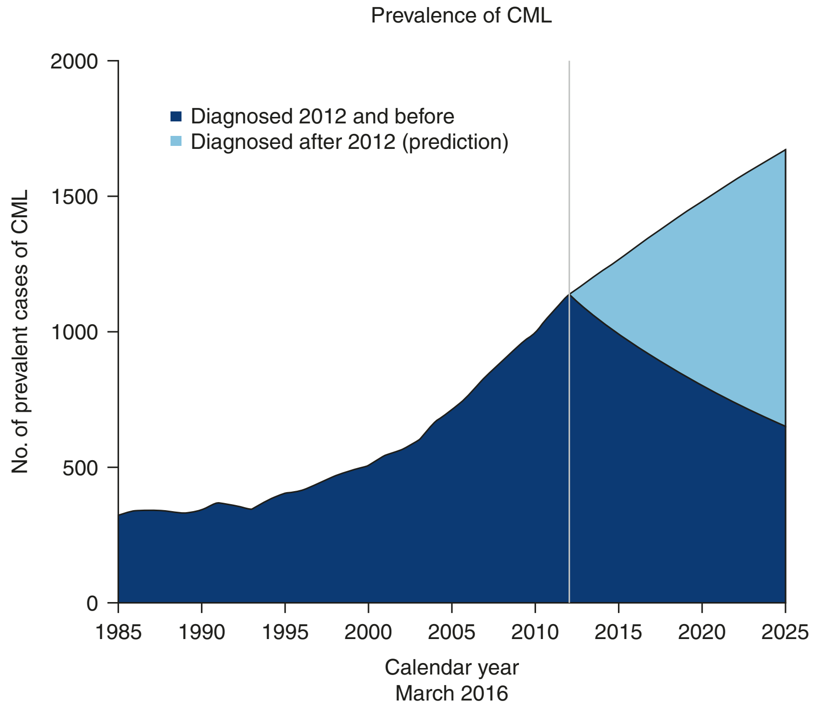


Fig. 3.3 Estimated prevalence of chronic myeloid leukaemia in Sweden by calendar year 1985–2016 and projected prevalence for 2017–2025. Based on data from the Swedish Cancer Registry and Statistics Sweden

(Fig. 3.3). In the United States, based on an excess annual mortality in CML of 1.53, and an annual incidence of approximately 1/100,000, Huang et al. estimated that the prevalence of CML will increase from approximately 70,000 in 2010 (corresponding to a prevalence of as high as 22/100,000) to 112,000 in 2020 and reach a near plateau of 35 times the annual incidence in 2050 [44]. Obviously, this trend will have profound pharmacoeconomic consequences [45, 46].

3.4 Risk Factors for Developing CML

The aetiology of CML is essentially unknown. Ionising radiation is the only established risk factor, having been linked to CML in atomic bomb survivors [47]. Results from a recent population-based case-control study suggested a weak association between smoking and CML [48], but whether tobacco use actually contributes to the aetiology of the disease is not unambiguous. Nevertheless, smokers seem to have a higher risk of disease progression compared with non-smokers [49]. Results from a study based on data from the Swedish Cancer Registry suggest that patients with CML have a moderately increased prevalence of other malignancies and autoimmune diseases, preceding the diagnosis of CML. These findings suggest that a more general predisposition to cancer and/or immunological mechanisms may be involved in the pathogenesis of CML [43, 50]. As for heredity, two studies based on the Swedish Cancer Registry and Multigeneration Registry were unable to find any significant familial aggregation of CML [51, 52].

3.5 Survival Rates and Non-disease-Related Prognostic Factors

3.5.1 Overall and Relative Survival in the Population-Based Setting

Results from a number of population-based studies have unanimously confirmed the significant

improvement in survival in patients with CML diagnosed since the introduction of TKIs at the turn of the century [3, 28, 29, 40, 53]. Previous studies suggested that the survival rate in patients treated within clinical trials, or in large referral centres, was significantly better than that of all patients with CML [54]. However, results from these large population-based studies have shown almost equal figures on survival with that obtained from the more selected materials, with an estimated 5-year overall survival of 85% for patients diagnosed in chronic phase with no difference between males and females [31, 37]. Data from the EUTOS registry, including patients diagnosed in chronic phase and treated outside clinical trials, the 5-year probability of dying because of CML was 3, 4 and 15% depending on the prognostic risk group (ELTS) at diagnosis [55].

A close to normal *relative survival* over an observation period of more than 10 years has been reported in 1536 patients of CML study IV [56]. This is not only in younger patients since age in the TKI era has a much smaller impact on CML-related death than in the pre-TKI era [57]. Similar observations on relative survival, though in a smaller cohort of patients, have been published by Sasaki [58]. In a study from the Swedish CML registry, relative survival was reported to be close to normal (i.e. 1) in younger CML patients but still reduced in the elderly population (Fig. 3.4). It may be concluded that in countries where TKIs are easily available, most patients with CML diagnosed in chronic phase (CP) have a life expectancy that is not identical but still close to that of the normal population [41, 53]. However, the small group of patients (5–7%) diagnosed in accelerated (AP) or blastic phase (BP) still have a less favourable prognosis (Fig. 3.5) [59].

3.5.2 Age and Comorbidity

Apart from disease-related pre-treatment factors (e.g. stage, Sokal and ELTS scores, aberrant cytogenetics), which are beyond the scope of this overview, several non-disease-related factors might have an impact on the prognosis of CML. Several studies indicate that, even after the introduction of imatinib in 2001–2002, elderly

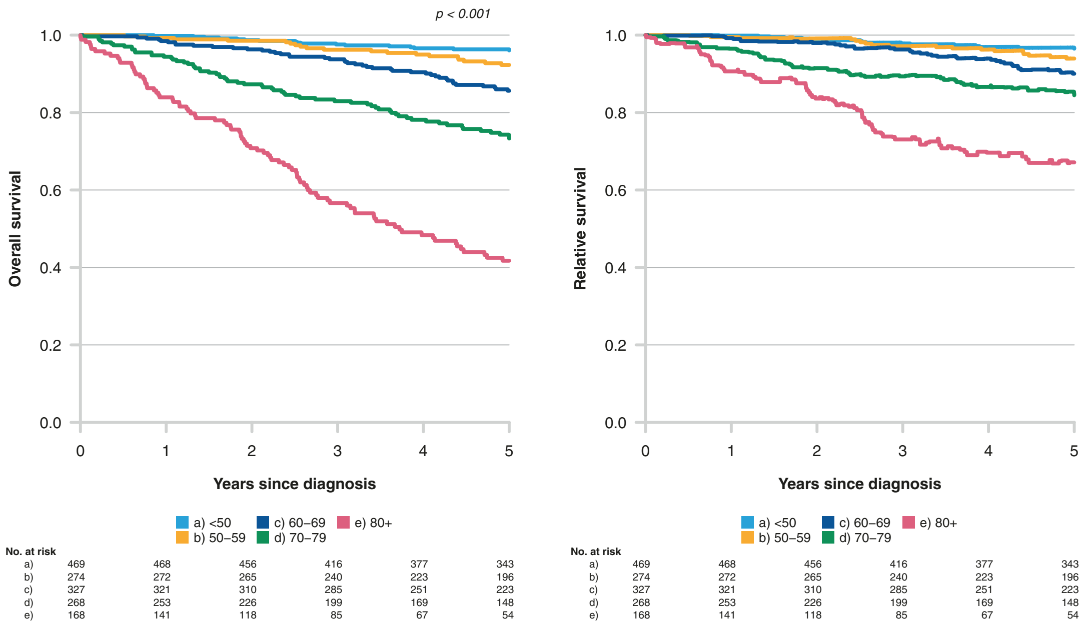


Fig. 3.4 Overall and relative survival by age in adult patients with CML diagnosed in 2002–2018. Data are obtained from the population-based Swedish Cancer Registry

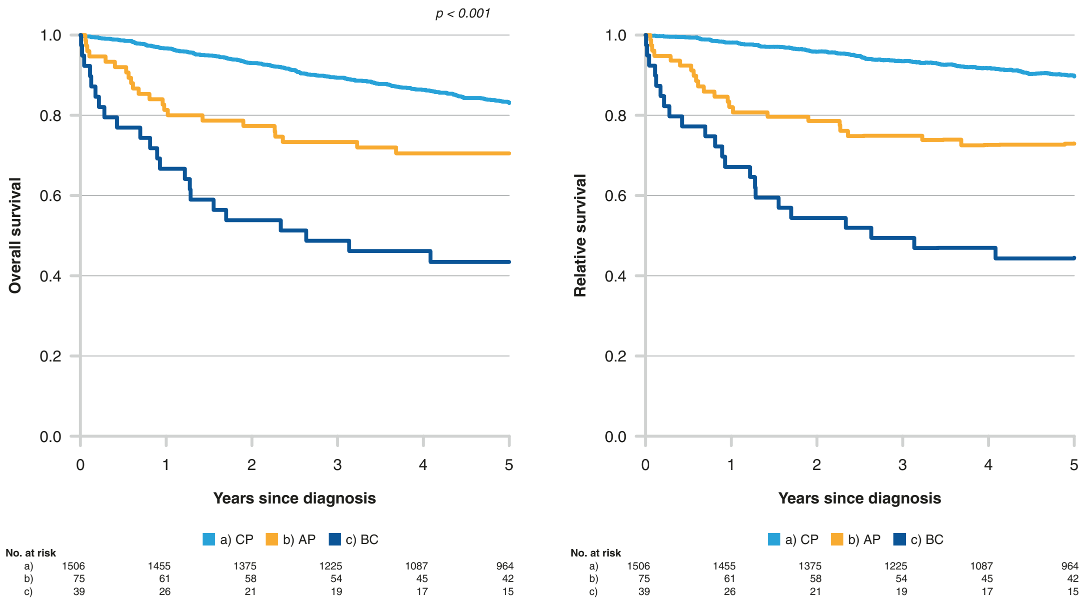


Fig. 3.5 Overall and relative survival by disease stage in adult patients with CML diagnosed in chronic, accelerated and blastic phase, respectively, 2002–2018. Data are obtained from the population-based Swedish CML Registry

CML patients (>70 years) have an inferior relative survival than younger ones [40, 60, 61]. Several reports show that elderly patients respond equally well as younger patients to treatment with imatinib [62, 63]. Possibly, a time lag in the introduction of imatinib and a persisting underuse TKIs in the elderly CML patients may explain the less impressive improvements in the elderly population [61, 64].

In another publication, based on patients participating in the German CML study IV, comorbidity, as measured by the Charlson comorbidity index [65] and separated from age in the analysis, was associated with worse survival but had no negative impact on response to imatinib [66]. However, comorbidities associated with significant organ failure or cognitive function may lead to lower treatment tolerability and, therefore, indirectly increase the risk of CML-related death [67].

3.5.3 Socioeconomy and CML

Even in economically more developed countries with equal availability to health care resources, socioeconomic factors may have an impact on the prognosis in patients with haematological cancers [68]. In CML, a population-based study from the UK showed that patients living in more deprived areas had poorer outcome in terms of relative survival, as well as a lower chance to obtain MMR, despite treatment with a TKI [31]. The authors speculate that non-adherence to TKI therapy may be the most important factor. However, in a later trial, based on linking the Swedish CML registry to several health databases, the authors concluded that the observed association between socioeconomic variables and survival could rather be explained by pretreatment factors (e.g. comorbidities) [69].

Previous publications suggested that centralised care of patients with CML is important for achieving results comparable with those of clinical trials [10]. More recently, Lauseker et al., analysing the outcome of 1491 patients included in the German CML study IV, observed a survival advantage for patients treated initially at a teaching hospital compared to those treated in municipal hospitals and by office-based physicians, respectively [70]. The difference remained when adjusted for age, performance status and EUTOS

score. Preliminary results from the Dutch registry suggest that patients with CML treated at smaller non-academic hospitals were less frequently monitored by cytogenetic and/or molecular assessments and were less often included in clinical trials [11, 12]. On the other hand, a report from the Swedish CML registry, based on 779 patients, was not able to find any difference in survival between patients living in university versus non-university catchment areas [13]. Apart from methodological issues, it may well be that the relative importance of centralised care in CML differs between countries due to differences in their health-care resources and organisation.

3.6 Do CML Patients Have an Increased Risk to Develop Other Cancers?

Studies on the risk of developing subsequent malignancies (other than MDS or acute leukaemia) after the diagnosis of CML have yielded conflicting results. Thus, in a study based on 1026 patients with CML, diagnosed in 1977–2008 and identified in the Danish Cancer Registry, Frederiksen et al. observed a 1.6-fold increased risk of developing a secondary malignancy as compared to the expected rate in the background population [71]. In a subsequent Swedish registry study, CML patients treated in the TKI era had a 1.5-fold increased risk of developing a secondary cancer as compared to the background population (matched by age, sex, health-care region and calendar year at diagnosis) [43, 50]. The authors speculated that this increased risk is more likely linked to the CML disease itself rather than to its treatment. However, other investigators, analysing different kinds of study populations, have found that patients with CML has only a borderline increased risk of secondary cancers [72] or no increased risk at all [73, 74]. Differences in patient numbers, selection, follow-up time and definition of 'secondary cancer' might explain these contradictory findings. Clearly, the question whether CML patients, nowadays mostly living an almost normal life span, have an increased risk of developing other malignancies needs to be further investigated.

Acknowledgements The authors appreciate the work of all clinicians reporting all newly diagnosed cases of CML to the Swedish CML registry.

Conflict of Interest Disclosures Nothing to disclose.

References

1. Socialstyrelsen (The National Board of Health and Welfare), Sweden, 2019. Statistics on Cancer Incidence. https://www.socialstyrelsen.se/globalassets/sharepoint-dokument/artikelkatalog/statistik/2020-12-7133.pdf
2. Storm HH, Michelsen EV, Clemmensen IH, Pihl J. The Danish cancer registry--history, content, quality and use. Dan Med Bull. 1997;44(5):535–9.
3. Thielen N, Visser O, Ossenkoppele G, Janssen J. Chronic myeloid leukemia in the Netherlands: a population-based study on incidence, treatment, and survival in 3585 patients from 1989 to 2012. Eur J Haematol. 2016;97(2):145–54.
4. Harrison SJ, Johnson PR, Holyoake TL. The Scotland leukaemia registry audit of incidence, diagnosis and clinical management of new patients with chronic myeloid leukaemia in 1999 and 2000. Scott Med J. 2004;49(3):87–90.
5. Maynadie M, Girodon F, Manivet-Janoray I, Mounier M, Mugneret F, Bailly F, Favre B, Caillot D, Petrella T, Flesch M, Carli PM. Twenty-five years of epidemiological recording on myeloid malignancies: data from the specialized registry of hematologic malignancies of Cote d'Or (Burgundy, France). Haematologica. 2011;96(1):55–61.
6. Osca-Gelis G, Puig-Vives M, Saez M, Gallardo D, Lloveras N, Marcos-Gragera R. Population-based incidence of myeloid malignancies: fifteen years of epidemiological data in the province of Girona, Spain. Haematologica. 2013;98(8):e95–7.
7. Barlow L, Westergren K, Holmberg L, Talback M. The completeness of the Swedish Cancer Register: a sample survey for year 1998. Acta Oncol. 2009;48(1):27–33.
8. SEER. SEER cancer statistics review (CSR) 1975–2017 2020.
9. Corm S, Roche L, Micol JB, Coiteux V, Bossard N, Nicolini FE, Iwaz J, Preudhomme C, Roche-Lestienne C, Facon T, Remontet L. Changes in the dynamics of the excess mortality rate in chronic phase-chronic myeloid leukemia over 1990-2007: a population study. Blood. 2011;118(16):4331–7.

10. Faber E, Muzik J, Koza V, Demeckova E, Voglova J, Demitrovicova L, Chudej J, Markuljak I, Cmunt E, Kozak T, Tothova E, Jarosova M, Dusek L, Indrak K. Treatment of consecutive patients with chronic myeloid leukaemia in the cooperating centres from the Czech Republic and the whole of Slovakia after 2000--a report from the population-based CAMELIA registry. Eur J Haematol. 2011;87(2):157–68.
11. Geelen IGP, Thielen N, Janssen J, Hoogendoorn M, Roosma TJA, Willemsen SP, Valk PJM, Visser O, Cornelissen JJ, Westerweel PE. Impact of hospital experience on the quality of tyrosine kinase inhibitor response monitoring and consequence for chronic myeloid leukemia patient survival. Haematologica. 2017a;102(12):e486–9.
12. Geelen IGP, Thielen N, Janssen J, Hoogendoorn M, Roosma TJA, Willemsen SP, Visser O, Cornelissen JJ, Westerweel PE. Treatment outcome in a population-based, 'real-world' cohort of patients with chronic myeloid leukemia. Haematologica. 2017b;102(11):1842–9.
13. Hoglund M, Sandin F, Hellstrom K, Bjoreman M, Bjorkholm M, Brune M, Dreimane A, Ekblom M, Lehmann S, Ljungman P, Malm C, Markevarn B, Myhr-Eriksson K, Ohm L, Olsson-Stromberg U, Sjalander A, Wadenvik H, Simonsson B, Stenke L, Richter J. Tyrosine kinase inhibitor usage, treatment outcome, and prognostic scores in CML: report from the population-based Swedish CML registry. Blood. 2013;122(7):1284–92.
14. Smith A, Roman E, Howell D, Jones R, Patmore R, Jack A, N. Haematological Malignancy Research. The Haematological Malignancy Research Network (HMRN): a new information strategy for population based epidemiology and health service research. Br J Haematol. 2010;148(5):739–53.
15. Hoffmann VS, Baccarani M, Hasford J, Lindoerfer D, Burgstaller S, Sertic D, Costeas P, Mayer J, Indrak K, Everaus H, Koskenvesa P, Guilhot J, Schubert-Fritschle G, Castagnetti F, Di Raimondo F, Lejniece S, Griskevicius L, Thielen N, Sacha T, Hellmann A, Turkina AG, Zaritskey A, Bogdanovic A, Sninska Z, Zupan I, Steegmann JL, Simonsson B, Clark RE, Covelli A, Guidi G, Hehlmann R. The EUTOS population-based registry: incidence and clinical characteristics of 2904 CML patients in 20 European countries. Leukemia. 2015;29(6):1336–43.
16. Chang CS, Lee K, Yang YH, Lin MT, Hsu CN. Estimation of CML incidence: disagreement between national cancer registry and health claims data system in Taiwan. Leuk Res. 2011;35(5):e53–4.
17. Foulon S, Cony-Makhoul P, Guerci-Bresler A, Delord M, Solary E, Monnereau A, Bonastre J, Tubert-Bitter P. Using healthcare claims data to analyze the prevalence of BCR-ABL-positive chronic myeloid leukemia in France: a nationwide population-based study. Cancer Med. 2019;8(6):3296–304.
18. Lauseker M, Gerlach R, Tauscher M, Hasford J. Improved survival boosts the prevalence of chronic myeloid leukemia: predictions from a population-based study. J Cancer Res Clin Oncol. 2016;142(7):1441–7.
19. Nguyen LT, Guo M, Naugler C, Rashid-Kolvear F. Incidence of chronic myeloid leukemia in Calgary, Alberta, Canada. BMC Res Notes. 2018;11(1): 780.
20. Castagnetti F, Di Raimondo F, De Vivo A, Spitaleri A, Gugliotta G, Fabbiano F, Capodanno I, Mannina D, Salvucci M, Antolino A, Marasca R, Musso M, Crugnola M, Impera S, Trabacchi E, Musolino C, Cavazzini F, Mineo G, Tosi P, Tomaselli C, Rizzo M, Siragusa S, Fogli M, Ragionieri R, Zironi A, Soverini S, Martinelli G, Cavo M, Vigneri P, Stagno F, Rosti G, Baccarani M. A population-based study of chronic myeloid leukemia patients treated with imatinib in first line. Am J Hematol. 2017;92(1):82–7.
21. Kurtovic-Kozaric A, Hasic A, Radich JP, Bijedic V, Nefic H, Eminovic I, Kurtovic S, Colakovic F, Kozaric M, Vranic S, Bovan NS. The reality of cancer treatment in a developing country: the effects of delayed TKI treatment on survival, cytogenetic and molecular responses in chronic myeloid leukaemia patients. Br J Haematol. 2015;172:420.
22. Geelen IGP, Thielen N, Janssen J, Hoogendoorn M, Roosma TJA, Valk PJM, Visser O, Cornelissen JJ, Westerweel PE. Omitting cytogenetic assessment from routine treatment response monitoring in chronic myeloid leukemia is safe. Eur J Haematol. 2018;100(4):367–71.
23. Berglund A, Holmberg L, Tishelman C, Wagenius G, Eaker S, Lambe M. Social inequalities in non-small cell lung cancer management and survival: a population-based study in Central Sweden. Thorax. 2010;65(4):327–33.
24. Gunnarsson N, Hoglund M, Stenke L, Wallberg-Jonsson S, Sandin F, Bjorkholm M, Dreimane A, Lambe M, Markevarn B, Olsson-Stromberg U, Wadenvik H, Richter J, Sjalander A. Increased prevalence of prior malignancies and autoimmune diseases in patients diagnosed with chronic myeloid leukemia. Leukemia. 2016;30(7):1562–7.
25. Dahlen T, Edgren G, Lambe M, Hoglund M, Bjorkholm M, Sandin F, Sjalander A, Richter J, Olsson-Stromberg U, Ohm L, Back M, Stenke L, Swedish CMLG, C. M. L. R. G. the Swedish. Cardiovascular events associated with use of tyrosine kinase inhibitors in chronic myeloid Leukemia: a population-based cohort study. Ann Intern Med. 2016;165(3):161–6.
26. Bray F, Parkin DM. Evaluation of data quality in the cancer registry: principles and methods. Part I: comparability, validity and timeliness. Eur J Cancer. 2009;45(5):747–55.
27. Parkin DM, Bray F. Evaluation of data quality in the cancer registry: principles and methods part II. Completeness. Eur J Cancer. 2009;45(5):756–64.
28. Beinortas T, Tavoriene I, Zvirblis T, Gerbutavicius R, Jurgutis M, Griskevicius L. Chronic myeloid leukemia incidence, survival and accessibility of tyrosine kinase inhibitors: a report from population-based

Lithuanian haematological disease registry 2000-2013. BMC Cancer. 2016;16:198.
29. Chen Y, Wang H, Kantarjian H, Cortes J. Trends in chronic myeloid leukemia incidence and survival in the United States from 1975 to 2009. Leuk Lymphoma. 2013;54(7):1411–7.
30. Rohrbacher M, Hasford J. Epidemiology of chronic myeloid leukaemia (CML). Best Pract Res Clin Haematol. 2009;22(3):295–302.
31. Smith AG, Painter D, Howell DA, Evans P, Smith G, Patmore R, Jack A, Roman E. Determinants of survival in patients with chronic myeloid leukaemia treated in the new era of oral therapy: findings from a UK population-based patient cohort. BMJ Open. 2014;4(1):e004266.
32. Mendizabal AM, Younes N, Levine PH. Geographic and income variations in age at diagnosis and incidence of chronic myeloid leukemia. Int J Hematol. 2016;103(1):70–8.
33. Rohrbacher M, Berger U, Hochhaus A, Metzgeroth G, Adam K, Lahaye T, Saussele S, Muller MC, Hasford J, Heimpel H, Hehlmann R. Clinical trials underestimate the age of chronic myeloid leukemia (CML) patients. Incidence and median age of Ph/BCR-ABL-positive CML and other chronic myeloproliferative disorders in a representative area in Germany. Leukemia. 2009;23(3):602–4.
34. McQuilten ZK, Wood EM, Polizzotto MN, Campbell LJ, Wall M, Curtis DJ, Farrugia H, McNeil JJ, Sundararajan V. Underestimation of myelodysplastic syndrome incidence by cancer registries: results from a population-based data linkage study. Cancer. 2014;120(11):1686–94.
35. Gale RP, Cozen W, Goodman MT, Wang FF, Bernstein L. Decreased chronic lymphocytic leukemia incidence in Asians in Los Angeles County. Leuk Res. 2000;24(8):665–9.
36. Matasar MJ, Ritchie EK, Consedine N, Magai C, Neugut AI. Incidence rates of the major leukemia subtypes among US Hispanics, Blacks, and non-Hispanic Whites. Leuk Lymphoma. 2006;47(11):2365–70.
37. Hoglund M, Sandin F, Simonsson B. Epidemiology of chronic myeloid leukaemia: an update. Ann Hematol. 2015;94(Suppl 2):S241–7.
38. de la Fuente J, Baruchel A, Biondi A, de Bont E, Dresse MF, Suttorp M, Millot F, B. F. M. G. S. G. C. M. L. C. International. Managing children with chronic myeloid leukaemia (CML): recommendations for the management of CML in children and young people up to the age of 18 years. Br J Haematol. 2014;167(1):33–47.
39. Radivoyevitch T, Jankovic GM, Tiu RV, Saunthararajah Y, Jackson RC, Hlatky LR, Gale RP, Sachs RK. Sex differences in the incidence of chronic myeloid leukemia. Radiat Environ Biophys. 2014;53(1):55–63.
40. Bjorkholm M, Ohm L, Eloranta S, Derolf A, Hultcrantz M, Sjoberg J, Andersson T, Hoglund M, Richter J, Landgren O, Kristinsson SY, Dickman PW. Success story of targeted therapy in chronic myeloid leukemia: a population-based study of patients diagnosed in Sweden from 1973 to 2008. J Clin Oncol. 2011;29(18):2514–20.
41. Bower H, Bjorkholm M, Dickman PW, Hoglund M, Lambert PC, Andersson TM. Life expectancy of patients with chronic myeloid Leukemia approaches the life expectancy of the general population. J Clin Oncol. 2016;34(24):2851–7.
42. Delord M, Foulon S, Cayuela JM, Rousselot P, Bonastre J. The rising prevalence of chronic myeloid leukemia in France. Leuk Res. 2018;69:94–9.
43. Gunnarsson N, Hoglund M, Stenke L, Wallberg Jonsson S, Sandin F, Bjorkholm M, Dreimane A, Lambe M, Markevarn B, Olsson-Stromberg U, Wadenvik H, Richter J, Sjalander A. Increased prevalence of prior malignancies and autoimmune diseases in patients diagnosed with chronic myeloid Leukemia. Blood. 2015a;126(23):1586.
44. Huang X, Cortes J, Kantarjian H. Estimations of the increasing prevalence and plateau prevalence of chronic myeloid leukemia in the era of tyrosine kinase inhibitor therapy. Cancer. 2012;118(12):3123–7.
45. Kantarjian HM, Fojo T, Mathisen M, Zwelling LA. Cancer drugs in the United States: Justum Pretium--the just price. J Clin Oncol. 2013;31(28):3600–4.
46. Ohm L, Lundqvist A, Dickman P, Hoglund M, Persson U, Stenke L, Carlsson KS, Bjorkholm M. Real-world cost-effectiveness in chronic myeloid leukemia: the price of success during four decades of development from non-targeted treatment to imatinib. Leuk Lymphoma. 2015;56(5):1385–91.
47. Heyssel R, Brill B, Woodbury LA, Nishimura ET, Ghose T, Hishino T, Yamasaki M. Leukemia in hiroshima atomic bomb survivors. Blood. 1960;15 (3):313–31.
48. Musselman JR, Blair CK, Cerhan JR, Nguyen P, Hirsch B, Ross JA. Risk of adult acute and chronic myeloid leukemia with cigarette smoking and cessation. Cancer Epidemiol. 2013;37(4):410–6.
49. Lauseker M, Hasford J, Saussele S, Kremers S, Kraemer D, Lindemann W, Hehlmann R, Pfirrmann M, German CMLSG. Smokers with chronic myeloid leukemia are at a higher risk of disease progression and premature death. Cancer. 2017;123(13): 2467–71.
50. Gunnarsson N, Stenke L, Hoglund M, Sandin F, Bjorkholm M, Dreimane A, Lambe M, Markevarn B, Olsson-Stromberg U, Richter J, Wadenvik H, Wallvik J, Sjalander A. Second malignancies following treatment of chronic myeloid leukaemia in the tyrosine kinase inhibitor era. Br J Haematol. 2015b;169(5):683–8.
51. Bjorkholm M, Kristinsson SY, Landgren O, Goldin LR. No familial aggregation in chronic myeloid leukemia. Blood. 2013;122(3):460–1.
52. Gunnarsson N, Hoglund M, Stenke L, Sandin F, Bjorkholm M, Dreimane A, Lambe M, Markevarn B, Olsson-Stromberg U, Wadenvik H, Richter J, Sjalander A. No increased prevalence of malignancies among first-degree relatives of 800 patients with chronic myeloid leukemia: a population-based study in Sweden. Leukemia. 2017;31(8):1825–7.

53. Bjorkholm M, Bower H, Dickman PW, Lambert PC, Höglund M, Andersson TM-L. Temporal trends in chronic myeloid Leukemia outcome using the loss in expectation of life: a Swedish population-based study. Blood. 2015;126(23):2779.
54. Pulte D, Gondos A, Redaniel MT, Brenner H. Survival of patients with chronic myelocytic leukemia: comparisons of estimates from clinical trial settings and population-based cancer registries. Oncologist. 2011;16(5): 663–71.
55. Pfirrmann M, Baccarani M, Saussele S, Guilhot J, Cervantes F, Ossenkoppele G, Hoffmann VS, Castagnetti F, Hasford J, Hehlmann R, Simonsson B. Prognosis of long-term survival considering disease-specific death in patients with chronic myeloid leukemia. Leukemia. 2015;30:48.
56. Hehlmann R, Lauseker M, Saussele S, Pfirrmann M, Krause S, Kolb HJ, Neubauer A, Hossfeld DK, Nerl C, Gratwohl A, Baerlocher GM, Heim D, Brummendorf TH, Fabarius A, Haferlach C, Schlegelberger B, Muller MC, Jeromin S, Proetel U, Kohlbrenner K, Voskanyan A, Rinaldetti S, Seifarth W, Spiess B, Balleisen L, Goebeler MC, Hanel M, Ho A, Dengler J, Falge C, Kanz L, Kremers S, Burchert A, Kneba M, Stegelmann F, Kohne CA, Lindemann HW, Waller CF, Pfreundschuh M, Spiekermann K, Berdel WE, Muller L, Edinger M, Mayer J, Beelen DW, Bentz M, Link H, Hertenstein B, Fuchs R, Wernli M, Schlegel F, Schlag R, de Wit M, Trumper L, Hebart H, Hahn M, Thomalla J, Scheid C, Schafhausen P, Verbeek W, Eckart MJ, Gassmann W, Pezzutto A, Schenk M, Brossart P, Geer T, Bildat S, Schafer E, Hochhaus A, Hasford J. Assessment of imatinib as first-line treatment of chronic myeloid leukemia: 10-year survival results of the randomized CML study IV and impact of non-CML determinants. Leukemia. 2017;31(11):2398–406.
57. Pfirrmann M, Baccarani M, Saussele S, Guilhot J, Cervantes F, Ossenkoppele G, Hoffmann VS, Castagnetti F, Hasford J, Hehlmann R, Simonsson B. Prognosis of long-term survival considering disease-specific death in patients with chronic myeloid leukemia. Leukemia. 2016;30(1):48–56.
58. Sasaki K, Strom SS, O'Brien S, Jabbour E, Ravandi F, Konopleva M, Borthakur G, Pemmaraju N, Daver N, Jain P, Pierce S, Kantarjian H, Cortes JE. Relative survival in patients with chronic-phase chronic myeloid leukaemia in the tyrosine-kinase inhibitor era: analysis of patient data from six prospective clinical trials. Lancet Haematol. 2015;2(5):e186–93.
59. Soderlund S, Dahlen T, Sandin F, Olsson-Stromberg U, Creignou M, Dreimane A, Lubking A, Markevarn B, Sjalander A, Wadenvik H, Stenke L, Richter J, Hoglund M. Advanced phase chronic myeloid leukaemia (CML) in the tyrosine kinase inhibitor era—a report from the Swedish CML register. Eur J Haematol. 2017;98(1):57–66.
60. Brunner AM, Campigotto F, Sadrzadeh H, Drapkin BJ, Chen YB, Neuberg DS, Fathi AT. Trends in all-cause mortality among patients with chronic myeloid leukemia: a surveillance, epidemiology, and end results database analysis. Cancer. 2013;119(14):2620–9.
61. Ector G, Visser O, Westerweel PE, Janssen J, Blijlevens NMA and Dinmohamed AG. Primary therapy and relative survival among elderly patients with chronic myeloid leukemia: a population-based study in the Netherlands, 1989–2017. Leukemia 2020.
62. Breccia M, Alimena G. The role of comorbidities in chronic myeloid leukemia. Leuk Res. 2013;37(7):729–30.
63. Gugliotta G, Castagnetti F, Palandri F, Breccia M, Intermesoli T, Capucci A, Martino B, Pregno P, Rupoli S, Ferrero D, Gherlinzoni F, Montefusco E, Bocchia M, Tiribelli M, Pierri I, Grifoni F, Marzocchi G, Amabile M, Testoni N, Martinelli G, Alimena G, Pane F, Saglio G, Baccarani M, Rosti G. Frontline imatinib treatment of chronic myeloid leukemia: no impact of age on outcome, a survey by the GIMEMA CML Working Party. Blood. 2011;117(21):5591–9.
64. Lauseker M, Gerlach R, Worseg W, Haferlach T, Tauscher M, Hasford J, Hoffmann VS. Differences in treatment and monitoring of chronic myeloid leukemia with regard to age, but not sex: results from a population-based study. Eur J Haematol. 2019;103(4):362–9.
65. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40(5):373–83.
66. Saussele S, Krauss MP, Hehlmann R, Lauseker M, Proetel U, Kalmanti L, Hanfstein B, Fabarius A, Kraemer D, Berdel WE, Bentz M, Staib P, de Wit M, Wernli M, Zettl F, Hebart HF, Hahn M, Heymanns J, Schmidt-Wolf I, Schmitz N, Eckart MJ, Gassmann W, Bartholomaus A, Pezzutto A, Leibundgut EO, Heim D, Krause SW, Burchert A, Hofmann WK, Hasford J, Hochhaus A, Pfirrmann M, Muller MC, K. Schweizerische Arbeitsgemeinschaft fur Klinische and C. M. L. S. G. the German. Impact of comorbidities on overall survival in patients with chronic myeloid leukemia: results of the randomized CML study IV. Blood. 2015;126(1):42–9.
67. Mohammadi M, Cao Y, Glimelius I, Bottai M, Eloranta S, Smedby KE. The impact of comorbid disease history on all-cause and cancer-specific mortality in myeloid leukemia and myeloma - a Swedish population-based study. BMC Cancer. 2015;15:850.
68. Bhayat F, Das-Gupta E, Smith C, McKeever T, Hubbard R. The incidence of and mortality from leukaemias in the UK: a general population-based study. BMC Cancer. 2009;9:252.
69. Larfors G, Sandin F, Richter J, Sjalander A, Stenke L, Lambe M, Hoglund M. The impact of socio-economic factors on treatment choice and mortality in chronic myeloid leukaemia. Eur J Haematol. 2017;98(4):398–406.
70. Lauseker M, Hasford J, Pfirrmann M, Hehlmann R, German CMLSG. The impact of health care settings on survival time of patients with chronic myeloid leukemia. Blood. 2014;123(16):2494–6.

71. Frederiksen H, Farkas DK, Christiansen CF, Hasselbalch HC, Sorensen HT. Chronic myeloproliferative neoplasms and subsequent cancer risk: a Danish population-based cohort study. Blood. 2011;118(25):6515–20.
72. Sasaki K, Kantarjian HM, O'Brien S, Ravandi F, Konopleva M, Borthakur G, Garcia-Manero G, Wierda WG, Daver N, Ferrajoli A, Takahashi K, Jain P, Rios MB, Pierce SA, Jabbour EJ, Cortes JE. Incidence of second malignancies in patients with chronic myeloid leukemia in the era of tyrosine kinase inhibitors. Int J Hematol. 2019;109(5):545–52.
73. Gugliotta G, Castagnetti F, Breccia M, Albano F, Iurlo A, Intermesoli T, Abruzzese E, Levato L, D'Adda M, Pregno P, Cavazzini F, Stagno F, Martino B, La Barba G, Sora F, Tiribelli M, Bigazzi C, Binotto G, Bonifacio M, Caracciolo C, Soverini S, Foa R, Cavo M, Martinelli G, Pane F, Saglio G, Baccarani M, Rosti G, P. Gruppo Italiano Malattie Ematologiche dell'Adulto - Chronic Myeloid Leukemia Working. Incidence of second primary malignancies and related mortality in patients with imatinib-treated chronic myeloid leukemia. Haematologica. 2017;102(9):1530–6.
74. Miranda MB, Lauseker M, Kraus MP, Proetel U, Hanfstein B, Fabarius A, Baerlocher GM, Heim D, Hossfeld DK, Kolb HJ, Krause SW, Nerl C, Brummendorf TH, Verbeek W, Fauser AA, Prummer O, Neben K, Hess U, Mahlberg R, Ploger C, Flasshove M, Rendenbach B, Hofmann WK, Muller MC, Pfirrmann M, Hochhaus A, Hasford J, Hehlmann R, Saussele S. Secondary malignancies in chronic myeloid leukemia patients after imatinib-based treatment: long-term observation in CML Study IV. Leukemia. 2016;30(6):1255–62.

4 Imatinib: The First-Line CML Therapy

Carmen Fava, Giovanna Rege-Cambrin, and Giuseppe Saglio

4.1 Introduction

Imatinib, the first TKI (tyrosine kinase inhibitor) of BCR-ABL1 introduced for the therapy of CML (chronic myelogenous leukemia), has profoundly changed the outcome perspectives of a disease previously fatal in the vast majority of the patients and which now shows an overall survival similar to that of a control population without leukemia. However, in addition to those who cannot tolerate the drug (approximately 10–15% of the total), 20–25% of the imatinib patients treated with the usual dosage of 400 mg do not reach an optimal response criteria according to the ELN (European Leukemia Net) recommendations. This has led to the exploration, as front-line therapy for CML, of second-generation tyrosine kinase inhibitors like nilotinib, dasatinib, and bosutinib, more powerful TKIs with respect to imatinib and initially registered as second-line therapy for the CML cases intolerant or resistant to imatinib. The clinical trials comparing imatinib versus the second generation TKIs have shown that the latter are able to induce faster and deeper molecular responses with respect to 400 mg imatinib, but these advantages are counterbalanced by a higher degree of immediate and long-term toxicities and by no improvement in the overall survival (OS) and progression-free survival (PFS) rates. In addition, more recently studies testing higher dosages of imatinib (800 mg per day) compared to standard dose imatinib or dose-adapted imatinib or imatinib plus interferon have been reported to be able to induce better cytogenetic and molecular responses, including the achievement of deep molecular responses like MR4 and MR4,5 which are needed to attempt treatment-free remission (TFR). Therefore, considering that imatinib has become a generic drug and that this has considerably lowered its cost allowing its use in patients all over the world, it is easy to understand why imatinib still represents the first-line therapy of choice for the majority of CML patients.

4.2 Imatinib and Response to Therapy

Imatinib was the first tyrosine kinase inhibitor (TKI) introduced in the therapy of chronic myeloid leukemia (CML), and it is still the standard of care and the most widely used frontline therapy for CML patients in chronic phase [1]. Indeed, the long-term overall survival (OS) observed in patients treated first line with imatinib has been matched but never overcome by other TKIs [2–4]. The most relevant data of the 8-year follow-up of the IRIS study that have also been confirmed by other studies and by

C. Fava · G. Rege-Cambrin · G. Saglio (✉)
Department of Clinical and Biological Sciences, University of Turin, Turin, Italy
e-mail: giuseppe.saglio@unito.it

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
R. Hehlmann (ed.), *Chronic Myeloid Leukemia*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-71913-5_4

independent retrospective analysis performed on patients outside clinical trials show a cumulative CCyR rate around 83–85% and an estimated OS rate of approximately 82–84% at 10 years, which is far better from what was observed before the introduction of this drug [5, 6]. This result may be ascribed to a substantial decrease in the number of the progressions to accelerated phase or blast crisis observed in patients treated with imatinib. Many records indeed suggest that progressions to a more advanced phase of the disease still represent a major cause of death for CML patients, still being incurable in most cases even in the tyrosine kinase inhibitor (TKI) era [7]. With imatinib therapy, the occurrence of progression drops from an expected rate of approximately 15% per year to a rate of 2–3% per year, and only for the first 2–3 years of treatment as during the subsequent years events of progression are really occasional [6]. This is certainly due to the great reduction of the leukemic mass observed in most of the imatinib-treated patients, which in few cases can also result in an apparent disappearance of the leukemic clone, but also to the fact that imatinib, inhibiting the BCR-ABL tyrosine kinase (TK) activity that plays a major role in determining the genomic instability of the leukemic cells, may per se be able to slow the propensity to progress [8].

It has been demonstrated that the patients who benefit from TKI therapy with imatinib are those who achieve and maintain CCyR for at least 2 years, as in these cases, the OS is similar to that of a control population without leukemia [9]. On the other side, various analyses have shown that patients who do not achieve good cytogenetic or molecular responses to imatinib at defined time points have a worse outcome, characterized by an increased risk of relapse, progression, and death [10, 11].

Based on these principles, a panel of CML experts from the European Leukemia Net (ELN) and members of the National Comprehensive Cancer Network (NCCN) have previously established and, more recently, revised treatment milestones to be achieved during CML treatment with TKIs [12, 13]. This obviously implies that to optimize CML treatment with TKIs an appropriate and timely follow-up with cytogenetic and standardized molecular methods of adequate reliability is needed. In particular, molecular monitoring of BCR-ABL transcript levels by real-time quantitative PCR (RQ PCR) has become the most useful and precise way to monitor CML patients, almost replacing the cytogenetic analysis during follow-up, although not at diagnosis as stated in the ELN 2020 recommendations [14–17]. Indeed, with respect to conventional cytogenetic analysis, RQ PCR not only allows monitoring the first steps of reduction of the leukemic burden occurring within the first months of TKI therapy but may also allow estimating the amount of the residual disease once CCyR is achieved, as the sensitivity that can be reached with the present RQ PCR procedures in a sample of good quality is in most cases between $10^{-4}/10^{-5}$, which corresponds to an amount between 2 and 3 logs below the threshold of the achievement of CCyR [14]. According to the established international scale (IS), the relevant BCR-ABL1% to be achieved are 1% (2-log reduction with respect to the median BCR-ABL1 amount present at diagnosis and that roughly corresponds to the threshold of CCyR), and 0.10% BCR-ABL1 (major molecular response (MMR)) and 0.01–0.0032% BCR-ABL1 corresponding, respectively, to MR4 (4-log reduction) and MR4.5 (4.5-log reduction) [18].

The attainment of CCyR or 1% BCR-ABL1 can still be considered the most significant response to target, as this goal has been demonstrated to be associated to the highest probability of long-term survival for CML patients [19–21]. On the other side, some data support the notion that deeper responses, as the achievement of level of $\text{BCR-ABL}^{\text{IS}} \leq 0.1\%$ (MMR), may indeed improve OS relative to achieve CCyR without MMR [21]. Indeed a 4-year landmark analysis performed within the context of the German CML-study IV suggests that patients who after 4 years were able to achieve a stable MR4.5 molecular response showed at 8 years a statistically significant better survival with respect to those patients who have simply achieved CCyR but not MMR [21]. If these results are confirmed, MR4.5 will represent a new molecular predictor

of long-term outcome. In any case, it has been clearly established by several clinical studies that a stable deep molecular response (at least MR4 or even better MR4.5) is required to obtain long-lasting treatment-free remission (TFR) that is progressively becoming the new treatment goal for CML patients [22–25]. Thus, the achievements of MMR and of MR4.5 in addition to CCyR and MMR are appealing targets to pursue, as they predict more durable and stable responses and can also open up the possibility to try stopping therapy.

It is noteworthy that many studies, particularly in more recent years, have indicated that early cytogenetic and molecular responses within the first year of therapy represent the strongest prognostic parameters not only in terms of OS, progression-free survival (PFS) or event-free survival (EFS) but also in terms of possibility of achieving deeper molecular responses and, therefore, the possibility of discontinuing treatment without molecular relapse (TFR) [26–28]. Based on these observations, some recent treatment recommendations as those of the GIMEMA group have been modified with respect to the past the time points at which the expected response goals should be met to match the criteria for optimal response and have also introduced MR4 (0.01% BCR-ABL1) in the optimal response requirements within the first 24 months of therapy [25].

Based on these parameters, it appears that approximately one third of CML patients do not show an optimal response to imatinib therapy, and they, therefore, face a statistically significantly higher risk of an inferior outcome in terms of EFS, PFS, and also OS (approximately 80% at 5 years with respect to >95% of those below 10% BCR-ABL at 3 months) [20, 27, 28]. Actually, it is true that with imatinib most of these patients will only show a delayed response and will not progress or die, but it should also be considered that approximately 15–20% of them in a short time will die and many of them because of CML and progression [20, 27, 28]. In addition to the cases of failure, progression, and death, the reasons for discontinuation also include 10–12% of patients who show adverse events (AEs) and are intolerant to imatinib treatment and should be moved to treatment with another TKI.

It is also noteworthy that the percentage of the patients who do not respond optimally to imatinib may vary according to the initial clinical and hematological features that determine their initial risk category, as established by Sokal and also by the more recent ELTS (EUTOS Long Term Survival) score, which appears to be even more precise than Sokal scores in predicting the outcome of the CML patients in terms of death by CML [29]. In the IRIS study, patients with low-, intermediate-, or high-risk Sokal scores showed significantly different response rates as 5-year CCyR (89, 82, and 69%, respectively: $P < 0.001$) and progression to advanced disease (3, 8, and 17%, respectively: $P = 0.002$).

Based on all these considerations, several clinical trials aiming to improve the first-line treatment of patients with chronic phase CML have been performed. These therapeutic strategies that have been tested include the first-line administration of the second-generation TKIs (originally used as second-line therapy) or higher dosages of imatinib from the start. Also combinations of imatinib with other drugs, namely interferon-alpha (IFN-α), have been tested and trials are still ongoing, but for the moment these therapeutic options remain investigational and are not used in normal clinical practice.

4.3 Imatinib Versus Second-Generation TKIs as First-Line Treatment

At present, the use of three second-generation TKIs (nilotinib at the dosage of 300 mg BID, dasatinib 100 mg OD and bosutinib at the dosage of 400 mg OD) has been approved and registered as first-line therapy [2, 3, 30]. As patients with CP CML have very long survival and very long follow-ups are, consequently, required before the efficacy of these alternative treatment options could be measured in terms of OS, important surrogate markers as the rates of CCyR, MMR, MR4, and MR4.5 achieved at relevant time-points and progression-free survival (PFS)

parameters have been frequently used as a way to evaluate the relative responses and to compare results. However, it is important to consider that the methods to assess and to report the rate of responses can sometimes vary and that also the definitions of the EFS and PFS may change according to the protocol in different trials and may, therefore, introduce bias in the comparative evaluation of the results obtained in different clinical studies.

The rationale to test the second-generation TKIs (i.e., nilotinib, dasatinib, and bosutinib) against imatinib as first-line therapy was due to the fact that they were more potent than imatinib in inhibiting BCR-ABL1 TK activity, and they were already approved as second-line therapy for imatinib-intolerant or imatinib-resistant patients, being able to induce a CCyR rate of approximately 40–50% in these patients also when the resistance was due to the presence of clones with BCR-ABL1 mutations (with the notable exception of the T315I mutation) poorly responsive to imatinib therapy [31–34].

The efficacy and the toxicity of nilotinib and dasatinib as first-line therapy were initially assessed in phase 2 studies [35–37]. The results obtained in 73 newly diagnosed CP-CML patients treated with nilotinib 400 mg twice a day by the GIMEMA CML working party showed CCyR achievement at 3 months in 78% of the patients and in 96% at 6 months, whereas the MMR rates observed were 52% and 66%, respectively, at the same time points and 85% at 12 months [35]. Similarly, results of 100 newly diagnosed CML patients treated at the MD Anderson Cancer Center with nilotinib 400 mg twice daily (BID) showed, with a median follow-up of 29 months (range 1–73), a cumulative CCyR rate of 93%, MMR rate of 73% and CMR rate (defined according to the previous ELN criteria as undetectable hybrid transcripts with a sensitivity of at least $10^{-4}/^{-5}$) of 33% [36]. At the same institution, 86 newly diagnosed patients were treated with dasatinib 50 mg twice daily (BID) or 100 mg QD [37]. With a median follow-up of 24 months, most patients achieved a rapid CCyR (94% at 6 months) with a cumulative CCyR ratio of 98%. After 12 and 18 months, MMR was achieved by 71 and 79% of patients. The toxicity profile with dasatinib was also favorable with a better tolerability with dasatinib QD vs. BID dosing.

ENESTnd is a phase 3, randomized, open-label, multicenter study comparing the efficacy and safety of nilotinib with imatinib in patients with newly diagnosed CML that has now completed the tenth year of follow-up [2, 38]. The trial included 846 patients randomly assigned 1:1:1 to nilotinib 300 mg BID (n = 282), nilotinib 400 mg BID (n = 281), or imatinib 400 mg/day (n = 283). MMR at 12 months was the primary endpoint. Patients were also stratified by Sokal risk score, which resulted in equal distributions of low-, intermediate-, and high risk Sokal scores in each arm of the trial. Efficacy results were presented in the intent-to-treat (ITT) population. The MMR rate at 12 months was significantly higher for nilotinib 300 mg BID (44%, $P < 0.0001$) and nilotinib 400 mg BID (43%, $P < 0.0001$) than for imatinib (22%). As this was the primary endpoint of the study, nilotinib 300 mg BID was approved by FDA and EMA and registered as the first-line therapy. Responses were rapidly achieved with nilotinib, with 6-month MMR rates of 33%, 30%, and 12% for nilotinib 300 mg BID, nilotinib 400 mg BID, and imatinib, respectively. These higher responses were also associated with fewer progressions to AP/BC with nilotinib than with imatinib as already observed during the follow-up at 5 years of the study [39]. Cumulative 10-year MMR rates of patients assigned to nilotinib 300, nilotinib 400, and imatinib are 82.6%, 80.4%, and 69.6% and cumulative MR4.5 rates are 63.8%, 61.6%, and 45.2%, respectively. The difference between MR4.5 rates achieved with nilotinib vs. imatinib by 10 years was similar to that observed after 5 years of treatment study [38, 39].

However, as already observed in the results of the 5-year follow-up, the occurrence of cardiovascular events (CVEs) was much more frequent with nilotinib than with imatinib and was dose dependent, being more frequent in the nilotinib 400 mg BID arm that in the nilotinib 300 mg BID [39]. They in addition continue to increase at similar rates during the subsequent 5 years [38]. Finally the observed 10-year OS rates are similar

between nilotinib and imatinib and in conclusion, the 10-year follow-up data confirm the sustained efficacy of frontline nilotinib in achieving earlier and deeper molecular responses but also underline the high risk of developing CVEs with prolonged nilotinib therapy.

Dasision is a phase 3, randomized, open-label, multicenter study comparing the efficacy and safety of dasatinib 100 mg OD as the first-line therapy with respect to that of imatinib [3, 40]. This study achieved a minimum follow-up of 5 years and was subsequently terminated [40]. Patients with newly diagnosed CML-CP were stratified according to the Euro Risk Score and randomly assigned to dasatinib 100 mg/day or imatinib 400 mg/day. Confirmed CCyR by 12 months was the primary endpoint of the study and by 12 months was significantly higher for dasatinib (83%, $P < 0.001$) than for imatinib (72%), allowing this drug also to be approved as the first-line therapy by FDA and EMA. The best cumulative MMR rate by 12 months was also significantly higher for dasatinib (46%, $P < 0.0001$) than for imatinib (28%) [40]. After 5 years, molecular response rates continue to be higher for dasatinib compared with imatinib (rates of MMR 76% vs. 64%, $P = 0.002$ and rates ofMR4.5 42% vs. 33%, $P = 0.025$). Transformations to AP/BC on study or after discontinuation were lower with dasatinib ($n = 12/259$; 4.6%) compared with imatinib ($n = 19/260$; 7.3%). However, 5-year PFS and OS rates were similar across treatment arms (PFS 85% dasatinib, 86% imatinib; OS 91% dasatinib, 90% imatinib) [40]. A higher proportion of patients on dasatinib achieved BCR-ABL ≤10% at 3 months (84%) compared with those on imatinib (64%). Patients who achieved BCR-ABL ≤10% versus >10% at 3 months showed improved PFS and OS and lower rates of transformation to AP/BP (PFS 89 vs. 72%, $P = 0.0014$; OS 94 vs. 81%, $P = 0.0028$; transformation $n = 6/198$ [3%] vs. $n = 5/37$ [14%]) than imatinib (PFS 93 vs. 72%, $P < 0.0001$; OS 95% vs. 81%, $P = 0.0003$; transformation $n = 5/154$ 3% vs. $n = 13/85$,15%) [28]. Concerning the AEs of dasatinib, the total incidence of pleural effusion after 5 years is 29%, but most cases were grade 1 or 2 (67 out of 74), and discontinuation of dasatinib due to pleural effusion occurred in only 15 patients (6% overall and 20% of patients who experienced a pleural effusion). Arterial ischemic events were not common, occurring in 12 patients (5%) on dasatinib and 6 patients (2%) on imatinib [40]. More recently, however, one investigator-initiated study comparing dasatinib 100 mg OD vs. imatinib 400 mg OD, although showing that the proportion of patients achieving CCyR was superior with dasatinib (84% vs. 69%) as well as the 12-month molecular responses (MMR 53 vs. 35%, $P = 0.049$; MR4 25 vs. 10%, $P = 0.038$), did not show any advantage in terms PFS as well as in terms of OS [41].

BELA is a phase 3 multicenter study comparing the efficacy and safety of bosutinib 500 mg OD with that of imatinib 400 mg OD [42]. In this study, CCyR by 12 months that was the primary endpoint of the study did not result to be significantly higher for bosutinib (70%), compared with imatinib (68%), and initially this did not allow bosutinib to be approved as the first-line therapy. These results have been jeopardized by the high rate of discontinuation mainly due to nonhematologic drug-related AEs that occurred in the bosutinib arm (19% rate of discontinuation in the bosutinib arm with respect to 5% in the imatinib arm) and, in particular, the high rates of discontinuation due to diarrhea on bosutinib. However, MMR rates by 12 months were significantly higher for bosutinib (39% bosutinib versus 26% imatinib, $P = 0.002$), and there were numerically fewer progressions to AP/BC with bosutinib (2%) than with imatinib (4%) [42].

Subsequently, bosutinib at a lower dosage of 400 mg OD was again tested vs. imatinib in the BFORE study, which showed a MMR rate at 12 months, the primary end-point significantly higher with bosutinib vs. imatinib (47.2% vs. 36.9%, respectively; $P = 0.02$) [30]. Also the complete cytogenetic response (CCyR) rate by 12 months (77.2% vs. 66.4%, respectively; $P = 0.0075$) was significantly higher with bosutinib. Disease progression to accelerated/blast phase was observed in four patients receiving bosutinib and in six patients receiving imatinib. Grade 3 diarrhea was observed in this trial in 7.8% of the cases treated with bosutinib, at a

lower incidence with respect to what was observed in the BELA trial. The results of this trial finally led to the registration of bosutinib as an additional option for first-line treatment.

In conclusion, because of their higher inhibition capacity of the BCR-ABL1 TK, second-generation TKIs demonstrate the achievement of faster molecular responses with respect to imatinib 400 mg, with more patients achieving BCR-ABL1 ≤ 10% at 3 months and higher rates of MMR and of deep molecular responses (DMR) like MR4 and MR4.5. Another clinical advantage of their use as front-line therapy could be represented by a trend toward a lower rate of transformation. On a longer run the advantage could be represented by a faster achievement of conditions allowing to try to discontinue the therapy. However, 5- and 10-year OS are not statistically different with respect to imatinib and some observed long-term toxicity effects, like a higher rate of cardiovascular events, could raise concerns for their use, particularly in some categories of patients.

4.4 High-Dose Imatinib

Current treatment guidelines for CML recommend first-line therapy with imatinib at a dose of 400 mg/day. However this dosage may not be optimal for patients characterized by a genetic predisposition to a lower efficiency of the OCT-1 transporter, a pump regulating the intracellular influx and concentration of imatinib, which, on the contrary, could significantly benefit from higher initial imatinib dose [43]. Furthermore, phase 1 dose-finding trials demonstrated no dose-limiting toxicities at imatinib doses up to 1000 mg/day, and a dose–response relationship was observed. The best results with imatinib 400 mg were obtained when imatinib plasma concentration was at least 1000 μM/L. This explains also why responses to imatinib are also dependent on a perfect adherence to dosage and to scheduled treatment [44].

Based on these considerations, shortly after the approval of imatinib, a number of studies were started to assess the efficacy and the safety of higher-dose imatinib (800 mg) administration [45–48]. The results of these studies generally showed that patients treated with 800 mg achieved more rapid cytogenetic and molecular responses, but no significant differences were reported in EFS, PFS, or OS. The lack of OS benefit with the higher dose could be due to the frequent dose reductions and treatment interruptions caused by a poor tolerance of 800 mg imatinib dosage.

This problem, as shown in the Study IV of the German CML Study Group, can be overcome by the use of a dose of imatinib adapted to allow a good tolerability by the individual patients. Comparing imatinib 400 mg/day with 800 mg/day alone, the rate of MMR at 12 months was 59% vs. 44% ($P < 0.001$) in favor of the arm in which the patients were starting with 800 mg per day but were allowed to adapt the dose. Indeed the median dose in the 800 mg arm was 628 mg/day, suggesting that treatment of early-phase CML with imatinib can be optimized and that early high-dose therapy followed by rapid adaptation to good tolerability can increase the rate of MMR at 12 months.

These data have been confirmed by a randomized study comparing the rates of molecular, hematological, and cytogenetic responses to imatinib 400 vs. imatinib 400 mg twice daily (imatinib 800) in which dose adjustments were allowed to maximize retention on study [49]. Molecular response at 12 months was deeper in the imatinib 800 arm (4-log reduction of BCR-ABL1 mRNA 25 vs. 10% of patients, $P = 0.038$; 3-log reduction 53% vs. 35%, $P = 0.049$). Furthermore, in both arms, few patients relapsed, progressed, or died, but both PFS ($P = 0.048$) and RFS (relapse-free survival) ($P = 0.031$) were superior for imatinib 800 [49].

Furthermore the 10-year follow-up of the German CML Study IV shows that very deep molecular responses can be obtained with prolonged imatinib therapy in the majority of CML patients [6, 21, 50]. Indeed after 10 years, 92% of patients in MMR reached $MR^{4.5}$, 88% in MR^4 reached MR^5, and, therefore, most imatinib-treated patients could become candidates for treatment discontinuation without the need to

switch to a second-generation TKI. These results were obtained by also continuing imatinib therapy in patients in CCyR but not in MMR and switching only the patients falling in the failure category according to the ELN recommendations. The switch occurred in 26.5% of the total population for resistance or intolerance, and the switched patients did worse than the rest and represented a poorer risk group [6].

4.5 Combination Therapy: Imatinib Plus Interferon-Alpha

Because of the established clinical benefit of IFN in CML treatment, combination therapy between this drug and imatinib always appeared appealing and it is under investigation in a number of clinical trials. In a phase 2 GIMEMA study of imatinib 400 mg/day plus pegylated interferon alpha (PEG–IFNα2a) 50–150 μg/week, CCyR and MMR rates were 70% and 47% at 12 months, with a probability of maintaining CCyR at 5 years in responding patients of 94% [51]. However, compliance to IFN was poor with 87% of patients discontinuing IFN within 2 years.

Some large randomized phase 3 trials are comparing imatinib monotherapy with combination treatment. In the open-label French SPIRIT trial, patients were randomized 1:1:1:1 to receive imatinib 400 mg/day, imatinib 600 mg/day, imatinib 400 mg/day plus cytarabine, or imatinib 400 mg/day plus PEG–IFNα2a [52]. A potential advantage for imatinib/IFN treatment was first observed in 18-month MMR (41 vs. 52 vs. 53 vs. 62%; $P = 0.0001$) along with deep molecular response (4-log reduction of BCR-ABL transcripts, CMR4) (4 vs. 7 vs. 5 vs. 15%; $P = 0.0013$) rates and reconfirmed at later times. However, further follow-up of SPIRIT is needed to establish whether these early differences confer a long-term survival advantage. Grade 3–4 neutropenia with or without thrombocytopenia during the first year was higher for combination arms (imatinib/cytarabine 41%, imatinib/IFN 40%) than in monotherapy arms (400 mg 8%, 600 mg 14%). Overall, 45% of the patients discontinued IFN during the first 12 months. Interestingly, the duration of treatment with IFN had an impact on responses: in patients who have been treated for less than 4 months as compared to more than 12 months, rate of MMR, optimal molecular response MR4, and undetectable minimal residual disease increased from 48% to 82%, 23% to 49%, and 8% to 20%, respectively. A rather similar comparison has been performed within the German CML Study Group (Study IV), with an arm in which patients were receiving imatinib 400 mg/day in combination with unpegylated IFNα2beta [53]. With respect to imatinib 400 mg/day alone, 12-month CCyR rates were similar, 52% for imatinib and 51% for imatinib plus IFN, and 12-month MMR rates were 30% and 35%, respectively. After 5 years of follow-up, no difference was reported between arms in progression-free survival (PFS) or overall survival (OS) [53]. In a third trial performed by the Nordic CML study group, newly diagnosed chronic-phase CML patients with a low or intermediate Sokal risk score and in imatinib-induced complete hematologic remission were randomized either to continue imatinib 400 mg/day or to receive a combination of PEG-IFN-α2b 50 μg weekly and imatinib 400 mg/day [54]. In the combination arm, 34 patients (61%) discontinued PEG-IFN-α2b, most because of toxicity. The MMR rate at 12 months was significantly higher in the imatinib plus PEG-IFN-α2b arm (82%) compared with the imatinib monotherapy arm (54%; intention-to-treat, $P = 0.002$), and the MMR rate increased with the duration of PEG-IFN-α2b treatment (<12-week MMR rate 67%, >12-week MMR rate 91%) [54]. Finally, to determine whether adding PEG-IFN-α2b and GM-CSF to high-dose imatinib may further improve the cytogenetic and molecular response rates in CML patients, 94 patients were treated with imatinib 800 mg/day for the first 6 months and then randomized to continue high-dose imatinib alone or in combination with PEG-IFN-α2b at the dosage of 0.5 μg/kg per week and GM-CSF 125 mg/m2 three times weekly [55]. With a median follow-up of 54 months, no differences in the CCyR, MMR, and CMR rates were observed. However, the potential benefit of adding

PEG-IFN-α2b and GM-CSF to imatinib may have been limited by the fact that, due to adverse events, all patients enrolled in the PEG-IFN-α2b arm discontinued this drug.

Reasons for these different findings between the French SPIRIT trial and the Nordic trial on one side and the German CML Study IV and the MD Anderson trial on the other side are not clear at the moment; however, multiple differences present in the protocols (i.e., the type of IFN used, patient populations, and trial designs) need to be considered.

In conclusion, although literature data are still rather controversial on the real efficacy of the association of imatinib plus IFN and higher rates of discontinuation are recorded due to IFN toxicity, the association of IFN and TKIs still appears particularly appealing for many investigators in view of the potential long-term effect on the higher rate of TFR [56].

4.6 Conclusions

The choice of the best first-line treatment of CML in chronic phase, in particular if imatinib or second-generation TKIs, has been a frequently discussed and controversial issue even among specialists. On the second-generation side there were mainly the rapidity and the depth of the response observed, whereas on the imatinib side there was the long-term safety and the cost. Now this equilibrium has been changed by two factors: (a) imatinib has become a generic drug and its cost is really affordable in most countries of the world and (b) the German CML study IV has demonstrated that over a period of 10 years the molecular responses that can be obtained by imatinib, in particular if dose adapted, are similar to those that can be obtained by second-generation TKIs and with less toxicity. So, with the exception of specific cases in which the achievement of a very rapid deep response is desired, imatinib can still represent the first choice for the vast majority of the patients.

In addition, the cost and the safety profile of imatinib make this drug the ideal partner of other drugs able to inhibit BCR-ABL1 TK activity, like the recently developed asciminib, a highly specific inhibitor of the BCR-ABL1 TK of the new category STAMP (Selectively Targeting the ABL Myristilation Pocket) compounds [57].

References

1. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2003;348(11):994–1004.
2. Saglio G, LeCoutre PD, Pasquini R, Jootar S, Nakamae H, Flinn IW, et al. Nilotinib versus Imatinib in patients (pts) with newly diagnosed Philadelphia chromosome-positive (Ph+) chronic myeloid Leukemia in chronic phase (CML-CP): ENESTnd 36-month (mo) follow-up. ASH Annual Meeting Abstracts. 2011;118(21):452.
3. Kantarjian H, Shah NP, Hochhaus A, Cortes J, Shah S, Ayala M, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2010;362(24):2260–70.
4. Yin OQ, Giles FJ, Baccarani M, le Coutre P, Chiparus O, Gallagher N, et al. Concurrent use of proton pump inhibitors or H2 blockers did not adversely affect nilotinib efficacy in patients with chronic myeloid leukemia. Cancer Chemother Pharmacol. 2012;70(2):345–50.
5. Hochhaus A, Larson RA, Guilhot F, Radich JP, Branford S, Hughes TP, et al. Long-term outcomes of Imatinib treatment for chronic myeloid Leukemia. N Engl J Med. 2017;376(10):917–27.
6. Hehlmann R, Lauseker M, Saussele S, Pfirrmann M, Krause S, Kolb HJ, et al. Assessment of imatinib as first-line treatment of chronic myeloid leukemia: 10-year survival results of the randomized CML study IV and impact of non-CML determinants. Leukemia. 2017;31(11):2398–406.
7. Pfirrmann M, Baccarani M, Saussele S, Guilhot J, Cervantes F, Ossenkoppele G, et al. Prognosis of long-term survival considering disease-specific death in patients with chronic myeloid leukemia. Leukemia. 2016;30(1):48–56.
8. Bolton-Gillespie E, Schemionek M, Klein HU, Flis S, Hoser G, Lange T, et al. Genomic instability may originate from imatinib-refractory chronic myeloid leukemia stem cells. Blood. 2013;121(20):4175–83.
9. Gambacorti-Passerini C, Antolini L, Mahon FX, Guilhot F, Deininger M, Fava C, et al. Multicenter independent assessment of outcomes in chronic myeloid leukemia patients treated with imatinib. J Natl Cancer Inst. 2011;103(7):553–61.
10. Kantarjian H, O'Brien S, Shan J, Huang X, Garcia-Manero G, Faderl S, et al. Cytogenetic and molecular responses and outcome in chronic myelogenous

leukemia: need for new response definitions? Cancer. 2008;112(4):837–45.

11. Marin D, Milojkovic D, Olavarria E, Khorashad JS, de Lavallade H, Reid AG, et al. European LeukemiaNet criteria for failure or suboptimal response reliably identify patients with CML in early chronic phase treated with imatinib whose eventual outcome is poor. Blood. 2008;112(12):4437–44.
12. Hochhaus A, Baccarani M, Silver RT, Schiffer C, Apperley JF, Cervantes F, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020;34(4):966–84.
13. Deininger MW, Shah NP, Altman JK, Berman E, Bhatia R, Bhatnagar B, et al. Chronic Myeloid Leukemia, version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Cancer Netw. 2020;18(10):1385–415.
14. Hughes T, Deininger M, Hochhaus A, Branford S, Radich J, Kaeda J, et al. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. Blood. 2006;108(1):28–37.
15. Muller MC, Hanfstein B, Lauseker M, Erben P, Proetel U, Schnittger S, et al. Time-related interpretation of molecular response levels according to long term overall and progression-free survival of CML patients on first-line Imatinib treatment. ASH Annual Meeting Abstracts. 2011;118(21):1681.
16. Cross NC, White HE, Ernst T, Welden L, Dietz C, Saglio G, et al. Development and evaluation of a secondary reference panel for BCR-ABL1 quantification on the International Scale. Leukemia. 2016;30(9):1844–52.
17. Möbius S, Schenk T, Himsel D, Maier J, Franke GN, Saussele S, et al. Results of the European survey on the assessment of deep molecular response in chronic phase CML patients during tyrosine kinase inhibitor therapy (EUREKA registry). J Cancer Res Clin Oncol. 2019;145(6):1645–50.
18. Cross NC, Hochhaus A. Deep molecular response in chronic myeloid leukemia. Leukemia. 2016;30(7):1632.
19. Jabbour E, Kantarjian H, O'Brien S, Shan J, Quintas-Cardama A, Faderl S, et al. The achievement of an early complete cytogenetic response is a major determinant for outcome in patients with early chronic phase chronic myeloid leukemia treated with tyrosine kinase inhibitors. Blood. 2011;118(17):4541–6. quiz 759
20. Hanfstein B, Muller MC, Hehlmann R, Erben P, Lauseker M, Fabarius A, et al. Early molecular and cytogenetic response is predictive for long-term progression-free and overall survival in chronic myeloid leukemia (CML). Leukemia. 2012;26:2096.
21. Hehlmann R, Muller MC, Lauseker M, Hanfstein B, Fabarius A, Schreiber A, et al. Deep molecular response is reached by the majority of patients treated with imatinib, predicts survival, and is achieved more quickly by optimized high-dose imatinib: results from the randomized CML-study IV. J Clin Oncol. 2014;32(5):415–23.
22. Mahon FX, Rea D, Guilhot J, Guilhot F, Huguet F, Nicolini F, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. Lancet Oncol. 2010;11(11):1029–35.
23. Ross DM, Branford S, Seymour JF, Schwarer AP, Arthur C, Bartley PA, et al. Patients with chronic myeloid leukemia who maintain a complete molecular response after stopping imatinib treatment have evidence of persistent leukemia by DNA PCR. Leukemia. 2010;24(10):1719–24.
24. Shah NP. NCCN guidelines updates: discontinuing TKI therapy in the treatment of chronic myeloid Leukemia. J Natl Compr Cancer Netw. 2019;17(5.5):611–3.
25. Baccarani M, Abruzzese E, Accurso V, Albano F, Annunziata M, Barulli S, et al. Managing chronic myeloid leukemia for treatment-free remission: a proposal from the GIMEMA CML WP. Blood Adv. 2019;3(24):4280–90.
26. Marin D, Ibrahim AR, Lucas C, Gerrard G, Wang L, Szydlo RM, et al. Assessment of BCR-ABL1 transcript levels at 3 months is the only requirement for predicting outcome for patients with chronic myeloid leukemia treated with tyrosine kinase inhibitors. J Clin Oncol. 2012;30(3):232–8.
27. Hughes TP, Saglio G, Kantarjian HM, Guilhot F, Niederwieser D, Rosti G, et al. Early molecular response predicts outcomes in patients with chronic myeloid leukemia in chronic phase treated with frontline nilotinib or imatinib. Blood. 2014;123(9):1353–60.
28. Jabbour E, Kantarjian HM, Saglio G, Steegmann JL, Shah NP, Boque C, et al. Early response with dasatinib or imatinib in chronic myeloid leukemia: 3-year follow-up from a randomized phase 3 trial (DASISION). Blood. 2014;123(4):494–500.
29. Pfirrmann M, Clark RE, Prejzner W, Lauseker M, Baccarani M, Saussele S, et al. The EUTOS long-term survival (ELTS) score is superior to the Sokal score for predicting survival in chronic myeloid leukemia. Leukemia. 2020;34(8):2138–49.
30. Cortes JE, Gambacorti-Passerini C, Deininger MW, Mauro MJ, Chuah C, Kim DW, et al. Bosutinib versus Imatinib for newly diagnosed chronic myeloid Leukemia: results from the randomized BFORE trial. J Clin Oncol. 2018;36(3):231–7.
31. Kantarjian HM, Giles F, Gattermann N, Bhalla K, Alimena G, Palandri F, et al. Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is effective in patients with Philadelphia chromosome-positive chronic myelogenous leukemia in chronic phase following imatinib resistance and intolerance. Blood. 2007;110(10):3540–6.

32. Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL. Overriding imatinib resistance with a novel ABL kinase inhibitor. Science. 2004;305(5682):399–401.
33. Khoury HJ, Cortes JE, Gambacorti-Passerini C, Kim D-W, Zaritskey A, Hochhaus A, et al. Activity of Bosutinib by baseline and emergent mutation status in Philadelphia chromosome-positive Leukemia patients with resistance or intolerance to other tyrosine kinase inhibitors. ASH Annual Meeting Abstracts. 2011;118(21):110.
34. Jabbour E, Branford S, Saglio G, Jones D, Cortes JE, Kantarjian HM. Practical advice for determining the role of BCR-ABL mutations in guiding tyrosine kinase inhibitor therapy in patients with chronic myeloid leukemia. Cancer. 2011;117(9):1800–11.
35. Rosti G, Palandri F, Castagnetti F, Breccia M, Levato L, Gugliotta G, et al. Nilotinib for the frontline treatment of Ph(+) chronic myeloid leukemia. Blood. 2009;114(24):4933–8.
36. Cortes JE, Jones D, O'Brien S, Jabbour E, Konopleva M, Ferrajoli A, et al. Nilotinib as front-line treatment for patients with chronic myeloid leukemia in early chronic phase. J Clin Oncol. 2010;28(3):392–7.
37. Cortes JE, Jones D, O'Brien S, Jabbour E, Ravandi F, Koller C, et al. Results of dasatinib therapy in patients with early chronic-phase chronic myeloid leukemia. J Clin Oncol. 2010;28(3):398–404.
38. Hughes TP, Saglio G, Larson RA, Kantarjian HM, Kim D-W, Issaragrisil S, et al. Long-term outcomes in patients with chronic myeloid Leukemia in chronic phase receiving frontline Nilotinib versus Imatinib: Enestnd 10-year analysis. Blood. 2019;134(Supplement_1):2924.
39. Hochhaus A, Saglio G, Hughes TP, Larson RA, Kim DW, Issaragrisil S, et al. Long-term benefits and risks of frontline nilotinib vs imatinib for chronic myeloid leukemia in chronic phase: 5-year update of the randomized ENESTnd trial. Leukemia. 2016;30(5):1044–54.
40. Cortes JE, Saglio G, Kantarjian HM, Baccarani M, Mayer J, Boque C, et al. Final 5-year study results of DASISION: the Dasatinib versus Imatinib study in treatment-naive chronic myeloid Leukemia patients trial. J Clin Oncol. 2016;34(20):2333–40.
41. Radich JP, Kopecky KJ, Appelbaum FR, Kamel-Reid S, Stock W, Malnassy G, et al. A randomized trial of dasatinib 100 mg versus imatinib 400 mg in newly diagnosed chronic-phase chronic myeloid leukemia. Blood. 2012;120(19):3898–905.
42. Cortes JE, Maru A, Souza CAAD, Guilhot F, Duvillie L, Powell C, et al. Bosutinib versus Imatinib in newly diagnosed chronic phase chronic myeloid Leukemia—BELA trial: 24-month follow-up. ASH Annual Meeting Abstracts. 2011;118(21):455.
43. White DL, Radich J, Soverini S, Saunders VA, Frede A, Dang P, et al. Chronic phase chronic myeloid leukemia patients with low OCT-1 activity randomised to high-dose imatinib achieve better responses, and lower failure rates, than those randomized to standard-dose. Haematologica. 2011;97:907.
44. Larson RA, Druker BJ, Guilhot F, O'Brien SG, Riviere GJ, Krahnke T, et al. Imatinib pharmacokinetics and its correlation with response and safety in chronic-phase chronic myeloid leukemia: a subanalysis of the IRIS study. Blood. 2008;111(8):4022–8.
45. Baccarani M, Rosti G, Castagnetti F, Haznedaroglu I, Porkka K, Abruzzese E, et al. Comparison of imatinib 400 mg and 800 mg daily in the front-line treatment of high-risk, Philadelphia-positive chronic myeloid leukemia: a European LeukemiaNet study. Blood. 2009;113(19):4497–504.
46. Cortes JE, Kantarjian HM, Goldberg SL, Powell BL, Giles FJ, Wetzler M, et al. High-dose imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: high rates of rapid cytogenetic and molecular responses. J Clin Oncol. 2009;27(28):4754–9.
47. Vieira SA, Deininger MW, Sorour A, Sinclair P, Foroni L, Goldman JM, et al. Transcription factor BACH2 is transcriptionally regulated by the BCR/ABL oncogene. Genes Chromosomes Cancer. 2001;32(4):353–63.
48. Baccarani M, Druker BJ, Branford S, Kim DW, Pane F, Mongay L, et al. Long-term response to imatinib is not affected by the initial dose in patients with Philadelphia chromosome-positive chronic myeloid leukemia in chronic phase: final update from the tyrosine kinase inhibitor optimization and selectivity (TOPS) study. Int J Hematol. 2014;99(5):616–24.
49. Deininger MW, Kopecky KJ, Radich JP, Kamel-Reid S, Stock W, Paietta E, et al. Imatinib 800 mg daily induces deeper molecular responses than imatinib 400 mg daily: results of SWOG S0325, an intergroup randomized PHASE II trial in newly diagnosed chronic phase chronic myeloid leukaemia. Br J Haematol. 2014;164(2):223–32.
50. Kalmanti L, Saussele S, Lauseker M, Müller MC, Dietz CT, Heinrich L, et al. Safety and efficacy of imatinib in CML over a period of 10 years: data from the randomized CML-study IV. Leukemia. 2015;29(5):1123–32.
51. Palandri F, Iacobucci I, Castagnetti F, Testoni N, Poerio A, Amabile M, et al. Front-line treatment of Philadelphia positive chronic myeloid leukemia with imatinib and interferon-alpha: 5-year outcome. Haematologica. 2008;93(5):770–4.
52. Preudhomme C, Guilhot J, Nicolini FE, Guerci-Bresler A, Rigal-Huguet F, Maloisel F, et al. Imatinib plus peginterferon alfa-2a in chronic myeloid leukemia. N Engl J Med. 2010;363(26):2511–21.
53. Hehlmann R, Lauseker M, Jung-Munkwitz S, Leitner A, Muller MC, Pletsch N, et al. Tolerability-adapted imatinib 800 mg/d versus 400 mg/d versus 400 mg/d plus interferon-alpha in newly diagnosed chronic myeloid leukemia. J Clin Oncol. 2011;29(12):1634–42.
54. Simonsson B, Gedde-Dahl T, Markevarn B, Remes K, Stentoft J, Almqvist A, et al. Combination of pegylated IFN-alpha2b with imatinib increases molecular response rates in patients with low- or

intermediate-risk chronic myeloid leukemia. Blood. 2011;118(12):3228–35.
55. Cortes J, Quintas-Cardama A, Jones D, Ravandi F, Garcia-Manero G, Verstovsek S, et al. Immune modulation of minimal residual disease in early chronic phase chronic myelogenous leukemia: a randomized trial of frontline high-dose imatinib mesylate with or without pegylated interferon alpha-2b and granulocyte-macrophage colony-stimulating factor. Cancer. 2011;117(3):572–80.
56. Heibl S, Buxhofer-Ausch V, Schmidt S, Webersinke G, Lion T, Piringer G, et al. A phase 1 study to evaluate the feasibility and efficacy of the addition of ropeginterferon alpha-2b to imatinib treatment in patients with chronic phase chronic myeloid leukemia (CML) not achieving a deep molecular response (molecular remission 4.5)-AGMT_CML 1. Hematol Oncol. 2020;38:792.
57. Hughes TP, Mauro MJ, Cortes JE, Minami H, Rea D, DeAngelo DJ, et al. Asciminib in chronic myeloid Leukemia after ABL kinase inhibitor failure. N Engl J Med. 2019;381(24):2315–26.

Check for updates

5 CML Therapy: A Focus on Second- and Third-Generation Tyrosine Kinase Inhibitors

Alexandra Lovell, Elias Jabbour, Jorge Cortes, and Hagop Kantarjian

Abbreviations

ABL1	Abelson gene
AOE	arterial occlusive event
AP	accelerated phase
BC	blast crisis
BCR	breakpoint cluster region
CCyR	complete cytogenetic response
CML	chronic myeloid leukemia
CP CML	chronic phase chronic myeloid leukemia
DMR	deep molecular response
EFS	event-free survival
MCyR	major cytogenetic response
MMR	major molecular response
OS	overall survival
PFS	progression-free survival
TFR	treatment-free remission
TKI	tyrosine kinase inhibitor

5.1 Introduction

Chronic myeloid leukemia (CML) represents 15% of newly diagnosed leukemia cases. In the United States, about 9000 new CML cases are diagnosed annually [1]. BCR-ABL1-targeted tyrosine kinase inhibitors (TKIs) have vastly changed the treatment and prognosis of CML. Before the introduction of TKIs, allogeneic hematopoietic stem cell transplantation (SCT) was the only known cure for CML. However, due to the advanced age of most patients with CML, this treatment modality was utilized in a small proportion of patients and mortality remained high. The TKIs have transformed CML into a manageable chronic disease state and have decreased the annual mortality to less than 2%. Thus, the prevalence of CML will continue to increase annually until the annual incidence equals the annual mortality, with an estimated potential plateau prevalence of 300,000–500,000 cases in the United States. There are currently five approved TKIs in the United States and in Europe. These include imatinib, dasatinib, nilotinib, bosutinib, and ponatinib. While imatinib changed the landscape of CML treatment drastically, the low rates of molecular response in addition to the high rates of discontinuation due to adverse effects led to the development of second- and third-generation TKIs. These TKIs are more potent than imatinib, have unique adverse effect profiles, and have activity against different molecular mutations, all of which must be considered when choosing a therapeutic agent. This chapter discusses the second- and third-generation TKIs and their role in CML therapy.

A. Lovell · E. Jabbour · J. Cortes · H. Kantarjian (✉)
MD Anderson Cancer Center, Houston, TX, USA
e-mail: ejabbour@mdanderson.org; Jorge.cortes@augusta.edu; hkantarjian@mdanderson.org

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
R. Hehlmann (ed.), *Chronic Myeloid Leukemia*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-71913-5_5

5.2 Dasatinib

Dasatinib is a second-generation TKI that is 325 times more potent than imatinib. Dasatinib binds to both the active and inactive conformations of the ABL kinase with activity against many mutations conferring resistance to imatinib, excluding the T315I mutation [2, 3]. Aside from the BCR-ABL1 kinase, dasatinib also inhibits KIT, SRC family, EPHA2, and PDGFR [4].

Dasatinib was first approved in adult patients in all phases of CML with resistance or intolerance to prior therapy [4]. In a phase II trial, patients with chronic phase CML (CP CML) and resistance or intolerance to imatinib were treated with dasatinib 70 mg twice daily. With a median follow-up of 8 months, complete hematologic response and major cytogenetic response (MCyR) were achieved in 90% and 52% of patients, respectively [5]. The median transcript ratios fell from 66% at baseline to 3% at 9 months. After a median of 15 months, 10% of patients experienced disease progression, with 93% of them having developed imatinib resistant disease [6]. The CA180-034 trial investigated multiple dosing regimens of dasatinib (100 mg daily, 50 mg twice daily, 140 mg daily, and 70 mg twice daily) in patients with CP CML and resistance or intolerance to imatinib [7]. All four dosing strategies produced similar rates of hematologic and cytogenetic response as well as similar progression-free survival (PFS) rates. Dasatinib 100 mg daily was more tolerable with lower rates of pleural effusion and thrombocytopenia compared with 70 mg twice daily. This trial led to a change in the recommended dose to 100 mg daily in CP CML. Improved tolerance with dasatinib 100 mg daily translated to more patients remaining on trial in that arm as shown in the 7-year follow up data. Rates of major molecular response (MMR), PFS, and overall survival (OS) were 46%, 42%, and 65%, respectively [8].

The activity of dasatinib in the second-line setting led to the large phase III DASISION trial comparing dasatinib with imatinib in newly diagnosed CP CML [9]. Patients were randomized to receive dasatinib 100 mg daily or imatinib 400 mg daily. The rate of complete cytogenetic response (CCyR) at 12 months was 77% with dasatinib versus 66% with imatinib ($P < 0.007$). The MMR rate at 12 months was 46% with dasatinib compared with 28% with imatinib, with responses with dasatinib achieved in a shorter time. Dasatinib was then approved as frontline therapy for CML. In the 5-year update of the DASISION trial, the rates of MMR were durable and remained significantly higher with dasatinib compared with imatinib (76% versus 64%; $P = 0.0022$) [10].

The SPIRIT2 trial was a similar phase III trial comparing frontline dasatinib 100 mg daily to imatinib 400 mg daily in CP CML [11]. More patients on dasatinib achieved deep molecular responses compared with imatinib. The MMR and MR4 (*BCR-ABL1* transcripts ≤0.01%) within 5 years was achieved in 83% and 78% on dasatinib compared with 63% and 57% on imatinib, respectively ($P < 0.001$ for both analyses). Similarly, the CCyR rate at 24 months was significantly higher among patients receiving dasatinib (43% versus 32%; $P = 0.001$). The probability of treatment-failure-free survival at 5 years was higher with dasatinib compared with imatinib. However, there were no differences in the rates of event-free survival (EFS) or OS.

Data reporting a median follow-up of 6.5 years is available in a smaller trial of 150 patients receiving dasatinib 100 mg daily or 50 mg twice daily [12]. The data confirm the DASISION trial results, indicating that dasatinib produces rapid and durable rates of MMR, translating into increased OS and EFS among patients achieving MMR. The 5-year cumulative rate of sustained MR4.5 (≥ 4.5-log reduction in *BCR-ABL1* transcripts) was 67% in patients who completed at least 27 months of therapy with dasatinib. Among those patients, 21% discontinued therapy, with 10% lost MR4.5 after a median time of 3.2 months. When TKI therapy was resumed, all patients achieved MR4.5 again after a median of 3.8 months.

Dasatinib inhibits multiple kinases which may cause off-target adverse effects. Common adverse effects include myelosuppression, fluid retention, gastrointestinal disturbances, rash, and arthralgia [4]. All grade pleural effusions have been reported in 10–28% of patients [6, 9, 10, 12]. The manufacturer labeling recommends dasatinib 100 mg daily for CP CML. However, dose optimization studies have shown that the incidence of pleural effusions is correlated with higher doses [13–15]. Other risk factors for pleural effusion include age ≥ 65 years and lymphocytosis during treatment with dasatinib [10, 13].

Earlier studies demonstrated that dasatinib was better tolerated at lower doses and still retained efficacy [8, 10, 16]. In an effort to reduce the incidence of adverse effects, minimize drug interruptions, and improve cost-effectiveness, dasatinib 50 mg daily was studied in 83 patients with newly diagnosed CP CML [17]. Patients could escalate to dasatinib 100 mg if they did not achieve *BCR-ABL1* transcript level ≤ 10% by 3 months, a CCyR by 6 months, or an MMR by 12 months and had no grade 3 toxicity. After a median follow-up of 24 months, 90% of patients achieved *BCR-ABL1* transcript levels ≤10% at 3 months. The cumulative CCyR rates by 6 and 12 months were 77% and 95%, respectively. The cumulative MMR rates were 53% at 6 months and 81% at 12 months. Only four patients required dose escalation to 100 mg daily due to failing to achieve CCyR at 6 months. None of these patients developed kinase domain mutations while on dasatinib 50 mg, but one patient developed a T315I mutation after starting dasatinib 100 mg. During follow up, no patient experienced disease transformation to accelerated phase (AP) or blast crisis (BC). Dose interruptions occurred in 25% of patients and only 6% had pleural effusions, a much lower rate than previously reported rates with standard dosing (28% in the DASISION trial) [10]. It is important to note that only 9% of patients had high-risk disease by Sokal risk score. The efficacy of lower dose dasatinib in the high-risk population remains to be determined, but dasatinib 50 mg daily appears to be an effective treatment strategy that minimizes adverse effects (pleural effusion, myelosuppression).

5.3 Nilotinib

Nilotinib was the next second-generation TKI to be approved [18]. Nilotinib is a structural derivative of imatinib that is 30 times more potent. It has activity against many of imatinib-resistant BCR-ABL1 mutations; however, resistant mutations exist, most notably, the T315I mutation [19, 20]. It binds to the inactive conformation of the ABL1 kinase domain and retains activity against KIT and PDGFR. However, it does not have activity against SRC family [21].

Like dasatinib, nilotinib was originally approved for CP CML in the second line setting after failure or intolerance to prior therapy that included imatinib, based on a single-arm open label multicenter phase II study [22]. In the 4-year follow-up data, 59% and 45% of patients treated with nilotinib 400 mg twice daily achieved a MCyR and CCyR, respectively [23]. The pivotal phase III ENESTnd trial evaluated nilotinib in the frontline setting [24]. Patients with CP CML were randomized to receive nilotinib 300 mg or 400 mg twice daily, or imatinib 400 mg daily. The rates of MMR at 12 months were significantly higher with nilotinib at both doses (44% for the nilotinib 300 mg dose and 43% for the 400 mg dose) compared with imatinib (22%; $P < 0.001$ for both comparisons). The CCyR rate at 12 months was also higher with nilotinib (80% with nilotinib 300 mg twice daily, 78% with nilotinib 400 mg twice daily, 65% for imatinib; $P < 0.001$ for both comparisons). There were similar response rates between the 300 mg or 400 mg twice daily dosing, which led to the approval of nilotinib 300 mg twice daily in newly diagnosed CP CML. Responses deepened after 5 years of follow-up. Patients receiving nilotinib 300 mg twice daily and 400 mg twice daily achieved deep responses more frequently than those receiving imatinib with 54%, 52%, and

31%, respectively, achieving MR4.5 [25]. After 10 years of follow-up, the EFS remained high at 92% and 96% for nilotinib 300 mg twice daily and 400 mg twice daily, respectively [26]. Progression to AP or BC occurred in 11 patients on nilotinib 300 mg twice daily, seven patients on nilotinib 400 mg twice daily, and 24 patients on imatinib 400 mg daily, with most events occurring within the first 5 years of treatment. OS was similar among the three groups ranging from 88 to 90%. The incidence of cardiovascular events with nilotinib was 21% and the exposure adjusted overall incidence was 34% per 10-patient years. The time on therapy did not correlate with an increase in cardiovascular events, with similar rates occurring within and beyond 5 years of treatment.

Nilotinib carries a black box warning for QTc prolongation and sudden deaths. It is recommended to avoid other QTc prolonging agents and strong CYP3A4 inhibitors while on nilotinib and to correct electrolyte abnormalities to reduce the risk of sudden death. Other warnings and precautions include myelosuppression, cardiac and arterial vascular occlusive events, pancreatitis, elevated lipase, hepatotoxicity, increased blood glucose, and fluid retention. Common adverse effects reported are gastrointestinal upset, rash, fatigue, and headache [18]. In the 5-year follow up data, ischemic heart disease, ischemic cerebrovascular events, and/or peripheral artery disease occurred in 7%, 13%, and 2% of patients in the nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, and imatinib arms respectively [25]. Nilotinib should be avoided in patients with preexisting cardiovascular disease.

5.4 Bosutinib

Bosutinib is a second-generation TKI that has 20–200 times the inhibitory effect as imatinib; however, it does not confer activity against T315I and V299L mutations [27]. It retains activity against the SRC family but has minimal activity against PDGFR and KIT [28, 29].

Bosutinib was approved based on early-phase data evaluating the efficacy of bosutinib 500 mg daily in patients with CML who had CML and failed at least one prior TKI [30, 31]. The phase III BELA trial evaluated 502 patients with newly diagnosed CP CML randomized to receive bosutinib 500 mg daily or imatinib 400 mg daily [32]. The primary outcome, the rate of CCyR at 12 months, was similar, but the time to CCyR was faster with bosutinib (12.9 weeks versus 24.6 weeks; $P < 0.001$). The MMR rate at 12 months favored bosutinib (41% versus 27%; $P < 0.001$). In the 24-month update, significantly more patients in the bosutinib arm achieved *BCR-ABL1* transcripts ≤10% at 3 months compared with imatinib (86% versus 66%; $P < 0.001$) [33]. The BFORE trial was a similar phase III trial evaluating bosutinib 400 mg daily versus imatinib 400 mg daily in newly diagnosed CP CML [34]. The primary outcome of MMR at 12 months was significantly better with bosutinib (47% versus 37%; $P = 0.0075$), as was CCyR rate at 12 months (77% versus 66%; $P = 0.0173$), leading to the approval of bosutinib 400 mg daily for frontline therapy of CML.

Bosutinib exhibits an acceptable safety profile. Common adverse effects (≥ 10%) include gastrointestinal events, transaminitis, and rash [35]. Bosutinib has minimal activity on PDGFR and KIT, which may explain lesser rates of fluid retention and myelosuppression. Diarrhea is common with most patients experiencing diarrhea in the first 4 weeks after starting therapy. This can be managed with medications such as loperamide or diphenoxylate/atropine, and the incidence of diarrhea decreases after the first few months on therapy [32]. Renal dysfunction may occur.

With its limited toxicity profile, bosutinib is an attractive option for patients with comorbidities that preclude the use of other TKIs or those with intolerance to previous TKI therapy. Small studies suggest that bosutinib is safe to use after the development of pleural effusions on dasatinib and can provide benefit when used even in the fourth-line setting, with the greatest benefit seen in patient who initiate therapy already having attained CCyR [36, 37]. Among the TKIs, bosutinib and imatinib are associated with the lowest risk of cardiovascular adverse effects.

5.5 Ponatinib

Ponatinib is a third-generation TKI that has a greater affinity for the BCR-ABL1 kinase and is about 500 times more potent than imatinib [2]. Ponatinib is the only TKI that has activity against the T315I mutation located in the gatekeeper region of the ATP-binding site. This mutation alters the ATP-binding pocket and eliminates a hydrogen binding site necessary for all other approved TKIs to retain activity [21, 38]. Ponatinib has activity against the VEGF receptor, TIE2, PDGFR, and FGFR which may be responsible for some of its adverse effects.

Ponatinib is the only third-generation TKI approved for use in the United States. It is indicated in all phases of CML where no other TKI is indicated or in patients who harbor a T315I mutation [39]. It was approved in 2012 for use in both CML and Philadelphia chromosome positive acute lymphoblastic leukemia based off the Phase II PACE trial [40]. Two hundred and sixty-seven patients with CP CML and resistance or intolerance to prior TKIs were treated with ponatinib 45 mg daily. More than 80% of patients had previously been treated with two TKIs, and 52% had received three TKIs prior to enrollment. After a median of 15 months, 56% of patients achieved a MCyR, 46% a CCyR, and 34% a MMR. Among patients with a T315I mutation, 70%, 66%, and 56% achieved MCyR, CCyR, and MMR, respectively. Responses remained durable throughout the study period with 91% sustaining MCyR at 12 months.

Like newer-generation TKIs, ponatinib induces responses quickly. Among patients who achieved a response, a CCyR was achieved after a median of 2.9 months, and MMR after a median of 5.5 months. In the long-term follow-up data of the PACE trial, 40% of patients achieved MMR and 24% achieved MR4.5 [41]. These responses remained durable, and the probability of sustaining MMR at 5 years was 59%. The 5-year PFS and OS were 53% and 75%, respectively. No mutations conferring resistance to ponatinib were identified.

A small single-arm phase II trial evaluated ponatinib 45 mg daily in frontline CP CML [42]. The study was amended later to start patients on ponatinib 30 mg daily due to a high frequency of dose reductions. Additionally, aspirin 81 mg daily was started on all patients after a warning was issued for vascular complications. The rate of CCyR at 6 months was 94%. After a median of 21 months, the rate of MMR was 80% and MR4.5 was 55%. *BCR-ABL1* transcripts ≤10% at 3 months occurred in 94% of patients. The randomized phase III EPIC trial evaluated ponatinib 45 mg daily versus imatinib 400 mg daily in frontline CP CML [43]. The trial was terminated early due to concerns for increased vascular adverse events seen with ponatinib. The rates of MMR at 12 months were 80% with ponatinib and 38% with imatinib, although this was not significant due to the small patient numbers and limited follow-up. The data suggest that ponatinib may provide earlier and deeper responses compared with imatinib in the frontline setting, but whether ponatinib is superior to second-generation TKIs in the frontline setting remains unknown.

Common adverse effects include myelosuppression, rash, dry skin, and abdominal pain. Most adverse effects occur within the first 1–2 months of treatment [39, 40]. Additionally, ponatinib carries black box warnings for heart failure, hepatotoxicity, venous thromboembolism, and arterial occlusion. Other serious adverse effects include pancreatitis and treatment-emergent hypertension, which occurred in 7% and 68% of patients, respectively. Ponatinib is active against the VEGF receptor, TIE2, PDGFR, and FGFR which may be implicated in its vascular adverse effects. In earlier phase data, any grade heart failure or left ventricular dysfunction was noted in 9% and arterial occlusion in 35% of patients. In the PACE trial, cardiovascular, cerebrovascular, and peripheral vascular events occurred in 7%, 3%, and 5% of patients, respectively. Among patients who had arterial occlusive events (AOE), 55% had a history of ischemic disease and 95% had risk factors for ischemic disease (hypertension, diabetes, hypercholesterolemia, or obesity). The rates of AOE appear to be dose related: among patients who had an event, 42% were treated with ponatinib 45 mg, 24% with 30 mg, and 26% with 15 mg [41].

Dose reductions occurred in 55% of all patients in the PACE trial with a median time to dose reduction of 2.3 months [40]. In the 5-year follow up of the PACE trial, among patients who had a dose

reduction after achieving MCyR or MMR, greater than 90% were able to sustain their response for 40 months after the dose reduction [41]. Dose reduction after achieving the desired response may be a therapeutic option to mitigate adverse events.

The OPTIC trial is an ongoing randomized phase II trial evaluating the efficacy, safety, and response-based dose reduction in patients with CP CML receiving ponatinib who had CML and resistance or intolerance to ≥ two TKIs or those who harbored a T315I mutation [44]. Over 200 patients were randomized to three different cohorts: 45 mg (cohort A), 30 mg (cohort B), and 15 mg (cohort C). The ponatinib dose was reduced to 15 mg in the first two cohorts once patients achieved *BCR-ABL1* transcripts ≤1%. At the interim analysis cutoff at 12 months, 39%, 27%, and 26% in cohorts A, B, and C, respectively, achieved *BCR-ABL1* transcripts ≤1%. Cohorts A, B, and C achieved MMR at 12 months in 15%, 18%, and 19%, respectively. More patients in cohort A than cohort B were reduced to 15 mg due to achievement of *BCR-ABL1* transcripts ≤1% (35% versus 21%); however, cohort A did require more dose reductions due to adverse effects than cohorts B and C (44% versus 31% versus. 28%). The AOEs were reported in 5%, 4%, and 1% of cohorts A, B, and C, respectively, but no deaths on study were attributable to AOEs. While the interim analysis of the OPTIC trial suggests dose-dependent efficacy and adverse effect profiles, longer follow up will further elucidate the safety and efficacy of different doses and may guide ponatinib dosing in the future.

Ponatinib is the only approved TKI with activity against the T315I mutation and is the only oral therapy available to these patients. Until further therapeutic options against the T315I mutation are approved, the risks and benefits of ponatinib as well as the daily dose should be seriously considered in patients with heart failure and preexisting ischemic disease.

5.6 Choice of Frontline Therapy in CML

Imatinib, dasatinib, nilotinib, and bosutinib are all approved and are category 1 recommendations for frontline use by current guidelines [45, 46]. The choice of TKI depends largely on the comorbidities of the patient and the aims of therapy.

Patients with pulmonary disease should avoid dasatinib due to the risk for pleural effusions and pulmonary arterial hypertension. Dasatinib may have an increased risk of bleeding in those on anticoagulation due to its inhibitory effect on platelets [4].

Nilotinib has been shown to prolong the QTc and cause vascular occlusive events including myocardial infarction. In the 10-year follow up data of the ENESTnd trial, 21% of patients had a cardiovascular event, with an incidence rate of 34% per 10-patient years [26]. Additionally, nilotinib may increase blood sugar levels [18]. It should be avoided in patients with electrolyte abnormalities and those with significant cardiovascular disease or uncontrolled diabetes.

Imatinib can cause arthralgia, fatigue, edema, and weight gain while the predominant adverse effect of bosutinib is diarrhea, which occurs in up to 80% of patients [35, 47]. Bosutinib should be avoided in patients with inflammatory bowel disease. In addition, bosutinib has been shown to cause mild to moderate, moderate to severe, and severe renal dysfunction in 27%, 13%, and 5% of patients with long term use [35]. Kidney failure was reported in 1% of patients.

End organ function should be assessed prior to prescribing. Imatinib and bosutinib have manufacturer recommendations for upfront dose adjustment in renal dysfunction. All TKIs except dasatinib have recommended dose adjustments depending on the degree of hepatic dysfunction. Ease of administration should also be taken into consideration, especially since the efficacy of these agents depends heavily on adherence. Imatinib, dasatinib, and bosutinib are once daily medications; nilotinib is twice daily and needs to be taken on an empty stomach to avoid increased drug exposure and toxicity. Imatinib and bosutinib should be taken with food; dasatinib can be administered without regard to meals (refer to Table 5.1 for a comparison of drug characteristics and prescribing information for the TKIs).

In addition to patient comorbidities, the desired treatment outcome should be considered.

Table 5.1 Comparison of TKIs

Drug	Imatinib	Dasatinib	Nilotinib	Bosutinib	Ponatinib
Approved indications	Newly diagnosed chronic phase Ph + CML Ph + CML in blast crisis, accelerated phase, or in chronic phase after failure of interferon-alfa therapy	Newly diagnosed chronic phase Ph + CML Chronic, accelerated, or myeloid or lymphoid blast phase Ph + CML with resistance or intolerance to prior therapy	Newly diagnosed chronic phase Ph + CML Chronic phase CML and AP CML resistant or intolerant to prior therapy	Newly diagnosed Ph + chronic phase CML Chronic, accelerated, or blast phase Ph + CML with resistance or intolerance to prior therapy	Chronic phase, accelerated phase, or blast crisis CML in whom no other TKI is indicated T315I-positive CML (chronic phase, accelerated phase, or blast crisis)
Dosage and administration	Chronic phase: 400 mg PO daily Accelerated phase or blast crisis: 600 mg PO daily Take with food	Newly diagnosed: 100 mg PO daily Accelerated phase or myeloid or lymphoid blast phase CML: 140 mg PO daily Off-label: 50 mg PO daily (see above discussion to limit toxicity) Take with or without food	Newly diagnosed: 300 mg PO twice daily Resistant/intolerant: 400 mg PO twice daily Take on empty stomach to avoid increased absorption and toxicity	Newly diagnosed: 400 mg PO daily Resistant/intolerant or accelerated or blast phase: 500 mg PO daily Take with food	45 mg PO daily Take with or without food
Black box warnings (BWW) and warnings/precautions	Edema and severe fluid retention, cytopenia, congestive heart failure, hepatotoxicity, hemorrhage, gastrointestinal perforations, bullous dermatologic reactions, hypothyroidism, embryo-fetal toxicity	Myelosuppression and bleeding events, fluid retention (pleural effusions), cardiac dysfunction, pulmonary arterial hypertension, QT prolongation, severe dermatologic reactions, tumor lysis syndrome, embryo-fetal toxicity	BBW: QTc prolongation, sudden death Myelosuppression, cardiac and arterial vascular occlusive events, pancreatitis and elevated serum lipase, hepatotoxicity, electrolyte abnormalities, tumor lysis syndrome, hemorrhage, fluid retention, embryo-fetal toxicity	Gastrointestinal toxicity, myelosuppression, hepatic toxicity, cardiac failure, fluid retention, renal toxicity, embryo-fetal toxicity	BBW: Arterial occlusion, venous thromboembolism, heart failure, hepatotoxicity Hypertension, pancreatitis, neuropathy, ocular toxicity, hemorrhage, fluid retention, cardiac arrhythmias, myelosuppression, tumor lysis syndrome, reversible posterior leukoencephalopathy syndrome, impaired wound healing and gastrointestinal perforation, embryo-fetal toxicity
Common adverse effects (≥20%)	Edema, nausea, vomiting, muscle cramps, musculoskeletal pain, diarrhea, rash, fatigue, abdominal pain	Myelosuppression, fluid retention, diarrhea, headache, skin rash, hemorrhage, dyspnea, fatigue, nausea, musculoskeletal pain	Nausea, rash, headache, fatigue, pruritus, vomiting, diarrhea, cough, constipation, arthralgia, nasopharyngitis, pyrexia, night sweats	Diarrhea, nausea, thrombocytopenia, rash, increased alanine aminotransferase, abdominal pain, increased aspartate aminotransferase	Abdominal pain, rash, constipation, headache, dry skin, arterial occlusion, fatigue, hypertension, pyrexia, arthralgia, nausea, diarrhea, lipase increased, vomiting, myalgia, and pain in extremities

continued

Table 5.1 continued

Drug	Imatinib	Dasatinib	Nilotinib	Bosutinib	Ponatinib
Hepatic or renal dose considerations	Hepatic (severe dysfunction): 300 mg daily Renal: No dose adjustments recommended	Hepatic: No dose adjustments recommended Renal: No dose adjustments recommended	Hepatic: Reduce starting dose to 200 mg twice daily (newly diagnosed) or 300 mg twice daily (resistant/intolerant) and increase as tolerated Renal: No adjustments recommended	Hepatic: 200 mg daily (newly diagnosed or resistant/intolerant) Renal: -CrCl 30–50 mL/min: 300 mg daily (newly diagnosed) or 400 mg daily (resistant/intolerant) -CrCl <30 mL/min: 200 mg daily (newly diagnosed) or 300 md daily (resistant/intolerant)	Hepatic: 30 mg daily Renal: No dose adjustments recommended
Drug-drug interactions	CYP3A4 inducers may decrease concentrations CYP3A4 inhibitors may increase concentrations Use caution when administering imatinib with CYP3A4 substrates with a narrow therapeutic index Avoid warfarin	Dose reduction may be necessary when given with strong CYP3A4 inhibitors Dose decrease may be necessary when given with strong CYP3A4 inducers Avoid coadministration with H2 blockers and proton pump inhibitors)	Avoid use with strong CYP3A inhibitors or reduce dose Avoid strong CYP3A inducers Avoid proton pump inhibitors (use H2 blockers as an alternative)	Avoid use with strong and moderate CYP3A inhibitors Avoid use with strong CYP3A inducers Avoid proton pump inhibitors (use H2 blockers as an alternative)	Avoid use with strong CYP3A4 inhibitors or reduce dose Avoid use with strong CYP3A4 inducers
Contraindicated BCR/ABL1 mutations	N/A	T315I, F317L/V/I/C, V299L	T315I, Y253H, E255K/V, F359V/C/I, G250E	T315I, V299L, G250E, F317L	None

Achievement of CCyR is associated with EFS and OS benefit [48–51]. While most patients who receive a TKI will achieve a cytogenetic response, depth of response, time to response, and durability of response are becoming increasingly important, especially when considering therapy discontinuation.

Achievement of deep molecular responses has demonstrated long term benefits. The 10-year follow up data from the IRIS study comparing imatinib to interferon-alfa plus cytarabine found a statistically significant OS advantage in patients achieving an MMR at 18 months [52]. This survival advantage has not been observed with second-generation TKIs, which produce faster, deeper molecular responses than imatinib and prevent more patients from progressing to AP or BC [9, 24, 34]. The depth of molecular response among patients achieving CCyR has not been proven to correlate with longer survival. Among patients with CP CML treated with imatinib 400 mg daily, imatinib 800 mg daily, nilotinib, or dasatinib, the rates of >MR4.5 or deep molecular remission (DMR; *BCR-ABL1* transcripts ≤0.01%) at 36 months were 18%, 31%, 29%, and 29% for each arm, respectively [53]. While the depth of molecular response inversely correlated with the risk of losing CCyR, in the landmark analysis at 18 and 24 months, no advantage was seen in transformation-free survival or OS with undetectable transcripts compared to achievement of lesser molecular response. While no OS benefit was seen, the depth of response remains an important factor because the achievement of DMR allows consideration of treatment discontinuation.

Several studies have demonstrated that patients who achieve DMR can maintain a successful treatment-free remission (TFR) [54–58]. The largest of these studies is the EURO-SKI trial evaluating patients with CP CML who had received TKI therapy for at least 3 years and had confirmed DMR (*BCR-ABL1* transcripts <0.01% or undetectable) for at least 1 year prior to discontinuation [59]. Loss of molecular response was defined as *BCR-ABL1* transcripts >0.1% and occurred in 39% at 6 months and 50% at 24 months. Among the various trials, about 40–60% of patients who discontinue therapy will have molecular relapse within 2 years, but most patients will regain molecular response after the TKI is resumed. Further analyses have shown that the longer the patient remains on therapy and sustains MMR4.5 prior to discontinuation, the lower the rates of molecular relapse [59, 60]. The TFR rates of 92% were associated with patients who discontinued therapy after maintaining MR4.5 ≥ 5 years while on treatment [61]. More patients treated with imatinib had molecular relapse than those treated with second-generation TKIs, suggesting that those seeking TFR (e.g. young patients) could be treated with second-generation TKIs for a faster, deeper response to increase the likelihood of attaining TFR. The ability to achieve a DMR, and thus TFR, offers patients both financial benefits as well as freedom from adverse effects; however, patients must be monitored closely to evaluate for molecular relapse.

The time to molecular response has been shown to correlate with outcomes, with earlier responses predicting better outcomes, creating the milestones for evaluating effectiveness of therapy [62, 63]. The CML-IV trial established the optimal milestone of *BCR-ABL1* transcripts <1% at 6 months [64]. This conferred a survival benefit regardless of treatment. Among patients who achieved *BCR-ABL1* transcripts <1% at 6 months, 88% were alive at 10 years compared with 81% who did not achieve this. Earlier attainment of molecular responses has been studied as well [25, 33, 65]. In the DASISION trial, a higher rate of dasatinib-treated patients achieved *BCR-ABL1* transcripts ≤10% at 3 months (early molecular response) compared with imatinib (84% versus 64%, respectively) [9]. After 5 years, regardless of the treatment arm, patients who achieved early molecular response had higher rates of PFS, OS, and lower rates of transformation to AP or BC [10]. It remains unknown if there is a difference in long-term outcomes between patients who achieve an optimal molecular response at 3 months compared with 6 months. Smaller studies have demonstrated that patients who do not achieve *BCR-ABL1* transcripts ≤10% at 3 months but do

attain a response by 6 months have similar survival outcomes. In a study of 320 patients with CP CML receiving imatinib, patients who had *BCR-ABL1* transcripts >10% at 3 months, but <1% at 6 months had similar freedom from treatment failure at 3 years, and 10-year PFS and OS [66]. Patients who did not achieve *BCR-ABL1* transcripts ≤10% at 3 months and <1% at 6 months, however, had significantly worse PFS and OS. In another study, patients receiving imatinib who had *BCR-ABL1* transcripts >10% at 3 months but achieved a reduction to <10% at 6 months had similar OS and PFS at 4 years as patients who had achieved early molecular response at 3 months [67]. Patients who did not achieve *BCR-ABL1* transcripts <10% at 6 months had worse 4-year OS and PFS rates. In patients who have not achieved *BCR-ABL1* transcripts ≤10% at 3 months, it is reasonable to continue treatment with the same TKI and reassess molecular response at 6 months. However, if the *BCR-ABL1* transcripts are ≥10% at 6 months, a change in therapy is indicated.

Risk stratification such as using the Sokal score [68] or Hasford score [69] may be used to select frontline treatment. In the DASISION trial, the rates of MMR at any time were numerically higher with dasatinib compared with imatinib (90% versus 69% in low risk, 71% versus 65% in intermediate risk, and 67% versus 54% in high risk, respectively). In the ENESTnd trial, patients with high Sokal risk scores achieved MMR at 12 months more frequently with nilotinib than with imatinib (41% with nilotinib 300 mg, 32% with nilotinib 400 mg, and 17% with imatinib) [24]. Additionally, EMR was higher in those treated with nilotinib compared with imatinib, with the greatest difference seen in the high risk group (86% with nilotinib 300 mg, 82% with nilotinib 400 mg, and 44% with imatinib) [65]. In the BFORE trial, MMR at 12 months was higher across all Sokal risk groups when treated with bosutinib compared with imatinib with 34% versus 17%, 45% versus 39%, and 58% versus 46% in high-, intermediate-, and low-risk groups, respectively [34]. Generally, molecular responses are greater with second-generation TKIs across all risk groups, and high-risk groups may benefit more from newer agents in order to achieve early milestones and prevent progression to AP or BC.

TKI therapy remains long term, if not indefinite for many patients with CML, causing financial burden. The cost of these agents may play a role in selecting the appropriate TKI. Currently the only available generics are for imatinib. Treatment with generic imatinib can be as low as $400 per year in some countries while the cost of branded imatinib (Gleevec®) can be as high as $132,000 per year [61]. Studies have shown that upfront imatinib generics and switching from branded imatinib to generic have similar safety and efficacy [70–72]. All second-generation TKIs (dasatinib, nilotinib, and bosutinib) remain brand name only and cost more than $150,000 per year. Generic dasatinib may soon be available.

Although no large, prospective, randomized head-to-head trials have been conducted comparing second-generation TKIs as frontline therapy, one propensity score matching analysis compared 102 patients on dasatinib with 104 patients on nilotinib and found similar rates of early molecular response, MMR at 12 months, 3-year EFS, 3-year OS, and discontinuation rates [73]. A retrospective cohort analysis of patients with CP CML evaluated the outcomes of patients receiving imatinib 400 mg, imatinib 800 mg, dasatinib, and nilotinib [74]. Patients on imatinib 800 mg or a second-generation TKI had superior CCyR rates, MMR rates, and 5-year EFS rates. However no significant differences in 5-year failure-free survival, transformation-free survival, or OS were observed (refer to Table 5.2 for responses and outcomes from the large phase III trials of each drug in the frontline setting).

Generally, imatinib, dasatinib, nilotinib, and bosutinib can all be utilized in the frontline setting. Cost, comorbidities, patient specific factors (high risk disease), and the desire for treatment discontinuation (i.e., achieving a DMR) may drive the selection of the TKI. When considering frontline therapy, comorbidities, adverse event profile, patient age, risk stratification, and cost should be considered.

Table 5.2 Overview of responses and outcomes with TKIs in the frontline setting

	Imatinib (IRIS Trial)	Dasatinib (DASISION Trial)	Nilotinib (ENESTnd Trial)	Bosutinib (BFORE and BELA Trials)
EMR	N/A	84%	89–91%	75%
MMR at 12 months	28%	46%[a]	43–44%[a]	47%[a]
CCyR at 12 months	53%	77%[a]	78–80%[a]	77%[a]
OS	10-year: 83%	5-year: 91%	10-year: 88–90%	2-year: 97%
EFS	10-year: 80%	N/A	5-year: 95–97%	2-year: 95%
PFS	N/A	5-year: 85%	5-year: 97–98%	N/A

[a]Statistically significant

5.7 Response-Based Approach

Because second-generation TKIs remain expensive and can have more toxic adverse event profiles compared with imatinib, a response-based approach has been evaluated. In the open-label TIDEL-II trial, patients with CP CML were treated with imatinib 600 mg daily and could be escalated to imatinib 800 mg daily if plasma drug levels were subtherapeutic [75]. *BCR-ABL1* transcripts were assessed at 3, 6, and 12 months for molecular targets of ≤10%, ≤1%, and ≤0.1%, respectively. Patients were separated into two cohorts. In cohort 1, patients who failed to achieve the targets could be escalated to imatinib 800 mg daily and subsequently switched to nilotinib 400 mg twice daily for failing the same target 3 months later. Patients switched to nilotinib if they were unable to escalate to imatinib 800 mg daily due to intolerance or those already on the 800 mg dose based on drug levels. In cohort 2, patients were switched to nilotinib 400 mg twice daily for failing any target. At 12 months, the rate of MMR was 66% with cohort 1 and 62% with cohort 2, which is higher than 12 month MMR rates reported in the IRIS follow up data; however this could be due to a different imatinib dosing scheme [52]. Twenty five patients (12%) failed to achieve early molecular response and had inferior OS, transformation-free survival, and decreased probability of achieving MMR with subsequent therapy. Six of these patients were able to achieve MMR at 24 months with dose escalation or change in therapy.

The DASCERN trial evaluated the early switch to dasatinib 100 mg daily compared to continuing treatment (imatinib at any dose selected by the investigator) in patients with CP CML who had *BCR-ABL1* transcripts >10% after 3 months of treatment with imatinib 400 mg [76]. Cross over was allowed if patients met European LeukemiaNet (ELN) criteria for treatment failure, and 52% of the imatinib group crossed over to the dasatinib arm after a median of 9 months. In the intent-to-treat (ITT) population, the rate of MMR at 12 months was 29% and 13% with dasatinib and imatinib, respectively ($P = 0.005$). After censoring for cross over, 64% of patients in the dasatinib group and 41% of patients in the imatinib group achieved MMR by 24 months. Among patients who crossed over to dasatinib, 58% achieved MMR at 24 months. The time to MMR was significantly shorter with dasatinib (14 months) compared with imatinib (20 months).

5.8 Therapy of CML Post-Frontline TKI Failure

There is no standardized choice of therapy once patients have progressed on frontline therapy with TKIs [5, 31, 77–81]. TKI salvage therapy depends on patient specific factors, such as comorbidities, adherence, and cost, in addition to disease-related factors like mutation profile.

Although no head-to-head trials of second-line agents have been conducted, indirect comparisons have been made. Among patients who

failed imatinib, bosutinib was compared with nilotinib and found to have a lower hazard ratio (HR) for PFS. The HR was not statistically significant for OS [82]. In earlier trials of patients with CP CML after imatinib failure, the rates of PFS and OS at 24 months were 80% and 94% with dasatinib, respectively [83]. In a similar trial, the 24-month PFS was 64% and OS was 87% with nilotinib [84]. Although not directly compared, dasatinib and bosutinib may produce better outcomes compared with nilotinib after imatinib failure. At MD Anderson, we offer dasatinib 50 mg as the frontline approach. In patients failing dasatinib, ponatinib is the salvage approach except in patients with cardiovascular risk factors, or if there are any guiding mutations. In these patients bosutinib or nilotinib are also reasonable approaches. In patients with post imatinib frontline failure, dasatinib or bosutinib are reasonable first salvage options. In a meta-analysis of patients with CP CML who experienced resistance or intolerance to at least one second-generation TKI, sequential use of second-generation TKIs provided limited benefit [85]. The probability of attaining CCyR was 22–26% with a second-generation TKI compared with 60% with ponatinib. Ponatinib will be the drug of choice post dasatinib or bosutinib failure unless, again, there are guiding mutations.

As expected, the rates of response decrease with increasing number of lines of therapy, especially when resistance develops rather than intolerance. In patients receiving bosutinib after imatinib failure or intolerance, after a median of 24.2 months, MCyR and CCyR were seen in 53% and 41% of imatinib-resistant patients, respectively. MMR was seen in 64% of evaluable imatinib-resistant patients [31]. In patients who were treated with two TKIs before bosutinib, after a median of 28.5 months, MCyR was seen in 31–35% and CCyR was seen in 14–27% of resistant patients. MMR was seen in only 3%–11% of patients [30].

In the third-line setting for CP CML, a matching-adjusted comparison was conducted evaluating bosutinib against ponatinib [86]. The CCyR rates were 61% with ponatinib compared with 26% with bosutinib. These responses were maintained at 4 years in 89% and 54% of patients treated with ponatinib and bosutinib, respectively. Treatment failure due to death, disease progression, or unsatisfactory response led to discontinuation of bosutinib in 42% of patients compared with 9% of patients on ponatinib. The rates of discontinuation due to adverse effects were similar among the two groups, occurring in 24% and 19% of patients on bosutinib and ponatinib, respectively. Although data are limited, ponatinib is the preferred agent after treatment failure with a second-generation TKI. If patients cannot receive a TKI or have progressed on all approved therapies, a clinical trial, omacetaxine (if available) or stem cell transplant should be pursued. Combinations of a "best" TKI with other agents (azacytidine, cytarabine, hydroxyurea, omacetaxine) can also be considered among patients who failed all TKIs and are not considered for allogeneic SCT, to control and prolong a chronic phase status, without necessarily aiming for a CCyR or better responses.

5.9 Future Therapies

Combination therapy utilizing dasatinib with venetoclax is under investigation (NCT02689440). BCL-2 expression has been demonstrated in CML cells of mice and may be a resistance mechanism against traditional TKI therapy [87, 88] Combined inhibition of both the BCR-ABL1 kinase and BCL-2 may lead to increased depth of response.

Additional therapies are under development for newly diagnosed and relapsed CML. HQP1351 is a third-generation TKI in early phase trials. In the phase I dose-escalation trial, patients with CP CML or AP CML with resistance or intolerance to ≥2 TKIs or a T315I mutation after ≥1 TKI received HQP1351 every other day [89]. After a median follow-up of 13 months, 95% of patients with CP CML achieved complete hematologic response, 61% achieved CCyR, and 37% achieved MMR. HQP1351 was well tolerated, but common adverse effects included thrombocytopenia, skin pigmentation, and hypertriglyceridemia. HQP1351 exhibited potent activity, and

may be a useful agent, especially among patients with a T315I mutation.

K0706 is a third-generation TKI with significantly less off-target activity compared to available agents. Patients with CML or Philadelphia chromosome positive acute lymphoblastic leukemia with resistance or intolerance to ≥3 TKIs were evaluated in the phase I trial. After a median of 7 months follow-up, 25% of patients with CP CML achieved CCyR and 19% achieved MMR. Common adverse effects were gastrointestinal disturbances, myalgia, fatigue, neutropenia, and thrombocytopenia. Dose-limiting toxicities included dyspnea and non-cardiac chest pain but resolved with dose reduction.

Asciminib is a BCR-ABL1 inhibitor with a novel mechanism of action and activity against the T315I mutation. It binds the myristoyl site of the BCR-ABL1 protein and acts as an allosteric inhibitor of the kinase, locking it into an inactive formation. This agent recently showed efficacy in patients with CP CML who had resistance or intolerance to at least two previous TKIs [90]. In those with hematologic relapse at baseline, complete hematologic response was achieved in 92% of patients after a median follow-up of 14 months. In those without CCyR at baseline, 54% achieved CCyR in a median time of 24 weeks. Lastly, MMR was achieved or maintained by 48% of patients at 12 months.

5.10 Conclusion

TKIs have radically changed the treatment and outcomes of patients with CP CML. Multiple options exist for front line treatment, including imatinib, dasatinib, bosutinib, and nilotinib. The choice of initial TKI is dependent on patient specific factors including comorbidities, desired response, cost, and disease related factors such as risk score. In general, second-generation TKIs are more potent than imatinib and induce faster and deeper responses. Second-generation TKIs should be considered in patients with high-risk disease and those seeking treatment-free remission. There is no preferred agent in the relapsed setting, except in the case of a T315I mutation where ponatinib is the only available TKI that retains activity against this mutation, although other agents are in development. Stem cell transplantation remains a treatment option for patients with CML and failure of multiple TKIs.

References

1. American Cancer Society. Key statistics for chronic myeloid leukemia. https://www.cancer.org/cancer/chronic-myeloid-leukemia/about/statistics.html#references. Published 2020. Accessed June 21, 2020.
2. O'Hare T, Walters DK, Stoffregen EP, et al. In vitro activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. Cancer Res. 2005;65(11):4500–5.
3. Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL. Overriding imatinib resistance with a novel ABL kinase inhibitor. Science. 2004;305(5682):399–401.
4. Bristol-Myers Squibb Company. Sprycel (dasatinib) [package insert]. Princeton, NJ: Bristol-Myers Squibb Company; 2018.
5. Hochhaus A, Kantarjian HM, Baccarani M, et al. Dasatinib induces notable hematologic and cytogenetic responses in chronic-phase chronic myeloid leukemia after failure of imatinib therapy. Blood. 2007;109(6):2303–9.
6. Hochhaus A, Baccarani M, Deininger M, et al. Dasatinib induces durable cytogenetic responses in patients with chronic myelogenous leukemia in chronic phase with resistance or intolerance to imatinib. Leukemia. 2008;22(6):1200–6.
7. Shah NP, Kantarjian HM, Kim DW, et al. Intermittent target inhibition with dasatinib 100 mg once daily preserves efficacy and improves tolerability in imatinib-resistant and -intolerant chronic-phase chronic myeloid leukemia. J Clin Oncol. 2008;26(19):3204–12.
8. Shah NP, Rousselot P, Schiffer C, et al. Dasatinib in imatinib-resistant or -intolerant chronic-phase, chronic myeloid leukemia patients: 7-year follow-up of study CA180-034. Am J Hematol. 2016;91(9):869–74.
9. Kantarjian H, Shah NP, Hochhaus A, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2010;362(24):2260–70.
10. Cortes JE, Saglio G, Kantarjian HM, et al. Final 5-year study results of DASISION: the Dasatinib versus Imatinib study in treatment-naive chronic myeloid Leukemia patients trial. J Clin Oncol. 2016;34(20):2333–40.
11. O'Brien S, Cork L, Bandeira V, et al. Spirit 2: final 5 year analysis of the UK National Cancer Research Institute randomized study comparing Imatinib with Dasatinib in patients with newly diagnosed chronic phase CML. Blood. 2018;132(Supplement 1):457.

12. Maiti A, Cortes JE, Patel KP, et al. Long-term results of frontline dasatinib in chronic myeloid leukemia. Cancer. 2020;126(7):1502–11.
13. Porkka K, Khoury HJ, Paquette RL, Matloub Y, Sinha R, Cortes JE. Dasatinib 100 mg once daily minimizes the occurrence of pleural effusion in patients with chronic myeloid leukemia in chronic phase and efficacy is unaffected in patients who develop pleural effusion. Cancer. 2010;116(2):377–86.
14. Khoury HJ, Guilhot F, Hughes TP, Kim DW, Cortes JE. Dasatinib treatment for Philadelphia chromosome-positive leukemias: practical considerations. Cancer. 2009;115(7):1381–94.
15. Cortes JE, Hochhaus A, Kantarjian HM, et al. Impact of dose reductions on 5-year efficacy in newly diagnosed patients with chronic myeloid leukemia in chronic phase (CML-CP) from DASISION. In: American Society of Clinical Oncology; 2017.
16. Talpaz M, Shah NP, Kantarjian H, et al. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. N Engl J Med. 2006;354(24):2531–41.
17. Naqvi K, Jabbour E, Skinner J, et al. Long-term follow-up of lower dose dasatinib (50 mg daily) as frontline therapy in newly diagnosed chronic-phase chronic myeloid leukemia. Cancer. 2020;126(1):67–75.
18. Novartis Pharmaceuticals Corporation. Tasigna (nilotinib) [package insert]. East Hanover, NJ: Novartis Pharmaceuticals Corporation; 2019.
19. Weisberg E, Manley P, Mestan J, Cowan-Jacob S, Ray A, Griffin JD. AMN107 (nilotinib): a novel and selective inhibitor of BCR-ABL. Br J Cancer. 2006;94(12):1765–9.
20. Cowan-Jacob SW, Fendrich G, Floersheimer A, et al. Structural biology contributions to the discovery of drugs to treat chronic myelogenous leukaemia. Acta Crystallogr D Biol Crystallogr. 2007;63(Pt 1):80–93.
21. Weisberg E, Manley PW, Breitenstein W, et al. Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. Cancer Cell. 2005;7(2):129–41.
22. Kantarjian HM, Giles F, Gattermann N, et al. Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is effective in patients with Philadelphia chromosome-positive chronic myelogenous leukemia in chronic phase following imatinib resistance and intolerance. Blood. 2007;110(10):3540–6.
23. Giles FJ, le Coutre PD, Pinilla-Ibarz J, et al. Nilotinib in imatinib-resistant or imatinib-intolerant patients with chronic myeloid leukemia in chronic phase: 48-month follow-up results of a phase II study. Leukemia. 2013;27(1):107–12.
24. Saglio G, Kim DW, Issaragrisil S, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med. 2010;362(24):2251–9.
25. Hochhaus A, Saglio G, Hughes TP, et al. Long-term benefits and risks of frontline nilotinib vs imatinib for chronic myeloid leukemia in chronic phase: 5-year update of the randomized ENESTnd trial. Leukemia. 2016;30(5):1044–54.
26. Hughes TP, Saglio G, Larson RA, et al. Long-term outcomes in patients with chronic myeloid Leukemia in chronic phase receiving frontline Nilotinib versus Imatinib: Enestnd 10-year analysis. Blood. 2019;134(Supplement_1):2924.
27. Redaelli S, Piazza R, Rostagno R, et al. Activity of bosutinib, dasatinib, and nilotinib against 18 imatinib-resistant BCR/ABL mutants. J Clin Oncol. 2009;27(3):469–71.
28. Puttini M, Coluccia AM, Boschelli F, et al. In vitro and in vivo activity of SKI-606, a novel Src-Abl inhibitor, against imatinib-resistant Bcr-Abl+ neoplastic cells. Cancer Res. 2006;66(23):11314–22.
29. Remsing Rix LL, Rix U, Colinge J, et al. Global target profile of the kinase inhibitor bosutinib in primary chronic myeloid leukemia cells. Leukemia. 2009;23(3):477–85.
30. Khoury HJ, Cortes JE, Kantarjian HM, et al. Bosutinib is active in chronic phase chronic myeloid leukemia after imatinib and dasatinib and/or nilotinib therapy failure. Blood. 2012;119(15):3403–12.
31. Cortes JE, Kantarjian HM, Brummendorf TH, et al. Safety and efficacy of bosutinib (SKI-606) in chronic phase Philadelphia chromosome-positive chronic myeloid leukemia patients with resistance or intolerance to imatinib. Blood. 2011;118(17):4567–76.
32. Cortes JE, Kim DW, Kantarjian HM, et al. Bosutinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: results from the BELA trial. J Clin Oncol. 2012;30(28):3486–92.
33. Brummendorf TH, Cortes JE, de Souza CA, et al. Bosutinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukaemia: results from the 24-month follow-up of the BELA trial. Br J Haematol. 2015;168(1):69–81.
34. Cortes JE, Gambacorti-Passerini C, Deininger MW, et al. Bosutinib versus Imatinib for newly diagnosed chronic myeloid Leukemia: results from the randomized BFORE trial. J Clin Oncol. 2018;36(3):231–7.
35. Pfizer Inc. Bosulif (bosutinib) [package insert]. New York, NY: Pfizer Inc.; 2019.
36. Garcia-Gutierrez V, Milojkovic D, Hernandez-Boluda JC, et al. Safety and efficacy of bosutinib in fourth-line therapy of chronic myeloid leukemia patients. Ann Hematol. 2019;98(2):321–30.
37. Tiribelli M, Abruzzese E, Capodanno I, et al. Efficacy and safety of bosutinib in chronic phase CML patients developing pleural effusion under dasatinib therapy. Ann Hematol. 2019;98(11):2609–11.
38. Tokarski JS, Newitt JA, Chang CY, et al. The structure of Dasatinib (BMS-354825) bound to activated ABL kinase domain elucidates its inhibitory activity against imatinib-resistant ABL mutants. Cancer Res. 2006;66(11):5790–7.
39. ARIAD Pharmaceuticals Inc. Iclusig (ponatinib) [package insert]. Cambridge, MA: ARIAD Pharmaceuticals Inc.; 2020.
40. Cortes JE, Kim DW, Pinilla-Ibarz J, et al. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. N Engl J Med. 2013;369(19):1783–96.

41. Cortes JE, Kim DW, Pinilla-Ibarz J, et al. Ponatinib efficacy and safety in Philadelphia chromosome-positive leukemia: final 5-year results of the phase 2 PACE trial. Blood. 2018;132(4):393–404.
42. Jain P, Kantarjian H, Jabbour E, et al. Ponatinib as first-line treatment for patients with chronic myeloid leukaemia in chronic phase: a phase 2 study. Lancet Haematol. 2015;2(9):e376–83.
43. Lipton JH, Chuah C, Guerci-Bresler A, et al. Ponatinib versus imatinib for newly diagnosed chronic myeloid leukaemia: an international, randomised, open-label, phase 3 trial. Lancet Oncol. 2016;17(5):612–21.
44. Cortes JE, Lomaia E, Turkina A, et al. Interim analysis (IA) of OPTIC: a dose-ranging study of three ponatinib (PON) starting doses. J Clin Oncol. 2020;38(15_suppl):7502.
45. National Comprehensive Cancer Network. Chronic Myeloid Leukemia (Version 3.2020). https://www.nccn.org/professionals/physician_gls/pdf/cml.pdf. Published 2020. Accessed June 2, 2020.
46. Hochhaus A, Baccarani M, Silver RT, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020;34(4):966–84.
47. Novartix Pharmaceuticals Corporation. Gleevec (imatinib) [package insert]. East Hanover, NJ: Novartix Pharmaceuticals Corporation; 2020.
48. Kantarjian HM, O'Brien S, Cortes JE, et al. Complete cytogenetic and molecular responses to interferon-alpha-based therapy for chronic myelogenous leukemia are associated with excellent long-term prognosis. Cancer. 2003;97(4):1033–41.
49. Bonifazi F, de Vivo A, Rosti G, et al. Chronic myeloid leukemia and interferon-α: a study of complete cytogenetic responders. Blood. 2001;98(10):3074–81.
50. Kantarjian HM, Cortes JE, O'Brien S, et al. Long-term survival benefit and improved complete cytogenetic and molecular response rates with imatinib mesylate in Philadelphia chromosome-positive chronic-phase chronic myeloid leukemia after failure of interferon-alpha. Blood. 2004;104(7):1979–88.
51. Druker BJ, Guilhot F, O'Brien SG, et al. Five-year follow-up of patients receiving Imatinib for chronic myeloid Leukemia. N Engl J Med. 2006;355(23):2408–17.
52. Hochhaus A, Larson RA, Guilhot F, et al. Long-term outcomes of Imatinib treatment for chronic myeloid Leukemia. N Engl J Med. 2017;376(10):917–27.
53. Falchi L, Kantarjian HM, Wang X, et al. Significance of deeper molecular responses in patients with chronic myeloid leukemia in early chronic phase treated with tyrosine kinase inhibitors. Am J Hematol. 2013;88(12):1024–9.
54. Mahon FX, Réa D, Guilhot J, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. Lancet Oncol. 2010;11(11):1029–35.
55. Hochhaus A, Masszi T, Giles FJ, et al. Treatment-free remission following frontline nilotinib in patients with chronic myeloid leukemia in chronic phase: results from the ENESTfreedom study. Leukemia. 2017;31(7):1525–31.
56. Ross DM, Masszi T, Gómez Casares MT, et al. Durable treatment-free remission in patients with chronic myeloid leukemia in chronic phase following frontline nilotinib: 96-week update of the ENESTfreedom study. J Cancer Res Clin Oncol. 2018;144(5):945–54.
57. Rea D, Nicolini FE, Tulliez M, et al. Discontinuation of dasatinib or nilotinib in chronic myeloid leukemia: interim analysis of the STOP 2G-TKI study. Blood. 2017;129(7):846–54.
58. Okada M, Imagawa J, Tanaka H, et al. Final 3-year results of the Dasatinib discontinuation trial in patients with chronic myeloid Leukemia who received Dasatinib as a second-line treatment. Clin Lymphoma Myeloma Leuk. 2018;18(5):353–60.e351
59. Saussele S, Richter J, Guilhot J, et al. Discontinuation of tyrosine kinase inhibitor therapy in chronic myeloid leukaemia (EURO-SKI): a prespecified interim analysis of a prospective, multicentre, non-randomised, trial. Lancet Oncol. 2018;19(6):747–57.
60. Rousselot P, Loiseau C, Delord M, Besson C, Cayuela J-M, Spentchian M. A report on 114 patients who experienced treatment free remission in a single institution during a 15 years period: long term follow-up, late molecular relapses and second attempts. Blood. 2019;134(Supplement_1):27.
61. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2020 update on diagnosis, therapy and monitoring. Am J Hematol. 2020;95(6):691–709.
62. Hanfstein B, Müller MC, Hehlmann R, et al. Early molecular and cytogenetic response is predictive for long-term progression-free and overall survival in chronic myeloid leukemia (CML). Leukemia. 2012;26(9):2096–102.
63. Marin D, Ibrahim AR, Lucas C, et al. Assessment of BCR-ABL1 transcript levels at 3 months is the only requirement for predicting outcome for patients with chronic myeloid leukemia treated with tyrosine kinase inhibitors. J Clin Oncol. 2012;30(3):232–8.
64. Hehlmann R, Lauseker M, Saußele S, et al. Assessment of imatinib as first-line treatment of chronic myeloid leukemia: 10-year survival results of the randomized CML study IV and impact of non-CML determinants. Leukemia. 2017;31(11):2398–406.
65. Hughes TP, Saglio G, Kantarjian HM, et al. Early molecular response predicts outcomes in patients with chronic myeloid leukemia in chronic phase treated with frontline nilotinib or imatinib. Blood. 2014;123(9):1353–60.
66. Kim D, Hamad N, Lee HG, Kamel-Reid S, Lipton JH. BCR/ABL level at 6 months identifies good risk CML subgroup after failing early molecular response at 3 months following imatinib therapy for CML in chronic phase. Am J Hematol. 2014;89(6):626–32.
67. Branford S, Roberts N, Yeung DT, et al. Any BCR-ABL reduction below 10% at 6 months of therapy sig-

nificantly improves outcome for CML patients with a poor response at 3 months. Blood. 2013;122(21):254.
68. Sokal JE, Cox EB, Baccarani M, et al. Prognostic discrimination in "good-risk" chronic granulocytic leukemia. Blood. 1984;63(4):789–99.
69. Hasford J, Pfirrmann M, Hehlmann R, et al. A new prognostic score for survival of patients with chronic myeloid leukemia treated with interferon alfa. Writing Committee for the Collaborative CML prognostic factors project group. J Natl Cancer Inst. 1998;90(11):850–8.
70. Sacha T, Góra-Tybor J, Szarejko M, et al. A multicenter prospective study on efficacy and safety of imatinib generics: a report from polish adult Leukemia group imatinib generics registry. Am J Hematol. 2017;92(7):E125–e128.
71. Danthala M, Gundeti S, Kuruva SP, et al. Generic Imatinib in chronic myeloid Leukemia: survival of the cheapest. Clin Lymphoma Myeloma Leuk. 2017;17(7):457–62.
72. Abou Dalle I, Kantarjian H, Burger J, et al. Efficacy and safety of generic imatinib after switching from original imatinib in patients treated for chronic myeloid leukemia in the United States. Cancer Med. 2019;8(15):6559–65.
73. Takahashi K, Kantarjian HM, Yang Y, et al. A propensity score matching analysis of dasatinib and nilotinib as a frontline therapy for patients with chronic myeloid leukemia in chronic phase. Cancer. 2016;122(21):3336–43.
74. Jain P, Kantarjian H, Alattar ML, et al. Long-term molecular and cytogenetic response and survival outcomes with imatinib 400 mg, imatinib 800 mg, dasatinib, and nilotinib in patients with chronic-phase chronic myeloid leukaemia: retrospective analysis of patient data from five clinical trials. Lancet Haematol. 2015;2(3):e118–28.
75. Yeung DT, Osborn MP, White DL, et al. TIDEL-II: first-line use of imatinib in CML with early switch to nilotinib for failure to achieve time-dependent molecular targets. Blood. 2015;125(6):915–23.
76. Cortes JE, Jiang Q, Wang J, et al. Dasatinib vs. imatinib in patients with chronic myeloid leukemia in chronic phase (CML-CP) who have not achieved an optimal response to 3 months of imatinib therapy: the DASCERN randomized study. Leukemia. 2020;34:2064.
77. Cortes JE, Khoury HJ, Kantarjian HM, et al. Long-term bosutinib for chronic phase chronic myeloid leukemia after failure of imatinib plus dasatinib and/or nilotinib. Am J Hematol. 2016;91(12):1206–14.
78. Ongoren S, Eskazan AE, Suzan V, et al. Third-line treatment with second-generation tyrosine kinase inhibitors (dasatinib or nilotinib) in patients with chronic myeloid leukemia after two prior TKIs: real-life data on a single center experience along with the review of the literature. Hematology. 2018;23(4):212–20.
79. Giles FJ, Abruzzese E, Rosti G, et al. Nilotinib is active in chronic and accelerated phase chronic myeloid leukemia following failure of imatinib and dasatinib therapy. Leukemia. 2010;24(7):1299–301.
80. Kantarjian H, Pasquini R, Hamerschlak N, et al. Dasatinib or high-dose imatinib for chronic-phase chronic myeloid leukemia after failure of first-line imatinib: a randomized phase 2 trial. Blood. 2007;109(12):5143–50.
81. Kantarjian H, Giles F, Wunderle L, et al. Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. N Engl J Med. 2006;354(24):2542–51.
82. Cortes JE, Muresan B, Mamolo C, et al. Matching-adjusted indirect comparison of bosutinib, dasatinib and nilotinib effect on survival and major cytogenetic response in treatment of second-line chronic phase chronic myeloid leukemia. Curr Med Res Opin. 2019;35(9):1615–22.
83. Mauro MJ, Baccarani M, Cervantes F, et al. Dasatinib 2-year efficacy in patients with chronic-phase chronic myelogenous leukemia (CML-CP) with resistance or intolerance to imatinib (START-C). J Clin Oncol. 2008;26(15_suppl):7009.
84. Kantarjian HM, Giles FJ, Bhalla KN, et al. Nilotinib is effective in patients with chronic myeloid leukemia in chronic phase after imatinib resistance or intolerance: 24-month follow-up results. Blood. 2011;117(4):1141–5.
85. Lipton JH, Bryden P, Sidhu MK, et al. Comparative efficacy of tyrosine kinase inhibitor treatments in the third-line setting, for chronic-phase chronic myelogenous leukemia after failure of second-generation tyrosine kinase inhibitors. Leuk Res. 2015;39(1):58–64.
86. Levy MY, McGarry LJ, Huang H, Lustgarten S, Chiroli S, Iannazzo S. Benefits and risks of ponatinib versus bosutinib following treatment failure of two prior tyrosine kinase inhibitors in patients with chronic phase chronic myeloid leukemia: a matching-adjusted indirect comparison. Curr Med Res Opin. 2019;35(3):479–87.
87. Carter BZ, Mak PY, Mu H, et al. Combined targeting of BCL-2 and BCR-ABL tyrosine kinase eradicates chronic myeloid leukemia stem cells. Sci Transl Med. 2016;8(355):355ra117.
88. Elrick LJ, Jorgensen HG, Mountford JC, Holyoake TL. Punish the parent not the progeny. Blood. 2005;105(5):1862–6.
89. Jiang Q, Huang X, Chen Z, et al. An updated safety and efficacy results of phase 1 study of HQP1351, a novel 3rd generation of BCR-ABL tyrosine kinase inhibitor (TKI), in patients with TKI resistant chronic myeloid Leukemia. Blood. 2019;134(Supplement_1):493.
90. Hughes TP, Mauro MJ, Cortes JE, et al. Asciminib in chronic myeloid Leukemia after ABL kinase inhibitor failure. N Engl J Med. 2019;381(24):2315–26.

6 Adverse Events Associated with ATP-Competitive BCR-ABL1 Tyrosine Kinase Inhibitors in Chronic Myeloid Leukemia

Delphine Rea

6.1 Introduction

ATP-competitive tyrosine kinase inhibitors (TKIs) of the BCR-ABL1 oncoprotein revolutionized the prognosis of chronic myeloid leukemia (CML). Indeed, TKIs confer a high degree of protection against progression to blast crisis, of overall survival, and a near-to-normal life expectancy in most patients diagnosed with chronic phase (CP)-CML [1–5]. Some patients with deep molecular responses may even discontinue TKIs and achieve treatment-free remission [6, 7]. Five TKIs received worldwide approval for use against CML. The first-generation TKI, imatinib, is licensed after failure of interferon-alpha therapy since 2001 and for newly diagnosed CML in all phases since 2002 (Table 6.1) [3]. Dasatinib, nilotinib, and bosutinib are second-generation TKIs with greater potency for native BCR-ABL1 than imatinib and activity against many imatinib-resistant mutants forms of BCR-ABL1 except the gatekeeper BCR-ABL1 T315I mutation. Dasatinib is approved since 2006 for CML in all phases after resistance or intolerance to imatinib and since 2010 for newly diagnosed CP-CML (Table 6.1) [8–10]. Nilotinib is approved for CP- or accelerated phase-CML after imatinib failure since 2007 and is licensed for newly diagnosed CP-CML since 2010 (Table 6.1) [11–14]. Bosutinib is indicated since 2012 for all phases CML in patients with intolerance or resistance to prior therapy and since 2017 for newly diagnosed CP-CML (Table 6.1) [15, 16]. The third-generation TKI ponatinib received marketing authorization for use since 2012 in patients with all phases CML and resistance or intolerance to prior therapy including patients who have the BCR-ABL1 T315I mutation (Table 6.1) [17, 18].

These five TKIs target the ATP-binding site of BCR-ABL1 and exert their antileukemic effect through the inhibition of the catalytic activation of the tyrosine kinase. They also bind to other protein kinases and non-kinases with different degrees of specificity and selectivity (Table 6.2). Imatinib and nilotinib inhibit PDGF-R and c-KIT. Nilotinib also blocks the DDR1 kinase and the oxidoreductase NQO2 [19]. Dasatinib is a dual SRC/ABL inhibitor and an inhibitor of PDGR-R, c-KIT, and the ephrin receptor [20]. Bosutinib is a potent SRC and ABL inhibitor; it has many other targets such as CAMK2G and STE20 and does not inhibit PDGR-R and c-KIT [20]. Ponatinib inhibits FLT3, RET, c-KIT, and the members of the FGF-R, PDGF-R, and

D. Rea (✉)
Service d'Hématologie Adulte, Hôpital Saint-Louis, AP-HP, Paris, France

France Intergroupe des Leucémies Myéloïdes chroniques, Fi-LMC, Lyon, France
e-mail: delphine.rea@aphp.fr

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
R. Hehlmann (ed.), *Chronic Myeloid Leukemia*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-71913-5_6

Table 6.1 The ATP-competitive arsenal in CML

TKI type	Year of approval	Indication
Imatinib	– 2001 – 2002	– CML after failure of IFN-α – Newly diagnosed all phases CML
Dasatinib	– 2006 – 2010	– All phases of CML after resistance or intolerance to imatinib – Newly diagnosed CP-CML
Nilotinib	– 2007 – 2010	– CP- or AP-CML after resistance or intolerance to imatinib – Newly diagnosed CP-CML
Bosutinib	– 2012 – 2017	– All phases of CML after resistance or intolerance to prior therapy – Newly diagnosed CP-CML
Ponatinib	– 2012	– All phases of CML with resistance or intolerance to prior therapy or who have the gatekeeper BCR-ABL1 T315I mutation

Table 6.2 ATP-competitive TKIs: targets

	Imatinib	Dasatinib	Nilotinib	Bosutinib	Ponatinib
Therapeutic kinase target					
BCR-ABL1	x	x	x	x	x
Non-therapeutic kinase targets					
ABL/ARG	x	x	x	x	x
Src family kinases		x		x	x
c-kit	x	x	x		x
PDGF-R	x	x	x		x
Ephrin-R		x	x		
DDR1	x	x	x		
TEC family kinases		x		x	
CAMKG2, STE20				x	
FLT3, RET, FGF-R, VEGF-R					x x
Non-therapeutic non-kinase targets	x	x	x	x	x

VEGF-R families of kinases [21]. As a result, TKIs are associated with a wide variety of off-target adverse events. In addition, they are all CYP3A4 substrates and inhibitors and have relevant drug interactions that may induce or aggravate toxicities. Finally, TKI safety may be influenced by age and comorbidities [22].

CML care must not only integrate optimal disease control based on treatment goals but also management of the iatrogenic risk, which depends on each TKI safety profile, dose, and on patient personal background [23]. The purpose of this chapter is to provide an updated overview of adverse events related to the use of TKIs and to discuss short- and long-term clinical management, focusing on patients with CP-CML.

6.2 Myelosuppression

Myelosuppression is a common and anticipated adverse event of TKIs, especially in patients with a high leukemic burden [24]. It is often limited to the first weeks or months of therapy, and it may thus be due to a toxic effect of TKIs combined to a reduced reserve or a delayed recovery of polyclonal Ph-negative hematopoietic stem cells [25].

In patients with newly diagnosed CP-CML treated with first-line imatinib at 400 mg QD in the IRIS trial, incidences of all grade neutropenia, thrombocytopenia, and anemia by 12 months were 60.8%, 56.6%, and 44.6%, respectively, and those of grade 3–4 were 14.3%, 7.8%, and 3.1%, respectively [26]. At later time points, newly occurring or worsening cytopenia were rare [27].

In patients with newly diagnosed CP-CML in the ENESTnd trial, cytopenia was less frequent in the nilotinib 300 mg BID arm than in the imatinib 400 mg QD arm. Incidences of all grade neutropenia, thrombocytopenia, and anemia by 12 months in the nilotinib 300 mg BID arm were 43%, 48%, and 38%, respectively, and those of grade 3–4 were 12%, 10%, and 3%, respectively [12]. First-line dasatinib in CP-CML at 100 mg QD in the DASISION study was more hematotoxic than imatinib at 400 mg QD. Incidences of all grades and grade 3–4 neutropenia, thrombocytopenia, and anemia by 12 months in the dasatinib arm were 65% and 21%, 70%, and 19% and 90% and 10%, respectively [9]. First-line bosutinib in CP-CML at 400 mg QD in the BFORE study was slightly more hematotoxic than imatinib at 400 mg QD. Incidences of all grades and grade 3–4 neutropenia, thrombocytopenia, and anemia by 12 months in the bosutinib arm were 11.2% and 6.7%, 35.1% and 13.8%, and 18.7% and 3.4%, respectively [16]. In patients in whom treatment is changed due to failure of prior TKIs, cytopenia tend to be more profound than in the first-line setting.

Although cytopenia with severe consequences such as bleeding or infections are rare in CP-CML, it is necessary to monitor blood cell counts weekly during the first month of treatment or until normalization, monthly during month 2 and 3, and then every 3 to 6 months unless otherwise indicated. In case of grade 1–2 cytopenia, interruption or dose reduction of TKIs are not necessary. In case of neutropenia or thrombocytopenia of grade 3–4, treatment may be interrupted until resolution. Anemia may be corrected using recombinant erythropoietin or red blood cell transfusion whenever needed. Long-lasting and recurrent cytopenia resulting in prolonged or repeated treatment interruptions and dose reductions may compromise TKI efficacy [28]. In these situations, supportive care with myeloid growth factors such as recombinant erythropoietin, thrombopoietin receptor agonists, or granulocyte-stimulating factor may be helpful although these agents are not approved in this indication [29–31]. A change in TKI therapy may also be envisaged but hematologic cross-intolerance between TKIs cannot be ruled out. Late development of severe cytopenia is rather unusual, and warrants investigations in search of progression to advanced phase CML, myelodysplasia, or other diseases.

6.3 Dermatologic Adverse Events

Dermatologic adverse events are most of the time mild to moderate and generally arise during the first months of therapy. Occurrence and severity are usually dose related and TKI treatment may be pursued in most cases, arguing in favor of direct toxic pharmacological effects rather than immunogenic or allergic mechanisms. Skin rashes induced by imatinib usually consist in maculopapular eruptions. Other and more heterogeneous patterns have been reported such as pigmentary changes, photosensitization, lichenoid reactions, psoriasiform eruptions, pseudoporphyria, exanthematous pustulosis, neutrophilic dermatosis, panniculitis, and Stevens Johnson syndrome [32]. In patients with newly diagnosed CP-CML treated with imatinib at 400 mg QD in the IRIS study, all grade and grade 3–4 cutaneous rashes by 12 months were reported in 33.9% and 2% of patients, respectively [26]. With nilotinib, perifollicular hyperkeratotic and erythematous maculopapular eruptions occur frequently. Incidences by 12 months of all grade and grade 3–4 cutaneous rashes were 31% and <1%, respectively, in patients with newly diagnosed CP-CML treated with nilotinib at 300 mg BID in the ENESTnd study, and were higher than those observed with imatinib [12]. Other frequent dermatologic adverse events associated with nilotinib include mild to moderate pruritus, skin xerosis, alopecia, and body hair loss. Dasatinib is frequently associated with perifollicular hyperkeratotic eruptions. Anecdotal dermatologic side effects of dasatinib include acneiform skin rash, hair depigmentation, vitiligo-like lesions, and panniculitis [33]. During first-line treatment at 100 mg QD in the DASISION trial, all grade and

grade 3–4 skin rashes were observed in 17% and 0% of patients by 12 months, respectively [9]. During first-line bosutinib evaluation at 400 mg QD in the BFORE trial, all grade and grade 3–4 skin rash occurred in 19.8% and 0.4% of patients by 12 months, respectively [16]. With ponatinib at 45 mg QD following intolerance or resistance to prior TKIs in CP-CML, all grade and grade 3–4 erythematous, macular and papular rashes occurred in 47% and 4% of patients by 5 years, respectively, and all grade skin xerosis occurred in 42% of patients [18]. Lichenoid skin damage may also occur [34].

Grade 1–2 skin rash and pruritus can be easily managed with symptomatic measures such as emollients and histamine antagonists with or without a short course of topical steroids. Application of non-cosmetic moisturizers may improve skin xerosis. Dose reduction or interruption of TKI may be indicated in some cases. Severe cutaneous reactions require expert advice from a dermatologist and most of the time alternative TKI treatment.

6.4 Gastrointestinal Adverse Events

Mild to moderate nausea, vomiting, diarrhea, constipation, and abdominal pain are frequent during treatment with all TKIs and represent a substantial source of discomfort. These symptoms usually have short time-to-onset and are dose related. In patients with CP-CML treated with first-line imatinib at 400 mg QD in the IRIS trial, nausea, vomiting, and diarrhea were experienced by 43.7%, 16.9%, and 32.8% of patients by 12 months, respectively [26]. In ENESTnd, the gastrointestinal safety profile of nilotinib at 300 mg QD was more favorable than that of imatinib, with respective incidences of nausea, vomiting, and diarrhea by 12 months of 11%, 5% and 8%, respectively [12]. Similar observations were made with 100 mg QD of dasatinib in DASISION, with the exception of diarrhea which was as frequent as in the imatinib arm with a 12 months incidence of 17% [9]. Diarrhea, nausea, and vomiting were very common in patients with CP-CML receiving bosutinib at 400 mg QD in BFORE, with respective all grade 12-month incidences of 70.1%, 35.1%, and 17.9% [16]. Symptoms usually resolved after supportive care, dose adjustments, or treatment interruptions (Table 6.3). With ponatinib at 45 mg QD in CP-CML, patients frequently complained of abdominal pain and constipation, with respective incidences by 5 years of 46% and 41% [18].

Nausea and vomiting may cease upon TKI intake during the largest meal of the day. This does not apply to patients treated with nilotinib, labeled to be taken fasting due to high-fat food effects on the drug bioavailability [35]. Whenever needed, antiemetics or drugs regulating gastrointestinal transit time may be effective. Other management strategies for nausea or vomiting may consist in splitting the daily dose into two—once in the morning and once in the evening or taking TKIs at bedtime to avoid the burden of nausea during waking hours. In case of persistent or severe events not responding to supportive care and TKI dose reduction, a change of TKI is indicated.

The use of dasatinib requires special attention in light of an increased risk of gastrointestinal bleeding attributed to drug-induced gastritis, ulcers of the digestive tract or ulcerative colitis, and platelet dysfunction [36–38]. Risk factor screening for bleeding such as prior history of gastrointestinal ulcer and low platelet count, and use of antiplatelet drugs or anticoagulants are warranted in dasatinib-treated patients, as well as early recognition and management of bleeding complications. Imatinib may also in rare cases induce gastric antral ectasia leading to intestinal bleeding [39].

6.5 Hepatic and Pancreatic Toxicity

Liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) frequently rise during TKI therapy. Elevation usually starts during the first few months of treatment in a mild to moderate fashion and may spontaneously regress. In case of true hepatic dysfunction, it is

Table 6.3 Main safety concerns with new generation TKIs and management

TKI	Safety concerns	Comments and management
Dasatinib	Pleural effusion	– Dasatinib interruption until spontaneous resolution – Oral corticosteroids may accelerate recovery – switch to another TKI or reintroduction with caution at a lower dose – Thoracocenthesis in severe cases
	Pulmonary arterial hypertension	– assess pulmonary arterial pressure upon development of suggestive symptoms – discontinue dasatinib, switch to another TKI – specific management by PAH specialists
Nilotinib	Hyperglycemia	– institute lifestyle modifications – start or adjust anti-diabetic medications per standard clinical practice
	Hypercholesterolemia	– Instruct for healthy lifestyle – Start statins based on LDL-C levels and CVD risk
	Arterial occlusion	– Discontinue nilotinib and switch to another TKI – Specific management by cardiologists
Bosutinib	Diarrhea	– Initiate antidiarrheal medications – Interrupt bosutinib in severe cases and resume at a lower dose upon recovery
	Liver injury	– Discontinue bosutinib
	Transaminase elevation >5 upper limit of normal (ULN)	– Withhold bosutinib and consider dose reduction after recovery to less than or equal to 2.5 ULN
Ponatinib	Arterial hypertension	– Initiate anti-hypertensive therapy, preferably with ACE inhibitors. Target BP is <140/90 mm hg – Interrupt ponatinib in case of severe hypertension or if BP is not medically controlled and resume at a lower dose upon improvement – Discontinue ponatinib in case of life-threatening symptoms or persistently uncontrolled hypertension despite antihypertensive medications
	Arterial occlusion	– Discontinue ponatinib and search for alternative CML therapy – Specific management by cardiologists
	Acute pancreatitis	– Withhold ponatinib and resume at a lower dose upon resolution – Discontinue ponatinib in case of life-threatening pancreatitis

PAH pulmonary arterial hypertension, *LDL-C* low-density lipoprotein cholesterol, *CVD* cardiovascular diseases, *BP* blood pressure, *ACE* angiotensin converting enzyme

important to exclude other diseases such as other drug usage, cholecystitis, viral infection, alcohol abuse, and endocrine or autoimmune disorders. Clinically significant hepatotoxicity is rare except for bosutinib (Table 6.3). In the BFORE trial, all grade increase in ALT and AST was observed in 30.6% and 22.8% of patients by 1 year and grade 3–4 elevation occurred in 19% and 9.7% of cases, respectively [16]. It is recommended to monitor liver enzymes at least every 2 weeks during the first month of TKI treatment, monthly during month 2 and 3, and then every 1–3 months unless otherwise indicated. In case of grade 1–2 AST or ALT elevation, interruption or dose reduction of TKIs is not required. In case of grade 3–4 elevation, TKI treatment has to be interrupted until resolution and resumed at the same dose or at a reduced dose in case of recurrence. In case of true TKI-induced hepatitis, a change of TKI is mandatory. Caution should be exercised when using paracetamol in combination with TKIs since rare cases of fatal hepatic failure have been reported in imatinib-treated patients [40]. Bilirubin increase is most commonly described in nilotinib-treated patients. Upon first-line treatment with nilotinib at 300 mg BID in the ENESTnd trial, all grade and grade 3–4 hyperbilirubinemia occurred in 53% and 4% of the cases by 1 year, respectively

[12]. Generally, nilotinib treatment can be maintained as unconjugated bilirubin is involved due to inhibition of the uridine diphosphate glucuronosyltransferase (UGT1A1) activity and polymorphisms within the promoter of UGT1A1 gene [41, 42].

TKIs may also trigger dose-dependent pancreatic enzymes elevation, although generally not always indicative of pancreatitis. In ENESTnd, the cumulative incidence of pancreatitis by 5 years was 1.8% in the nilotinib 300 mg BID arm and 0.7% in the imatinib 400 mg QD arm, while grade 3–4 lipase increase was reported in 9% and 4.3% of each arm, respectively [13]. Special attention is required when ponatinib is used since pancreatitis was a dose-limiting toxic effect during the phase I evaluation trial of the drug [43]. During subsequent phase 2 study, 7% of patients with CP-CML treated at a dose of 45 mg QD developed acute pancreatitis by 1 year, among which 6% were severe or life threatening [17].

In case of grade 3–4 elevations in lipase in otherwise asymptomatic patients, it is recommended to interrupt TKI until resolution and to restart treatment at the previous dose or at a lower dosage. In case of concomitant acute abdominal pain, imaging must be performed in order to exclude or to support a diagnosis of acute pancreatitis. In case of confirmed diagnosis of grade 3 acute pancreatitis, a change in therapy is recommended for nilotinib-treated patients while in ponatinib-treated patients who may not have any other treatment option, interrupting ponatinib until resolution and restarting treatment at a lower dosage may be discussed. In case of recurrent pancreatitis or grade 4 pancreatitis, a change in therapy is mandatory (Table 6.3).

6.6 Musculoskeletal Symptoms

Musculoskeletal symptoms include cramps, myalgia, bone pain, and arthralgia. Nocturnal or exercise-associated muscle cramps predominating in the distal part of the limbs are commonly experienced by patients receiving imatinib and tend to be chronic. Bone pain and arthralgia generally decrease overtime. Cramps were reported by 38.3% of patients with CP-CML treated with imatinib at 400 mg QD in the IRIS trial but grade 3–4 events were infrequent [26]. Of note, creatine kinase increase is a common finding during imatinib treatment and imatinib is a rare but possible cause of rhabdomyolysis [44]. Cramps and myalgia are less problematic in patients treated with other TKIs.

Although musculoskeletal disorders associated with TKIs are usually of mild or moderate intensity, they may substantially impact on everyday life activities. These disorders being also one of the most common complaints in the general population; other possible diagnoses must be ruled out. Muscle cramps may be attenuated by calcium and magnesium supplementation as well as quinine or TKI dose reduction [22]. Pain relievers may be useful to control bone pain and arthralgia.

6.7 Fluid Retention

Dose-related fluid retention is extremely frequent in patients treated with imatinib. It usually manifests as weight gain, periorbital edema, or lower limb edema. Rarely, retinal edema, cerebral edema, joint effusions, and pleural or pericardial involvement have been described in patients treated with high doses of the drug [45–47]. All grade and grade 3–4 peripheral edema were reported in, respectively, 55.5% and 0.9% of imatinib-treated patients in the IRIS trial [26]. Fluid retention can also be observed in patients treated with other TKIs, although to a lesser extent.

Mild and isolated periorbital edema does not necessitate medical intervention. Whenever moderate or extensive, reduction in salt intake and diuretics may be beneficial. In severe cases, TKI treatment should be suspended until resolution and restarted at a reduced dose or changed. Caution is required in patients with a history of cardiac dysfunction or renal insufficiency and in case of concomitant treatment with other drugs involved in edema formation such as calcium channel blockers since in these, potentially life-threatening generalized fluid retention may occur.

6.8 Pulmonary Toxicity

Pulmonary toxicity is most frequently observed in patients treated with dasatinib, whose commonest risk of non-hematologic adverse events is represented by unilateral or bilateral pleural effusions, sometimes associated to lung parenchymal infiltrates and pericarditis. The risk of dasatinib-induced pleural effusion does not decrease overtime and mainly depends on age and dasatinib dose [48]. In patients with newly diagnosed CP-CML treated with dasatinib at 100 mg QD, the incidence of pleural effusions was 10% after 1 year, and 28% after 5 years [9, 10]. Bosutinib is also associated with a substantial risk of pleural effusion in patients with prior history of pleural effusion on dasatinib [49, 50]. The pathogenesis of dasatinib- or bosutinib-associated pleural effusion is unclear. In dasatinib-treated patients, pleural effusions consist in the vast majority of the cases in lymphocyte-predominant exudates with the presence of chyle, arguing against drug-induced fluid retention as an explanation of effusion formation and supporting the hypothesis of a dysregulation of the lymphatic network and of lymphocyte trafficking [51, 52].

The management of dasatinib-induced pleural effusions depends on their severity (Table 6.3). Symptomatic effusions require dasatinib interruption until spontaneous resolution followed by switch to another TKI or reintroduction with caution at a lower dose since recurrence is possible, especially in elderly patient populations. A short course of oral corticosteroids may be beneficial in the absence of rapid clinical improvement. Thoracocentesis may be necessary in severe cases.

A very rare but potentially fatal complication of dasatinib or bosutinib therapy is represented by precapillary pulmonary arterial hypertension (PAH) [53–55]. TKI-associated PAH may be difficult to prove since early symptoms such as fatigue, dyspnea, or chest pain are not very specific and diagnosis requires right-heart catheterization, an invasive procedure that is not systematically performed. PAH may be totally or partially reversible after dasatinib withdrawal but some patients require treatment with vasoactive drugs such as sildenafil [53, 56]. In case of dasatinib-associated PAH, dasatinib should be permanently discontinued (Table 6.3). No specific patient characteristics appear to be associated with an increased risk of developing PAH while receiving dasatinib [56].

6.9 Cardiac and Cardiovascular Toxicity

A wide array of cardiac and cardiovascular side effects has been described during TKI development and in the post-marketing setting. Unfortunately, these side effects are not always reversible upon drug discontinuation.

6.9.1 Arrhythmia and QT Interval Prolongation

All TKIs except imatinib have undergone preapproval analysis of their effects on electrocardiogram (ECG). All TKIs including imatinib have the potential to prolong the QT interval due to direct interactions with myocardial hERG channels. However, clinically symptomatic or asymptomatic but relevant QTc prolongation above 500 ms or more than 60 ms from baseline is uncommon at clinical doses, and there is no evidence of fatal ventricular tachyarrythmias. Nevertheless, it is strongly advised to correct any electrolyte imbalance that may aggravate the QTc prolongation potential of TKIs prior to therapy initiation and to monitor ECG at baseline and during therapy in case of clinical symptoms or arrhythmogen risk factors such as congenital long QT interval, underlying cardiac disease, concomitant treatment with strong CYP3A4 inhibitors, or QT interval prolonging medications.

6.9.2 Systemic Arterial Hypertension

Newly occurring or worsening hypertension is a common side effect of ponatinib at 45 mg QD, with an incidence of 9% by 12 months and 37%

by 5 years in patients with CP-CML failing prior TKIs, including 2% of severe or life-threatening cases [17, 18]. It is interesting to note that ponatinib is a potent inhibitor of VEGF-R family kinases and that hypertension is the most frequently observed toxicity of VEGF-R inhibitors [57]. Pretreatment assessment of blood pressure and early detection and management of ponatinib-associated hypertension are important together with avoidance of other medications known to raise blood pressure such as non-steroidal anti-inflammatory drugs or recombinant erythropoietin, since hypertension is a well-known predisposing factor for atherosclerosis, heart failure and cerebral hemorrhage. In case of hypertension during ponatinib treatment, renal artery stenosis has to be ruled out. Dose reduction of ponatinib may be beneficial to reduce the incidence and severity of hypertension [58]. In the hypothesis where hypertension relates to VEGF-R blockade, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers might be indicated (Table 6.3) [58].

6.9.3 Congestive Heart Failure

In 2006, the description of congestive heart failure in 10 patients treated with imatinib and experiments performed in mice led to the conclusion that ABL1 inhibition by TKIs was toxic for cardiomyocytes [59]. In fact, imatinib is a cause of ventricular dysfunction or congestive heart failure in less than 1% of patients, and these events are more likely to occur in elderly patients or with pre-existing myocardial diseases [60, 61]. Congestive heart failure is also a rare event in patients treated with newer-generation TKIs with a frequency likely ranging from <1% to 5% of patients. As a result, it is advisable to closely monitor patients with known cardiac disease, especially those with a decreased left ventricular function. Patients developing any warning signs of heart failure should consult rapidly to increase chances of successful management and avoid fatal outcome.

6.9.4 Arterial Occlusion

Ponatinib and nilotinib are associated with a substantial risk of arterial occlusion [13, 18]. The incidence of cardiac, cerebrovascular, or peripheral artery occlusion in patients treated with ponatinib at 45 mg QD in the PACE trial was 19% by 12 months and increased up to 31% by 5 years [17, 18]. Patients with classical major risk factors for CVD including older age, diabetes, or hypertension and those with prior history of ischemic disease were at particularly high risk of arterial occlusion [18]. In addition, an association between ponatinib dose intensity and the risk of arterial occlusive events was found [62]. Recommendations to minimize the risk of arterial occlusion in ponatinib-treated patients include a careful assessment of the cardiovascular status prior to ponatinib initiation, the set-up of adequate CVD prevention measures, and dose adjustment according to ponatinib efficacy [22, 58, 63]. It is also advised to avoid ponatinib in patients with prior history of myocardial infarction or stroke unless absolutely necessary, and to stop ponatinib in patients experiencing arterial occlusion (Table 6.3). Of course, other important factors need to be taken into account to allow the best decision making such as CML status and possibility for alternative therapy [63]. Whether dose-optimization strategies will further help in reducing the risk of arterial occlusive events is being investigated in the setting of the OPTIC randomized study (ClinicalTrials.gov Identifier: NCT02467270).

Nilotinib-associated increased risk of arterial occlusion was revealed late after first marketing authorization [64, 65]. The cumulative incidence of arterial occlusive events in the ENESTnd trial including ischemic heart disease, ischemic cerebrovascular events, and peripheral artery disease was 10.6% by 5 years and 24.8% by 10 years in patients treated with nilotinib at 300 mg BID and 17.9% by 5 years and 33.4% by 10 years in patients treated with nilotinib at 400 mg BID, indicating a dose-dependent toxicity [13, 14]. As observed with ponatinib, patients with several risk factors for CVD or underlying CVD are at

highest risk but events tend to occur later than in ponatinib-treated patients [13, 66]. A risk management plan to minimize the incidence and severity of arterial ischemic events in nilotinib-treated CML patients may include determination of the global cardiovascular risk, delivery of risk-adapted CVD prevention through healthy lifestyle behaviors instructions or medications like statins or aspirin, and rapid detection of clinical symptoms compatible with arterial stenosis or occlusion [22, 67]. Nilotinib should be stopped in case of arterial occlusion and switched in favor of an alternative TKI with a more favorable cardiovascular safety profile (Table 6.3) [22, 67].

6.10 Metabolic and Endocrine Side Effects

Effects of TKIs on bone mineral metabolism were first described during imatinib treatment in the form of early-onset and persistent mild to moderate hypophosphatemia in almost 50% of patients. Mild to moderate hypophosphatemia is also observed during treatment with nilotinib, dasatinib, bosutinib, and ponatinib. Hypophosphatemia is often accompanied by increased phosphaturia, low to normal levels of calcium, secondary hyperparathyroidism, and biphasic modifications of markers of bone formation and resorption [68, 69]. In imatinib-treated adult patients, bone mineral density remains stable or slightly increases overtime [69, 70]. Growth retardation has been described in prepubertal patients receiving TKIs, and this effect occurs through disturbances in the growth hormone/insulin-like growth factor-1 axis [71, 72].

Nilotinib has deleterious effects on glucose and lipid metabolism. Increase in fasting glucose levels is frequent during nilotinib therapy, especially in patients with pre-existing risk factors for type 2 diabetes [13]. A likely mechanism involves a decrease in plasma adiponectin levels, tissue insulin resistance, and hyperinsulinemia [73]. These effects warrant a careful evaluation of glucose metabolism before and during nilotinib therapy, as diabetic patients may need therapeutic adjustments (Table 6.3). Those with newly occurring prediabetes or diabetes require adequate glycemic control through weight reduction, exercise, and/or oral glucose-lowering agents (Table 6.3). Early onset LDL-cholesterol elevation is also frequently observed during nilotinib treatment [13, 74]. Therefore lipid disorders should be detected prior to and during nilotinib therapy. Given the key role of LDL-cholesterol in atherogenesis, lifestyle intervention or lipid-lowering agents may be necessary (Table 6.3). With imatinib, improvement in fasting blood glucose in diabetic patients, regression of type 2 diabetes, and spontaneous disappearance of hypercholesterolemia have been described [75, 76].

Abnormal thyroid function tests have been frequently observed during treatment with TKIs, although a causal relationship with TKI treatment is difficult to establish given the high frequency of thyroid diseases in the general population. In most cases, hypothyroidism or hyperthyroidism are subclinical and transient, and specific treatment is not necessary [77, 78]. Thyroidectomized patients receiving levothyroxine may show increased needs in hormone replacement therapy and must be carefully monitored [79].

6.11 Infections and Second Malignancies

All TKIs have the potential to inhibit cellular immunity in vitro but this effect is usually observed at higher concentrations than those measured in patients, and such experiments seem poor predictors of human reactions as well-controlled TKI-treated CP-CML patients are not immunocompromised. There has been no evidence of an increased incidence rate of TKI-related secondary malignancies in CP-CML patients [80]. However with dasatinib 100 mg QD in the frontline setting, the incidence of infections is slightly increased as compared to imatinib-treated patients [22, 81]. Of note, few cases of hepatitis B virus (HBV) reactivation sometimes leading to severe hepatic dysfunction or even fatal fulminant hepatitis have been described during imatinib, dasatinib,

and nilotinib treatment in chronically infected patients [82–84]. Although the exact frequency of such events is unknown, it is recommended to test all patients for HBV infection prior to TKI treatment initiation and to closely monitor liver function tests, serological markers, and viral burden in chronically infected patients in order to assess disease activity, candidacy for, or response to antiviral therapy. Regarding the new severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) responsible for the COVID-19 pandemic, some hypothesize that imatinib might be protective due to its antiviral properties against other coronaviruses such as SARS-CoV and MERS-CoV in vitro and its antiinflammatory action but to date, there are no strong clinical data supporting this hypothesis [85].

6.12 Other Side Effects

With TKIs, fatigue and headache are very frequent, the latter being managed with standard analgesics. Ocular side effects are mainly represented by benign conjunctival hemorrhages in imatinib-treated patients. Neurological side effects are rare and must be distinguished from other causes of neurological disorders. TKI-associated acute kidney injury is rare, but long-term increase of creatinine and decrease in the estimated glomerular filtration rate (eGFR) has been described in imatinib-treated patients [86]. The underlying mechanism of creatinine elevation and eGFR decline may be a functionally reversible inhibition of creatinine tubular secretion by imatinib and not a true drug-induced decrease of glomerular filtration [87]. Long-term increased creatinine has also been described in 13% of patients treated with bosutinib at 500 mg QD in clinical trials, and it is advised to avoid nephrotoxic agents and check for other causes of renal dysfunction [88, 89].

6.13 Conclusion

TKIs used to treat CML are associated with a wide range of adverse events. Most are mild to moderate and cease either spontaneously, upon symptomatic treatment, or after TKI dose decrease. Whenever temporary treatment interruptions are necessary, the same TKI may be reintroduced at the prior dose or at a lower dose as long as efficacy is maintained. In some cases, toxicity-driven treatment change may be necessary. Severe complications of therapy have emerged with new generation TKIs, such as cardiovascular and pulmonary toxicities. The risk of developing such complications is influenced by the type and dose of TKI in use and by personal factors. Thus individualized risk assessment integrating CML, TKI, and patient characteristics should guide treatment choices to ensure minimal harmful side effects and maximal successful outcome.

References

1. Sasaki K, Strom SS, O'Brien S, Jabbour E, Ravandi F, Konopleva M, Borthakur G, Pemmaraju N, Daver N, Jain P, Pierce S, Kantarjian H, Cortes JE. Relative survival in patients with chronic-phase chronic myeloid leukemia in the tyrosine kinase inhibitor era: analysis of patient data from six prospective clinical trials. Lancet Haematol. 2015;2:e189–93.
2. Bower H, Björkholm M, Dickman PW, Höglund M, Lambert PC, Andersson TM. Life expectancy of patients with chronic myeloid leukemia approaches the life expectancy of the general population. J Clin Oncol. 2016;34:2851–7.
3. Hochhaus A, Larson RA, Guilhot F, Radich JP, Branford S, Hughes TP, Baccarani M, Deininger MW, Cervantes F, Fujihara S, Ortmann CE, Menssen HD, Kantarjian H, O'Brien SG, Druker BJ, IRIS Investigators. Long-term outcomes of imatinib treatment for chronic myeloid leukemia. N Engl J Med. 2017;376:917–27.
4. Kalmanti L, Saussele S, Lauseker M, Müller MC, Dietz CT, Heinrich L, Hanfstein B, Proetel U, Fabarius A, Krause SW, Rinaldetti S, Dengler J, Falge C, Oppliger-Leibundgut E, Burchert A, Neubauer A, Kanz L, Stegelmann F, Pfreundschuh M, Spiekermann K, Scheid C, Pfirrmann M, Hochhaus A, Hasford J, Hehlmann R. Safety and efficacy of imatinib in CML over a period of 10 years: data from the randomized CML-study IV. Leukemia. 2015;29:1123–32.
5. Hehlmann R, Lauseker M, Saußele S, Pfirrmann M, Krause S, Kolb HJ, Neubauer A, Hossfeld DK, Nerl C, Gratwohl A, Baerlocher GM, Heim D, Brümmendorf TH, Fabarius A, Haferlach C, Schlegelberger B, Müller MC, Jeromin S, Proetel U, Kohlbrenner K, Voskanyan A, Rinaldetti S, Seifarth W, Spieß B, Balleisen L, Goebeler MC, Hänel M, Ho A, Dengler

J, Falge C, Kanz L, Kremers S, Burchert A, Kneba M, Stegelmann F, Köhne CA, Lindemann HW, Waller CF, Pfreundschuh M, Spiekermann K, Berdel WE, Müller L, Edinger M, Mayer J, Beelen DW, Bentz M, Link H, Hertenstein B, Fuchs R, Wernli M, Schlegel F, Schlag R, de Wit M, Trümper L, Hebart H, Hahn M, Thomalla J, Scheid C, Schafhausen P, Verbeek W, Eckart MJ, Gassmann W, Pezzutto A, Schenk M, Brossart P, Geer T, Bildat S, Schäfer E, Hochhaus A, Hasford J. Assessment of imatinib as first-line treatment of chronic myeloid leukemia: 10-year survival results of the randomized CML study IV and impact of non-CML determinants. Leukemia. 2017;31:2398–406.

6. Hochhaus A, Baccarani M, Silver RT, Schiffer C, Apperley JF, Cervantes F, Clark RE, Cortes JE, Deininger MW, Guilhot F, Hjorth-Hansen H, Hughes TP, Janssen JJWM, Kantarjian HM, Kim DW, Larson RA, Lipton JH, Mahon FX, Mayer J, Nicolini F, Niederwieser D, Pane F, Radich JP, Rea D, Richter J, Rosti G, Rousselot P, Saglio G, Saußele S, Soverini S, Steegmann JL, Turkina A, Zaritskey A, Hehlmann R. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020;34:966–84.
7. Rea D, Cayuela JM. Treatment-free remission in patients with chronic myeloid leukemia. Int J Hematol. 2018;108:355–64.
8. Shah NP, Kantarjian HM, Kim DW, Réa D, Dorlhiac-Llacer PE, Milone JH, Vela-Ojeda J, Silver RT, Khoury HJ, Charbonnier A, Khoroshko N, Paquette RL, Deininger M, Collins RH, Otero I, Hughes T, Bleickardt E, Strauss L, Francis S, Hochhaus A. Intermittent target inhibition with dasatinib 100 mg once daily preserves efficacy and improves tolerability in imatinib-resistant and -intolerant chronic-phase chronic myeloid leukemia. J Clin Oncol. 2008;26:3204–12.
9. Kantarjian H, Shah NP, Hochhaus A, Cortes J, Shah S, Ayala M, Moiraghi B, Shen Z, Mayer J, Pasquini R, Nakamae H, Huguet F, Boqué C, Chuah C, Bleickardt E, Bradley-Garelik MB, Zhu C, Szatrowski T, Shapiro D, Baccarani M. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2010;362:2260–70.
10. Cortes JE, Saglio G, Kantarjian HM, Baccarani M, Mayer J, Boqué C, Shah NP, Chuah C, Casanova L, Bradley-Garelik B, Manos G, Hochhaus A. Final 5-year study results of DASISION: The dasatinib versus imatinib study in treatment-naïve chronic myeloid leukemia patients trial. J Clin Oncol. 2016;34:2333–40.
11. Kantarjian HM, Giles FJ, Bhalla KN, Pinilla-Ibarz J, Larson RA, Gattermann N, Ottmann OG, Hochhaus A, Radich JP, Saglio G, Hughes TP, Martinelli G, Kim DW, Shou Y, Gallagher NJ, Blakesley R, Baccarani M, Cortes J, le Coutre PD. Nilotinib is effective in patients with chronic myeloid leukemia in chronic phase after imatinib resistance or intolerance: 24-month follow-up results. Blood. 2011;117:1141–5.
12. Saglio G, Kim DW, Issaragrisil S, le Coutre P, Etienne G, Lobo C, Pasquini R, Clark RE, Hochhaus A, Hughes TP, Gallagher N, Hoenekopp A, Dong M, Haque A, Larson RA, Kantarjian HM, ENESTnd Investigators. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med. 2010;362:2251–9.
13. Hochhaus A, Saglio G, Hughes TP, Larson RA, Kim DW, Issaragrisil S, le Coutre PD, Etienne G, Dorlhiac-Llacer PE, Clark RE, Flinn IW, Nakamae H, Donohue B, Deng W, Dalal D, Menssen HD, Kantarjian HM. Long-term benefits and risks of front-line nilotinib vs imatinib for chronic myeloid leukemia in chronic phase: 5-year update of the randomized ENESTnd trial. Leukemia. 2016;30:1044–54.
14. Hughes TP, Saglio G, Larson RA, Kantarjian HM, Kim D-W, Issaragrisil S, Le Coutre P, Etienne G, Boquimpani C, Clark RE, Dubruille V, Flinn IW, Kyrcz-Krzemien S, Medras E, Zanichelli M, Bendit I, Sondhi M, Titorenko K, Nourry-Boulot C, Aimone P, Hochhaus A. Long-term outcomes in patients with chronic myeloid leukemia in chronic phase receiving frontline nilotinib versus imatinib: Enestnd 10-year analysis. Blood. 2019;134(Supplement_1):2924.
15. Gambacorti-Passerini C, Cortes JE, Lipton JH, Kantarjian HM, Kim DW, Schafhausen P, Crescenzo R, Bardy-Bouxin N, Shapiro M, Noonan K, Leip E, DeAnnuntis L, Brümmendorf TH, Khoury HJ. Safety and efficacy of second-line bosutinib for chronic phase chronic myeloid leukemia over a five-year period: final results of a phase I/II study. Haematologica. 2018;103:1298–307.
16. Cortes JE, Gambacorti-Passerini C, Deininger MW, Mauro MJ, Chuah C, Kim DW, Dyagil I, Glushko N, Milojkovic D, le Coutre P, Garcia-Gutierrez V, Reilly L, Jeynes-Ellis A, Leip E, Bardy-Bouxin N, Hochhaus A, Brümmendorf TH. Bosutinib versus imatinib for newly diagnosed chronic myeloid leukemia: results from the randomized BFORE trial. J Clin Oncol. 2018;36:231–7.
17. Cortes JE, Kim DW, Pinilla-Ibarz J, le Coutre P, Paquette R, Chuah C, Nicolini FE, Apperley JF, Khoury HJ, Talpaz M, Di Persio J, DeAngelo DJ, Abruzzese E, Rea D, Baccarani M, Müller MC, Gambacorti-Passerini C, Wong S, Lustgarten S, Rivera VM, Clackson T, Turner CD, Haluska FG, Guilhot F, Deininger MW, Hochhaus A, Hughes T, Goldman JM, Shah NP, Kantarjian H, PACE Investigators. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. N Engl J Med. 2013;369:1783–96.
18. Cortes JE, Kim DW, Pinilla-Ibarz J, le Coutre PD, Paquette R, Chuah C, Nicolini FE, Apperley JF, Khoury HJ, Talpaz M, DeAngelo DJ, Abruzzese E, Rea D, Baccarani M, Müller MC, Gambacorti-Passerini C, Lustgarten S, Rivera VM, Haluska FG, Guilhot F, Deininger MW, Hochhaus A, Hughes TP, Shah NP, Kantarjian HM. Ponatinib efficacy and safety in Philadelphia chromosome-positive leukemia: final 5-year results of the phase 2 PACE trial. Blood. 2018;132:393–404.

19. Bantscheff M, Eberhard D, Abraham Y, Bastuck S, Boesche M, Hobson S, Mathieson T, Perrin J, Raida M, Rau C, Reader V, Sweetman G, Bauer A, Bouwmeester T, Hopf C, Kruse U, Neubauer G, Ramsden N, Rick J, Kuster B, Drewes G. Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. Nat Biotechnol. 2007;25:1035–44.
20. Remsing Rix LL, Rix U, Colinge J, Hantschel O, Bennett KL, Stranzl T, Müller A, Baumgartner C, Valent P, Augustin M, Till JH, Superti-Furga G. Global target profile of the kinase inhibitor bosutinib in primary chronic myeloid leukemia cells. Leukemia. 2009;23:477–85.
21. Huang WS, Metcalf CA, Sundaramoorthi R, Wang Y, Zou D, Thomas RM, Zhu X, Cai L, Wen D, Liu S, Romero J, Qi J, Chen I, Banda G, Lentini SP, Das S, Xu Q, Keats J, Wang F, Wardwell S, Ning Y, Snodgrass JT, Broudy MI, Russian K, Zhou T, Commodore L, Narasimhan NI, Mohemmad QK, Iuliucci J, Rivera VM, Dalgarno DC, Sawyer TK, Clackson T, Shakespeare WC. Discovery of 3-[2-(imidazo[1,2-b]pyridazin-3-yl)ethynyl]-4-methyl-N-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)phenyl}benzamide (AP24534), a potent, orally active pan-inhibitor of breakpoint cluster region-abelson (BCR-ABL) kinase including the T315I gatekeeper mutant. J Med Chem. 2010;53:4701–19.
22. Steegmann JL, Baccarani M, Breccia M, Casado LF, García-Gutiérrez V, Hochhaus A, Kim DW, Kim TD, Khoury HJ, Le Coutre P, Mayer J, Milojkovic D, Porkka K, Rea D, Rosti G, Saussele S, Hehlmann R, Clark RE. European LeukemiaNet recommendations for the management and avoidance of adverse events of treatment in chronic myeloid leukaemia. Leukemia. 2016;30:1648–71.
23. Rabian F, Lengline E, Rea D. Towards a personalized treatment of chronic myeloid leukemia. Curr Hematol Malig Rep. 2019;14:492–500.
24. Mustjoki S, Richter J, Barbany G, Ehrencrona H, Fioretos T, Gedde-Dahl T, Gjertsen BT, Hovland R, Hernesniemi S, Josefsen D, Koskenvesa P, Dybedal I, Markevärn B, Olofsson T, Olsson-Strömberg U, Rapakko K, Thunberg S, Stenke L, Simonsson B, Porkka K, Hjorth-Hansen H, Nordic CML Study Group (NCMLSG). Impact of malignant stem cell burden on therapy outcome in newly diagnosed chronic myeloid leukemia patients. Leukemia. 2013;27:1520–6.
25. Bumm T, Müller C, Al-Ali HK, Krohn K, Shepherd P, Schmidt E, Leiblein S, Franke C, Hennig E, Friedrich T, Krahl R, Niederwieser D, Deininger MW. Emergence of clonal cytogenetic abnormalities in Ph- cells in some CML patients in cytogenetic remission to imatinib but restoration of polyclonal hematopoiesis in the majority. Blood. 2003;101:1941–9.
26. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, Cornelissen JJ, Fischer T, Hochhaus A, Hughes T, Lechner K, Nielsen JL, Rousselot P, Reiffers J, Saglio G, Shepherd J, Simonsson B, Gratwohl A, Goldman JM, Kantarjian H, Taylor K, Verhoef G, Bolton AE, Capdeville R, Druker BJ. IRIS investigators. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2003;348:994–1004.
27. Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N, Deininger MW, Silver RT, Goldman JM, Stone RM, Cervantes F, Hochhaus A, Powell BL, Gabrilove JL, Rousselot P, Reiffers J, Cornelissen JJ, Hughes T, Agis H, Fischer T, Verhoef G, Shepherd J, Saglio G, Gratwohl A, Nielsen JL, Radich JP, Simonsson B, Taylor K, Baccarani M, So C, Letvak L, Larson RA, IRIS Investigators. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med. 2006;355:2408–17.
28. Sneed TB, Kantarjian HM, Talpaz M, O'Brien S, Rios MB, Bekele BN, Zhou X, Resta D, Wierda W, Faderl S, Giles F, Cortes JE. The significance of myelosuppression during therapy with imatinib mesylate in patients with chronic myelogenous leukemia in chronic phase. Cancer. 2004;100:116–21.
29. Quintas-Cardama A, Kantarjian H, O'Brien S, Garcia-Manero G, Rios MB, Talpaz M, Cortes J. Granulocyte-colony-stimulating factor (filgrastim) may overcome imatinib-induced neutropenia in patients with chronic-phase chronic myelogenous leukemia. Cancer. 2004;100:2592–7.
30. Cortes J, O'Brien S, Quintas A, Giles F, Shan J, Rios MB, Talpaz M, Kantarjian H. Erythropoietin is effective in improving the anemia induced by imatinib mesylate therapy in patients with chronic myeloid leukemia in chronic phase. Cancer. 2004;100:2396–402.
31. Shoukier M, Borthakur G, Jabbour E, Ravandi F, Garcia-Manero G, Kadia T, Matthews J, Masarova L, Naqvi K, Sasaki K, Verstovsek S, Cortes J. The effect of eltrombopag in managing thrombocytopenia associated with tyrosine kinase therapy in patients with chronic myeloid leukemia and myelofibrosis. https://doi.org/10.3324/haematol.2020.260125
32. Amitay-Maish I, Stemmer SM, Lacouture ME. Adverse cutaneous reactions secondary to tyrosine kinase inhibitors including imatinib mesylate, nilotinib and dasatinib. Dermatol Ther. 2011;24:386–95.
33. Brazzelli V, Grasso V, Borroni G. Imatinib, dasatinib and nilotinib: a review of adverse cutaneous reactions with emphasis on our clinical experience. J Eur Acad Dermatol Venereol. 2013;27:1471–80.
34. Patel AB, Solomon AR, Mauro MJ, Ehst BD. Unique cutaneous reaction to second- and third-generation tyrosine kinase inhibitors for chronic myeloid leukemia. Dermatology. 2016;232:122–5.
35. Tanaka C, Yin OQ, Sethuraman V, Smith T, Wang X, Grouss K, Kantarjian H, Giles F, Ottmann OG, Galitz L, Schran H. Clinical pharmacokinetics of the BCR-ABL tyrosine kinase inhibitor nilotinib. Clin Pharmacol Ther. 2010;87:197–203.
36. Quintás-Cardama A, Kantarjian H, Ravandi F, O'Brien S, Thomas D, Vidal-Senmache G, Wierda W,

Kornblau S, Cortes J. Bleeding diathesis in patients with chronic myelogenous leukemia receiving dasatinib therapy. Cancer. 2009;115:2482–90.
37. Patodi N, Sagar N, Rudzki Z, Langman G, Sharma N. Haemorrhagic colitis caused by dasatinib. Case Rep Hematol. 2012;2012:417106.
38. Quintás-Cardama A, Han X, Kantarjian H, Cortes J. Tyrosine kinase inhibitor-induced platelet dysfunction in patients with chronic myeloid leukemia. Blood. 2009;114:261–3.
39. Ong J, Yeung D, Filshie R, Hughes TP, Nandurkar H. Imatinib-induced gastric antral vascular ectasia in three patients with chronic myeloid leukaemia. Int J Hematol. 2015;102:639–42.
40. Deininger MW, O'Brien SG, Ford JM, Druker BJ. Practical management of patients with chronic myeloid leukemia receiving imatinib. J Clin Oncol. 2003;21:1637–47.
41. Singer JB, Shou Y, Giles F, Kantarjian HM, Hsu Y, Robeva AS, Rae P, Weitzman A, Meyer JM, Dugan M, Ottmann OG. UGT1A1 promoter polymorphism increases risk of nilotinib-induced hyperbilirubinemia. Leukemia. 2007;21:2311–5.
42. Rosti G, Castagnetti F, Gugliotta G, Palandri F, Baccarani M. Physician's guide to the clinical management of adverse events on nilotinib therapy for the treatment of CML. Cancer Treat Rev. 2012;38:241–8.
43. Cortes JE, Kantarjian H, Shah NP, Bixby D, Mauro MJ, Flinn I, O'Hare T, Hu S, Narasimhan NI, Rivera VM, Clackson T, Turner CD, Haluska FG, Druker BJ, Deininger MW, Talpaz M. Ponatinib in refractory Philadelphia chromosome-positive leukemias. N Engl J Med. 2012;367:2075–88.
44. Hohenegger M. Drug induced rhabdomyolysis. Curr Opin Pharmacol. 2012;12:335–9.
45. Breccia M, D'Elia GM, D'Andrea M, Latagliata R, Alimena G. Pleural-pericardic effusion as uncommon complication in CML patients treated with Imatinib. Eur J Haematol. 2005;74:89–90.
46. Esmaeli B, Prieto VG, Butler CE, Kim SK, Ahmadi MA, Kantarjian HM, Talpaz M. Severe periorbital edema secondary to STI571 (Gleevec). Cancer. 2002;95:881–7.
47. Moore JC, Dennehey CF, Anavim A, Kong KM, Tiong OS. Multiple joint effusions associated with high-dose imatinib therapy in a patient with chronic myelogenous leukaemia. Eur J Haematol. 2006;76:444–6.
48. Hughes TP, Laneuville P, Rousselot P, Snyder DS, Rea D, Shah NP, Paar D, Abruzzese E, Hochhaus A, Lipton JH, Cortes JE. Incidence, outcomes, and risk factors of pleural effusion in patients receiving dasatinib therapy for Philadelphia chromosome-positive leukemia. Haematologica. 2019;104:93–101.
49. Kantarjian HM, Cortes JE, Kim DW, Khoury HJ, Brümmendorf TH, Porkka K, Martinelli G, Durrant S, Leip E, Kelly V, Turnbull K, Besson N, Gambacorti-Passerini C. Bosutinib safety and management of toxicity in leukemia patients with resistance or intolerance to imatinib and other tyrosine kinase inhibitors. Blood. 2014;123:1309–18.
50. Khoury HJ, Cortes JE, Kantarjian HM, Gambacorti-Passerini C, Baccarani M, Kim DW, Zaritskey A, Countouriotis A, Besson N, Leip E, Kelly V, Brümmendorf TH. Bosutinib is active in chronic phase chronic myeloid leukemia after imatinib and dasatinib and/or nilotinib therapy failure. Blood. 2012;119:3403–12.
51. Bergeron A, Réa D, Levy V, Picard C, Meignin V, Tamburini J, Bruzzoni-Giovanelli H, Calvo F, Tazi A, Rousselot P. Lung abnormalities after dasatinib treatment for chronic myeloid leukemia: a case series. Am J Respir Crit Care Med. 2007;176:814–8.
52. Goldblatt M, Huggins JT, Doelken P, Gurung P, Sahn SA. Dasatinib-induced pleural effusions: a lymphatic network disorder? Am J Med Sci. 2009;338:414–7.
53. Montani D, Bergot E, Günther S, Savale L, Bergeron A, Bourdin A, Bouvaist H, Canuet M, Pison C, Macro M, Poubeau P, Girerd B, Natali D, Guignabert C, Perros F, O'Callaghan DS, Jaïs X, Tubert-Bitter P, Zalcman G, Sitbon O, Simonneau G, Humbert M. Pulmonary arterial hypertension in patients treated by dasatinib. Circulation. 2012;125:2128–37.
54. Montani D, Seferian A, Savale L, Simonneau G, Humbert M. Drug-induced pulmonary arterial hypertension: a recent outbreak. Eur Respir Rev. 2013;22:244–50.
55. Riou M, Seferian A, Savale L, Chaumais MC, Guignabert C, Canuet M, Magro P, Rea D, Sitbon O, Jaïs X, Humbert M, Montani D. Deterioration of pulmonary hypertension and pleural effusion with bosutinib following dasatinib lung toxicity. Eur Respir J. 2016;48:1517–9.
56. Shah NP, Wallis N, Farber HW, Mauro MJ, Wolf RA, Mattei D, Guha M, Rea D, Peacock A. Clinical features of pulmonary arterial hypertension in patients receiving dasatinib. Am J Hematol. 2015;90:1060–4.
57. des Guetz G, Uzzan B, Chouahnia K, Morère JF. Cardiovascular toxicity of anti-angiogenic drugs. Target Oncol. 2011;6:197–202.
58. Saussele S, Haverkamp W, Lang F, Koschmieder S, Kiani A, Jentsch-Ullrich K, Stegelmann F, Pfeifer H, La Rosée P, Goekbuget N, Rieger C, Waller CF, Franke GN, le Coutre P, Kirchmair R, Junghanss C. Ponatinib in the treatment of chronic myeloid leukemia and Philadelphia chromosome-positive acute leukemia: recommendations of a German expert consensus panel with focus on cardiovascular management. Acta Haematol. 2020;143:217–31.
59. Kerkelä R, Grazette L, Yacobi R, Iliescu C, Patten R, Beahm C, Walters B, Shevtsov S, Pesant S, Clubb FJ, Rosenzweig A, Salomon RN, Van Etten RA, Alroy J, Durand JB, Force T. Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. Nat Med. 2006;12:908–16.
60. Breccia M, Cannella L, Frustaci A, Stefanizzi C, Levi A, Alimena G. Cardiac events in imatinib mesylate-treated chronic myeloid leukemia patients: a single institution experience. Leuk Res. 2008;32:835–6.

61. Atallah E, Durand JB, Kantarjian H, Cortes J. Congestive heart failure is a rare event in patients receiving imatinib therapy. Blood. 2007;110:1233–7.
62. Dorer DJ, Knickerbocker RK, Baccarani M, Cortes JE, Hochhaus A, Talpaz M, Haluska FG. Impact of dose intensity of ponatinib on selected adverse events: multivariate analyses from a pooled population of clinical trial patients. Leuk Res. 2016;48:84–91.
63. Hochhaus A, Breccia M, Saglio G, García-Gutiérrez V, Réa D, Janssen J, Apperley J. Expert opinion-management of chronic myeloid leukemia after resistance to second-generation tyrosine kinase inhibitors. Leukemia. 2020;34:1495–502.
64. Aichberger KJ, Herndlhofer S, Schernthaner GH, Schillinger M, Mitterbauer-Hohendanner G, Sillaber C, Valent P. Progressive peripheral arterial occlusive disease and other vascular events during nilotinib therapy in CML. Am J Hematol. 2011;86:533–9.
65. Le Coutre P, Rea D, Abruzzese E, Dombret H, Trawinska MM, Herndlhofer S, Dörken B, Valent P. Severe peripheral arterial disease during nilotinib therapy. J Natl Cancer Inst. 2011;103:1347–8.
66. Rea D, Mirault T, Raffoux E, Boissel N, Andreoli AL, Rousselot P, Dombret H, Messas E. Usefulness of the 2012 European CVD risk assessment model to identify patients at high risk of cardiovascular events during nilotinib therapy in chronic myeloid leukemia. Leukemia. 2015;29:1206–9.
67. Rea D, Ame S, Charbonnier A, Coiteux V, Cony-Makhoul P, Escoffre-Barbe M, Etienne G, Gardembas M, Guerci-Bresler A, Legros L, Nicolini F, Tulliez M, Hermet E, Huguet F, Johnson-Ansah H, Lapusan S, Quittet P, Rousselot P, Mahon FX, Messas E. Management of the cardiovascular disease risk during nilotinib treatment in chronic myeloid leukemia: 2015 recommendations from the France Intergroupe des Leucémies Myéloïdes Chroniques. Bull Cancer. 2016;103:180–9.
68. Berman E, Nicolaides M, Maki RG, Fleisher M, Chanel S, Scheu K, Wilson BA, Heller G, Sauter NP. Altered bone and mineral metabolism in patients receiving imatinib mesylate. N Engl J Med. 2006;354:2006–13.
69. O'Sullivan S, Horne A, Wattie D, Porteous F, Callon K, Gamble G, Ebeling P, Browett P, Grey A. Decreased bone turnover despite persistent secondary hyperparathyroidism during prolonged treatment with imatinib. J Clin Endocrinol Metab. 2009;94:1131–6.
70. Alemán JO, Farooki A, Girotra M. Effects of tyrosine kinase inhibition on bone metabolism: untargeted consequences of targeted therapies. Endocr Relat Cancer. 2014;21:R247–59.
71. Narayanan KR, Bansal D, Walia R, Sachdeva N, Bhansali A, Varma N, Marwaha RK. Growth failure in children with chronic myeloid leukemia receiving imatinib is due to disruption of GH/IGF-1 axis. Pediatr Blood Cancer. 2013;60:1148–53.
72. Hijiya N, Suttorp M. How I treat chronic myeloid leukemia in children and adolescents. Blood. 2019;133:2374–84.
73. Racil Z, Koritakova E, Sacha T, Klamova H, Belohlavkova P, Faber E, Rea D, Malaskova L, Prochazkova J, Zackova D, Voglova J, Wącław J, Cetkovsky P, Zak P, Mayer J. Insulin resistance is an underlying mechanism of impaired glucose metabolism during nilotinib therapy. Am J Hematol. 2018;93:E342–5.
74. Rea D, Mirault T, Cluzeau T, Gautier JF, Guilhot F, Dombret H, Messas E. Early onset hypercholesterolemia induced by the 2nd-generation tyrosine kinase inhibitor nilotinib in patients with chronic phase-chronic myeloid leukemia. Haematologica. 2014;99:1197–203.
75. Veneri D, Franchini M, Bonora E. Imatinib and regression of type 2 diabetes. N Engl J Med. 2005;352:1049–50.
76. Gottardi M, Manzato E, Gherlinzoni F. Imatinib and hyperlipidemia. N Engl J Med. 2005;353:2722–3.
77. Kim TD, Schwarz M, Nogai H, Grille P, Westermann J, Plöckinger U, Braun D, Schweizer U, Arnold R, Dörken B, le Coutre P. Thyroid dysfunction caused by second-generation tyrosine kinase inhibitors in Philadelphia chromosome-positive chronic myeloid leukemia. Thyroid. 2010;20:1209–14.
78. Samis J, Lee P, Zimmerman D, Arceci RJ, Suttorp M, Hijiya N. Recognizing Endocrinopathies associated with tyrosine kinase inhibitor therapy in children with chronic myelogenous leukemia. Pediatr Blood Cancer. 2016;63:1332–8.
79. de Groot JW, Zonnenberg BA, Plukker JT, van Der Graaf WT, Links TP. Imatinib induces hypothyroidism in patients receiving levothyroxine. Clin Pharmacol Ther. 2005;78:433–8.
80. Miranda MB, Lauseker M, Kraus M-P, Proetel U, Hanfstein B, Fabarius A, Baerlocher GM, Heim D, Hossfeld DK, Kolb H-J, Krause SW, Nerl C, Brümmendorf TH, Verbeek W, Fauser AA, Prümmer O, Neben K, Hess U, Mahlberg R, Plöger C, Flasshove M, Rendenbach B, Hofmann W-K, Müller MC, Pfirrmann M, Hochhaus A, Hasford J, Hehlmann R, Saußele S. Secondary malignancies in chronic myeloid leukemia patients after imatinib-based treatment: long-term observation in CML study IV. Leukemia. 2016;30:1255–62.
81. Kantarjian HM, Shah NP, Cortes JE, Baccarani M, Agarwal MB, Undurraga MS, Wang J, Ipiña JJ, Kim DW, Ogura M, Pavlovsky C, Junghanss C, Milone JH, Nicolini FE, Robak T, Van Droogenbroeck J, Vellenga E, Bradley-Garelik MB, Zhu C, Hochhaus A. Dasatinib or imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: 2-year follow-up from a randomized phase 3 trial (DASISION). Blood. 2012;119:1123–9.
82. Ando T, Kojima K, Isoda H, Eguchi Y, Honda T, Ishigami M, Kimura S. Reactivation of resolved infection with the hepatitis B virus immune escape mutant G145R during dasatinib treatment for chronic myeloid leukemia. Int J Hematol. 2015;102:379–82.
83. Ikeda K, Shiga Y, Takahashi A, Kai T, Kimura H, Takeyama K, Noji H, Ogawa K, Nakamura A, Ohira

H, Sato Y, Maruyama Y. Fatal hepatitis B virus reactivation in a chronic myeloid leukemia patient during imatinib mesylate treatment. Leuk Lymphoma. 2006;47:155–7.

84. Lai G-M, Yan S-L, Chang C-S, Tsai C-Y. Hepatitis B reactivation in chronic myeloid leukemia patients receiving tyrosine kinase inhibitor. World J Gastroenterol. 2013;19:1318–21.
85. Bernal-Bello D, Jaenes-Barrios B, Morales-Ortega A, Ruiz-Giardin JM, García-Bermúdez V, Frutos-Pérez B, Farfán-Sedano AI, de Ancos-Aracil C, Bermejo F, García-Gil M, Zapatero-Gaviria A, San Martín-López JV. Imatinib might constitute a treatment option for lung involvement in COVID-19. Autoimmun Rev. 2020;19:102565.
86. Marcolino MS, Boersma E, Clementino NC, Macedo AV, Marx-Neto AD, Silva MH, van Gelder T, Akkerhuis KM, Ribeiro AL. Imatinib treatment duration is related to decreased estimated glomerular filtration rate in chronic myeloid leukemia patients. Ann Oncol. 2011;22:2073–9.
87. Vidal-Petiot E, Rea D, Serrano F, Stehlé T, Gardin C, Rousselot P, Peraldi M-N, Flamant M. Imatinib increases serum creatinine by inhibiting its tubular secretion in a reversible fashion in chronic myeloid leukemia. Clin Lymphoma Myeloma Leuk. 2016;16:169–74.
88. Cortes JE, Gambacorti-Passerini C, Kim DW, Kantarjian HM, Lipton JH, Lahoti A, Talpaz M, Matczak E, Barry E, Leip E, Brümmendorf TH, Khoury HJ. Effects of Bosutinib Treatment on Renal Function in Patients with Philadelphia Chromosome-Positive Leukemias. Clin Lymphoma Myeloma Leuk. 2017;17:684–95.
89. Cortes JE, Apperley JF, DeAngelo DJ, Deininger MW, Kota VK, Rousselot P, Gambacorti-Passerini C. Management of adverse events associated with bosutinib treatment of chronic-phase chronic myeloid leukemia: expert panel review. J Hematol Oncol. 2018;11:143.

7 Pharmacoeconomic Considerations for Tyrosine Kinase Inhibitors in the Treatment of Chronic Myeloid Leukemia

Benyam Muluneh, Rena M. Conti, Joehl Nguyen, Ashley Cole, Richard A. Larson, and Stacie B. Dusetzina

7.1 Introduction

The introduction and use of the tyrosine kinase inhibitor (TKI) drugs have markedly improved the overall survival and quality of life for patients with chronic myeloid leukemia (CML). There are currently five branded TKIs approved in the United States (US) and European Union (EU) for CML treatment, and one generic option (Gleevec's generic, imatinib). Imatinib, a first-generation TKI, was the first treatment approved for CML by the European Medicines Agency and the Food and Drug Administration in 2001. Second-generation TKIs dasatinib, nilotinib, and bosutinib have since been approved in the US and EU for both patients who are resistant or intolerant to prior therapy (in 2006 and 2007) as well as those with newly diagnosed disease. Ponatinib is a highly active but more toxic third-generation TKI approved in 2012 as a last option or for patients with T315I-mutated CML patients (Table 7.1). These TKIs are very effective as they inhibit the pathogenic mutation thereby inducing remission and significantly prolonged life expectancy of these patients.

Internationally, many countries have added CML TKIs to their national formularies; however, despite their clinical benefits for patients, affordability of TKIs remains a significant concern in the US [1]. Costs to payers and patients in the US far exceed costs in other high-income countries largely due to the absence of a nationalized healthcare system with concentrated bargaining power [1]. In the US, branded second-generation TKIs will remain on patent until at least mid-2023, with wholesale acquisition prices of these products exceeding $14,000 per 30 day supply in 2020 (Table 7.2). While Gleevec's generic (imatinib) has reduced costs to health plans, it remains expensive for many patients in the US [2]. In addition, a large proportion of newly treated patients in the US initiate treatment with second-generation drugs, limiting cost savings that typically come with generic drug use [3]. In contrast, in Europe most

B. Muluneh (✉)
Division of Pharmacotherapy and Experimental Therapeutics, University of North Carolina Eshelman School of Pharmacy, Chapel Hill, NC, USA
e-mail: bmuluneh@unc.edu

R. M. Conti
Questrom School of Business, Boston University, Boston, MA, USA

J. Nguyen · A. Cole
Division of Pharmaceutical Outcomes and Policy, University of North Carolina Eshelman School of Pharmacy, Chapel Hill, NC, USA

R. A. Larson
Department of Medicine and the Comprehensive Cancer Center, University of Chicago, Chicago, IL, USA

S. B. Dusetzina
Department of Health Policy and Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN, USA

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
R. Hehlmann (ed.), *Chronic Myeloid Leukemia*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-71913-5_7

Table 7.1 Original approval and patent expiration dates for CML TKIs in the US and the European Union [56–58]

Drug[a]	TKI generation	Manufacturer	Approval		Patent expiration	
			EMA	FDA	EU	USA
Gleevec (imatinib)	First	Novartis	11/2001	05/2001	12/2016	07/2015
Tasigna (nilotinib)	Second	Novartis	11/2007	10/2007	07/2023	07/2023
Sprycel (dasatinib)	Second	Bristol-Myers Squibb	11/2006	06/2006	04/2020	10/2025
Bosulif (bosutinib)	Second	Pfizer	03/2013	09/2012	09/2024	11/2026
Iclusig (ponatinib)	Third	Takeda	07/2013	12/2012	06/2028	12/2026

[a]All 30 tabs unless otherwise noted

Table 7.2 Current U.S. pricing for tyrosine kinase inhibitors used for chronic myeloid leukemia

Drug	Manufacturer	Wholesale acquisition cost (WAC)	Average wholesale price per package[a]	Average wholesale price per unit
Gleevec (400 mg)	Novartis	$10,122	$12,147	$405
Generic imatinib (400 mg)	Apotex Corp	$575	$10,932	$364
	Areva Pharmaceuticals	$302	$10,942	$365
	Armas Pharmaceuticals	$130	$10,932	$364
	Ascend Laboratories	$302	$10,919	$364
	Celltrion	$110	$132	$4
	Dr. Reddy's Laboratories	$410	$10,932	$364
	Hikma Pharmaceuticals USA	$1368	$10,932	$364
	Lupin Pharmaceuticals	$547	$10,932	$364
	Major Pharmaceuticals	$4631	$5558	$185
	Mylan Institutional	$3800	$4,56	$152
	Mylan Pharmaceuticals	$369	$442	$15
	Northstar Rx	$3480	$11,255	$375
	Sun Pharmaceuticals	$302	$11,840	$395
	Teva Pharmaceuticals	$437	$10,932	$364
Tasigna (150 mg)	Novartis	$14,716	$17,659 (112 caps)	$158
Tasigna (200 mg)	Novartis	$14,716	$17,659 (112 caps)	$158
Sprycel (100 mg)	Bristol-Myers Squibb	$14,508	$17,410	$580
Sprycel (140 mg)	Bristol-Myers Squibb	$14,508	$17,410	$580
Bosulif (400 mg)	Pfizer	$15,673	$18,808	$627
Bosulif (500 mg)	Pfizer	$15,673	$18,808	$627
Iclusig (15 mg)	Takeda	$16,561	$19,873	$662
Iclusig (45 mg)	Takeda	$16,561	$19,873	$662

[a]All 30 tabs unless otherwise noted

nationalized plans prefer imatinib over second-generation TKIs. In the United Kingdom, for example, the National Institute for Health and Care Excellence (NICE) recommend using dasatinib and nilotinib only if imatinib cannot be used or for imatinib-resistant disease [4]. As of 2019, NICE has yet to make a recommendation regarding bosutinib, and it reserves ponatinib as a last line treatment or in patients with T315I-mutated CML, similar to most health plans [5, 6].

Policies encouraging generic competition with branded prescription drugs are intended to

improve the affordability of prescription drugs for patients and payers. In the US and in the EU loss of patent exclusivity in the US opens the branded drug to potential competition from multiple generic manufacturers [7]. Research shows that after loss of patent exclusivity, prices for oral drugs initially fall quickly and steadily as additional generic manufacturers enter the market. However, preferences for the use of generic drug vary by country [8]. In addition, the timing of patent expiration and generic entry for specific members of the TKI therapeutic class also varies between countries. For example, there were at least 3 years between Gleevec's generic availability in Canada and the United States. In April 2013, Health Canada approved two generic formulations of imatinib. In comparison, in the US imatinib first became available to consumers in early 2016. The delayed entry was partly due to "pay-for-delay" schemes. Under these arrangements brand-name drug manufacturers typically pay the generic drug manufacturer to delay market entry, despite having regulatory approval to sell their product in the United States. Dasatinib, nilotinib, bosutinib, and ponatinib's patents will all expire in the EU and US within the coming decade (Table 7.2) [9]. There are several generic dasatinib applications that are under review by the European Medicine's Agency (EMA) [10], and several companies in India and South America are already manufacturing and marketing generic dasatinib within different markets [11].

7.2 System Affordability and Comparative Cost Analysis

7.2.1 Generic Imatinib Vs. Second-Generation TKIs in Europe

Because of the current differences in price between generic and brand-name TKIs, the mix of product use in a country is an important factor in understanding spending on CML. Specifically, the selection of first-line treatment is an important consideration as indications for second-generation drugs changed over time to also include use without prior treatment with Gleevec.

In Europe, use of imatinib compared to second-generation TKIs as first-line therapy is variable. Contemporary data are lacking on generic imatinib uptake following patent expiry. In the European arm of the SIMPLICTY observational study, a registry of patients with CML seen in routine clinical practice, 46% of patients initiated imatinib as first-line therapy compared to 54% who initiated dasatinib or nilotinib based on data between 2010 and 2016 [12]. However, in the Dutch national CML registry cohort, patients newly diagnosed between 2008 and 2013 were more likely to be treated with imatinib (77%) compared to nilotinib (17%) or dasatinib (6%) [13]. In a more recent study, patients diagnosed with CML between 2016 and 2018 in the five major European markets had higher rates of imatinib as the preferred first-line therapy compared to second-generation TKIs. The proportion of patients using a first-generation TKI across all five markets was 62% and ranged from 41% of patients in Germany to 80% of patients in France [14]. In this study, second-generation TKIs were overwhelmingly used among patients for second-line (88%) and third-line therapy (64%) [14]. As generic entry may have improved access to treatment for patients with CML due to lower costs, imatinib uptake could increase over time in Europe.

The annual cost of TKI treatment in Europe also varies across individual countries. Although the TKI-based treatment price has increased substantially above inflation since it was first introduced, the cost of treatment with branded imatinib has been generally lower compared to second-generation TKIs, even prior to generic availability [15–17]. Annual estimates in 2013 ranged from 31,000 USD in Italy to 54,500 USD in Germany for imatinib compared to up to 90,000 USD for second-generation TKIs [15]. In France, annual costs for treatment with imatinib, nilotinib, and dasatinib were around 40,000 USD, 51,500 USD, and 71,000 USD, respectively [15]. In an analysis of insurance claims data in Germany between 2012 and 2014, the annual median costs were reported to be around an

equivalent 80,000 USD for imatinib compared to 82,000 USD for nilotinib and 110,000 USD for dasatinib [16]. TKI treatment accounts for 60–70% of the total annual direct healthcare costs of care in CML [16]. After generic entry of imatinib, average costs decreased in Europe but variability in cost increased. In Austria, the price of generic imatinib was 52% of the branded product's price, while in Latvia, generic imatinib costs decreased by 96% following patent expiry [17, 18]. This amounts to an annual cost for imatinib treatment ranging from around 1500 to 19,000 USD compared to approximately 38,000 USD prior to generic entry [17, 18]. More recently, Shih et al. estimated that the average annual cost of treatment with generic imatinib was an equivalent of 4000 USD compared to 40,000 USD for second-generation TKIs, further reflecting the significant reduction in prices for generic imatinib in European markets [19].

The most recent treatment recommendations from the European LeukemiaNet in 2020 affirm that imatinib is a cost-effective treatment for chronic phase CML [20]. Evidence on the comparative cost-effectiveness of imatinib relative to second-generation TKIs in Europe vary depending on the year, country, model approach, and model assumptions. In particular, models estimating the impact of generic imatinib demonstrated that a first-line strategy with imatinib is more economically attractive given the current pricing of second-generation TKIs [17, 21]. An Austrian cost-effectiveness model concluded that imatinib followed by second-line nilotinib was the more cost-effective strategy compared to sequential treatment with nilotinib or dasatinib first in the context of the Austrian healthcare reimbursement system [17]. In a scenario analysis projecting a 52% decrease in generic imatinib costs, imatinib as first-line therapy followed by nilotinib after intolerance or failure remained the dominant or more cost-effective strategy compared to strategies starting with a second-generation TKI. Specifically, this study found that the incremental cost effectiveness ratio (a ratio that compares the difference in price as a function of the difference in clinical outcomes) was 125,400 EUR per quality-adjusted life year [17]. Generally, the threshold for deeming a product as cost-effective in European countries has been lower (around 20,000–30,000 EUR per quality-adjusted life year) [22]. More recently, a cost-effectiveness modeling study on imatinib as first-line therapy in the Netherlands found that imatinib had become increasingly more cost-effective over a 16-year period when accounting for increases in branded pricing, discounted outcomes, changes in willingness-to-pay, and patent expiry in 2016 [21].

7.2.2 Generic Imatinib Vs. Second-Generation TKIs in the US

After generic imatinib entered the U.S. market in 2016, prices for payers remained high over the first 2 years of generic availability. In one study of commercially insured patients, prices declined by only 8% and 10% compared to branded imatinib in 2016 and 2017, respectively [23]. Other estimates projected a 30% decrease in pricing based on data from other initial generic competitors [24]. This is in contrast to expectations of savings of 70–90% with generic entry, which has historically been achieved with small molecule drugs in the US [7]. More recently, as additional generic manufacturers entered the market, the price has continued to decline (Table 7.2).

Over time, generic imatinib uptake has increased in the US, accounting for 58% of all imatinib prescription fills in February 2017 and 74% of fills in September 2017 [23]. Some of the increased use of generic imatinib is due to expected brand-to-generic switching among patients already using Gleevec. For example, in one study 68% and 74% of commercially insured and Medicaid-insured patients, respectively, received at least one 30-day supply of generic imatinib after previously receiving the branded product [25]. In that study, generic availability was associated with decreases in the monthly health plan expenditures for imatinib in both the commercially insured and Medicaid samples.

While uptake of generic imatinib is encouraging as it could lead to substantial cost savings, there has also been a shift in the use of

first- versus branded second-generation products (dasatinib, nilotinib, and bosutinib) in the US over time, which may limit potential cost savings for payers. For example, first-line use of second-generation TKIs (nilotinib and dasatinib) increased from less than 10% of new TKI initiations in 2007 to over 60% in 2017 [23].

7.2.3 Strategies to Reduce Expenditures

Overall, imatinib is currently the most economically attractive treatment option among the TKI therapeutic class due to a combination of its favorable price, long-term safety profile, and lower overall healthcare costs. As patent expiries for second-generation TKIs are expected in 2023–2025 (U.S. Securities and Exchange Commission) and more generics become available, the focus of clinical decision making may shift to risk factors, adverse events, and safety.

In the US, strategies intended to reduce expenditures for payers may impact patient outcomes as well as system affordability of TKIs for the treatment of CML. For patients with resistance or who develop intolerance to imatinib, health plan benefits related to second-generation TKI access have important cost implications. In a decision analytic modeling study, health plans with open access to either second-generation TKI compared to restricted access to only one second-generation TKI, i.e., either only dasatinib or only nilotinib, were associated with improved clinical response outcomes when accounting for genetic variations with differential sensitivities to therapy. This study found that an open access benefit design resulted in lower annual drug costs per drug response compared to restricted access. The annual drug costs for an open access design was 120,700 USD per complete hematologic response and 198,300 USD per major cytogenetic response [26]. In the restricted access design with access to only one second-generation TKI, costs were 5–6% and 22–41% higher for dasatinib only and nilotinib only strategies, respectively [26].

In another pharmacoeconomic evaluation, investigators compared TKI treatment costs over a six-month period under an alternative payment model that encouraged generic substitution of branded medications (the Oncology Care Model developed by the Centers for Medicare and Medicaid Services in the US). Under this model, generic substitution with imatinib was associated with a 38,000 USD decrease in TKI treatment costs for both newly diagnosed and prevalent patients [27]. Approximately 67% of total savings under the model were attributed to generic substitution of branded imatinib and 33% attributed to switching from second-generation TKIs to generic imatinib [27].

Similar to studies in Europe, economic evaluations within the United States with various market and clinical assumptions have concluded that generic imatinib as a first-line strategy is cost-effective compared to other strategies. In 2016, Padula et al. showed that imatinib was the preferred and cost-effective strategy over a physician's choice strategy in chronic phase CML over a 5-year time horizon under the assumption that generic imatinib prices would be reduced by up to 90% relative to the brand's price [7]. In this model, the total annual costs of CML for an imatinib-first strategy were lower than a physician's choice strategy even with a slight higher quality of life-year improvement for the physician's choice strategy. The incremental cost-effectiveness ratio was approximately 70,000 USD/quality adjusted life year compared to 92,000 USD/quality adjusted life year for a physician's choice strategy [7]. In probabilistic sensitivity analyses, this study found that imatinib was cost-effective in 99.7% of simulations when accounting for uncertainty across multiple model parameters [7].

Another recent decision analytic model compared treatment strategies of initiating either imatinib, dasatinib, or nilotinib as first-line therapy and incorporated real-world costs to account for adverse events and treatment patterns such as switching to second-generation TKIs. This study showed that under Federal Supply Schedule prices, a generic imatinib first strategy costs 55,000 USD in the first year, inclusive of real-world switching patterns and all medical expenditures [3, 28]. The imatinib first strategy was also associated with

fewer hospitalizations or emergency department visits, but more switching to a second-line TKI and more cases of allogeneic hematopoietic stem cell transplantation [28]. Given the small differences in survival between the three TKIs under study, the imatinib-first strategy dominated all other alternatives. Other recent economic models have similarly concluded that the treatment value under current pricing in the US favors clinical strategies that include imatinib as first line in the absence of clinical reasons to avoid the first-generation TKI such as comorbidities or intolerance [19, 29]. These models have also been updated to consider the potential for treatment-free remission (TFR) and discontinuation of more potent and costly second-generation TKIs, which are further discussed below.

7.2.4 Additional Considerations beyond Treatment Selection: Cost-Effectiveness of Molecular Monitoring

The European LeukemiaNet guidelines currently recommend molecular monitoring via quantitative reverse transcriptase polymerase chain reaction every 3 months and adjust treatment based on the results [20]. Several economic models have analyzed the economic impact or cost-effectiveness of molecular monitoring in CML care. Closer alignment with the monitoring guidelines that recommend the frequency of quantitative RT-PCR testing is associated with lower healthcare expenditures and improved adherence. In a study using two large administrative healthcare databases in the US, around 36% of patients initiating imatinib, dasatinib, or nilotinib had no molecular monitoring tests in the first year, similar to findings in other studies [30]. The direct effect of one additional molecular monitoring test was associated with statistically significant decrease in inpatient admissions, inpatient days, and emergency department visits by 11.6%, 13.0%, and 8.3%, respectively [30]. Patients with a high frequency of molecular monitoring were also more likely to be adherent to TKI therapy [30]. The impact of molecular monitoring frequency on healthcare utilization and expenditures supports its use as a cost-effective component on long-term CML care strategies, as molecular monitoring is critical for identifying whether patients are meeting treatment milestones that predict outcome and whether certain patients may be at risk of treatment failure [31].

7.2.5 Cost-Effectiveness in the Era of TKI Discontinuation

Despite initial expectations that life-long treatment with TKI would be required, emerging evidence shows that TKIs can be safely discontinued in some patients with chronic phase CML who achieve and maintain deep molecular response [32, 33]. Thus, treatment goals may evolve, leading patients to initiate a more potent, second-generation TKI due to the higher probability of rapidly achieving a deep molecular response compared to imatinib. Given the potential for discontinuation and treatment-free remission, patients on second-generation TKIs may also be able to avoid long-term treatment costs and potentially toxicities. In the ENESTnd trial, among patients randomized to initial imatinib therapy, 42% achieved an MR [4] response (4-log reduction in BCR/ABL1 transcripts below a standardized baseline by quantitative RT-PCR assay) by 5 years, and 56% by 10 years. Among patients randomized to nilotinib, the corresponding fractions were 66% at 5 years, and 73% at 10 years [18].

However, the European LeukemiaNet treatment guidelines state that although treatment discontinuation can be considered for eligible patients, 80% of newly diagnosed patients are unlikely to achieve durable treatment free remission [20]. Among those who meet the molecular response criteria to discontinue safely, around 40–60% experience molecular recurrence within 6 months [32, 33]. Although this area has garnered great interest in recent years, several recently published modeling studies have shown that the potential for offsetting overall treatment costs by discontinuing a second-generation TKI may not be fully realized even for patients who are able to discontinue life-long treatment.

In a decision analytic study, Shih et al. concluded that initiating a second-generation TKI as first-line therapy at current list prices in the US is not a cost-effective strategy over generic imatinib for attaining treatment-free remission for a subset of patients. In this analysis, the additional gain in one quality-adjusted life year from achieving treatment free remission with second-generation TKIs would result in a societal cost of 22 million USD over 10 years in the US [19]. This base-case estimate was driven by a marginal improvement in effectiveness and large increases in additional drug costs. Assuming an unlikely near-perfect deep molecular response rate and a 200,000 USD/per quality adjusted life year willingness to pay, the highest annual cost for second-generation TKIs to be considered cost-effective ranged from 44,000 to 82,000 USD [19]. This estimate corresponds to a required reduction of 45–70% in annual costs based on U.S. prices [19]. The results were consistent in simulations using data for Japan, Europe, and developing countries.

Yamamoto et al. similarly found that imatinib was still more cost-effective in the US and Japan over 10 years after accounting for the probability of deep molecular response and discontinuation [29]. Investigators updated their original cost-effectiveness model that estimated the impact of generic imatinib entry in 2016 following a 70–90% price reduction [7]. In this updated model, the cumulative incidence of discontinuation at 10 years was 20.3%, 34.2%, 38.1%, and 30.9% for the imatinib first, dasatinib first, nilotinib first, and physician's choice strategies, respectively [29]. For the US, estimates comparing second-generation TKIs and physician's choice strategies to imatinib were around 1.3 million USD per quality adjusted life year gained for no discontinuation, and around 430,000–700,000 USD per quality adjusted life year gained for successful discontinuation after 2 years of deep molecular response [29]. By traditional willingness-to-pay threshold, the cost savings of discontinuation did not result in cost-effective strategies for using second-generations TKIs. Sensitivity analyses on cost-effectiveness acceptability showed that treatment with second-generation TKIs in the first line would be cost-effective in only 30–70% of simulations if the U.S. willingness-to-pay threshold was dramatically increased to one million USD per quality adjusted life year [29].

Another decision analytic model analyzed second-generation TKI discontinuation after 5 years compared to no discontinuation potential for imatinib as well as a scenario where imatinib and second-generation TKIs could be discontinued after 10 and 7 years, respectively based on reported patient characteristics in discontinuation trials [28, 34, 35]. These scenarios assessed the differences in total TKI costs between imatinib and second-generation TKIs at an annual discounted rate of 3%, and while assuming survival and constant non-TKI medical costs over the treatment period. Five years of treatment with a second-generation TKI followed by discontinuation was equivalent to approximately 40 additional years of treatment with generic imatinib without discontinuation [28]. Assuming discontinuation after 10 years of treatment, imatinib costs amounted to around 20% of the discounted costs accrued after 7 years on a second-generation TKI [28]. The cost difference between imatinib and second-generation TKIs measured in years of treatment would be longer than the average remaining life expectancy given the adult age of onset for CML. In addition, while it may eventually be a possibility to stratify treatment decisions at diagnosis based on an individual patient's likelihood of successful discontinuation, evidence in this area is lacking. These findings suggest that any potential cost savings associated with discontinuation is minimal on a population level, considering that only some patients will be eligible for treatment discontinuation and roughly half of those patients will relapse and need to resume treatment [32, 33, 36, 37].

7.3 Patient Affordability, Adherence, and Access

In addition to the cost of CML care to the healthcare system, there has been particular attention given to how the cost of these drugs impacts patients directly. Access to TKIs is widely

available in most high-income countries via health care payer markets that have bargaining power to reduce costs to patients. In contrast, TKIs remain costly for patients in the US, adversely contributing to patient outcomes. High rates of adherence (>90%) to CML treatment is critical to patient outcomes [38], and cost is one of the known barriers to adherence. Several analyses have illustrated the inverse correlation between cost of TKIs and adherence to CML treatment. It is estimated that nearly a third of CML patients may be nonadherent due to high out-of-pocket costs (OOP) of their TKI therapy [39, 40]. In an earlier work by Streeter et al., colleagues showed that in addition to polypharmacy, the most significant factor increasing the likelihood of abandonment of newly prescribed oral oncolytic therapy is high cost sharing. Claims with cost sharing greater than $500 were abandoned four times more often than claims with cost sharing of $100 or less [41]. Similarly, Doshi et al. found prescription abandonment rates were 41% when cost sharing exceeded $500 [42]. Specifically looking at CML adult patients, Dusetzina and colleagues used health insurance claims data and found that patients with high copayments for CML TKIs were 42% more likely to be nonadherent and 70% more likely to discontinue treatment (nonadherence defined as having less than 80% of days with medication available over a 180 day period following treatment initiation) [39]. Medicare, a national health insurance program for American adults aged 65 or older, includes an optional prescription drug benefit ("Part D") with high cost sharing for beneficiaries with CML. Within Medicare Part D a similar inverse relationship between adherence and OOP costs has been demonstrated [40, 43]. Using SEER registry data, Shen and colleagues showed that average OOP costs for nonadherent CML patients were $829 per 30 days in contrast to $567 for adherent individuals (nonadherence defined as having less than 80% of days with medication available over the 30 day period). Winn and colleagues also found that Medicare beneficiaries who were diagnosed with CML and did not have low income subsidies to reduce their OOP costs had longer times until initiation of TKIs than those with subsidies [44]. Despite the high OOP costs required by some insurance plans in the US, there is still a survival advantage to having insurance versus not having insurance for CML patients. The 5-year overall survival for patients under 65 with CML patients was reported in 2017 to be 73% in uninsured patients versus 87% in insured patients [45].

Despite the availability of some free-drug assistance programs from manufacturers, the application process can be quite burdensome to patients and not all patients may meet the eligibility criteria [46]. Participation in these programs must be renewed annually.

There have been several solutions proposed to improve patient affordability and access to CML TKIs, thereby improving adherence and outcomes, including potential for lowering overall medical costs [47]. One potential solution to improve affordability is to expand eligibility to low-income subsidies for older adults insured under Medicare Part D. A 2016 analysis by Winn et al. showed that having low-income subsidies was an important predictive factor for earlier initiation of TKIs. Specifically, those with subsidies had a 35% shorter time to initiate TKIs than patients without subsidies [44]. Another important option is to encourage generic drug utilization, given the potential for lower costs of these treatments to both health plans and patients, which would theoretically increase patient adherence. One analysis by Cole et al. showed that for newly diagnosed CML patients, initiating on generic imatinib had higher adherence (92%) compared with those initiating on branded imatinib (85%) [48]. In commercially insured patients, OOP costs do not appear to differ markedly between generic and brand imatinib. Analysis by Kim et al. showed median out OOP costs for all brand TKIs to be $35 per fill compared with $30 per fill for generic imatinib [49]. Of note, this is likely due to manufacturer patient assistance programs that cap copays to <$50 for commercially insured patients. More recently, these programs have closed for generic imatinib [50, 51]. Coverage on health plans may change over time with plans preferring generics and not covering branded drugs. For older adults, the

Medicare part D design is especially concerning as patients in the coverage gap may actually face higher cost sharing for generic compared with branded drugs and have minimal access to patient assistance programs [52, 53].

7.4 The Costs of Safety and Quality

When evaluating available TKIs for the treatment of CML and the costs associated with their use, the interplay between their individual safety profiles and health care costs must be considered. TKI treatment is not risk free, and adverse events can be both variable and serious among different TKIs. For example, among first-line TKIs, dasatinib is associated with risk of pleural effusion, nilotinib with vascular occlusive events and rash, and imatinib with fluid retention, GI symptoms, and fatigue. The economic burden of managing these adverse events can be costly. For example, average per patient costs of treating vascular occlusive events such as femoral arterial stenosis and peripheral arterial occlusive disease are $17,015 and $15,154 respectively [54]. Also, treatment costs of pleural effusions can average between $2062 and $2717 per patient, and if invasive procedures are required, such as chest tube placement, it can cost an average of $6394–$9013 per patient [55].

The first year of treatment is associated with the occurrence of most adverse events. Using emergency department and hospital admissions as a proxy for adverse events, CML patients treated with dasatinib and nilotinib had 17% and 7% higher risk of adverse events, respectively, compared with imatinib (but not statistically significant with nilotinib). Imatinib had the lowest incidence of safety events (imatinib 37%; dasatinib 44%; nilotinib 40%). Imatinib had lower 1-year all-cause healthcare expenditure costs compared to dasatinib and nilotinib (difference in medians [95% CI]: dasatinib vs. imatinib: $22,393 [$17,068–$27,718]; nilotinib vs. imatinib: $19,463 [$14,689–$24,236]). Even when outpatient pharmacy expenditures were excluded (median, $105,402; IQR $74,177, $129,819), median 1-year medical expenditures were higher among newly initiated nilotinib and dasatinib patients [3]. Other long-term adverse effects (i.e., cardiovascular with nilotinib) increase over time which could change produce-specific estimates of relative costs.

Overall, the healthcare resource utilization costs related to adverse events from TKIs need to be taken into consideration at the time of initiation. Experienced physicians assess concomitant risk factors of diabetes, hypertension, smoking, and vascular disease. Individual patient health factors that may predispose patients to serious adverse events resulting from these medications must be evaluated in the same manner as the possible cost savings and safety benefit of TKI discontinuation.

7.5 Conclusion

Globally, system and patient-level costs and affordability play a significant role in treatment selection for CML patients. Given imatinib's generic availability, favorable safety profile, long-term efficacy, and similar survival benefits in comparison to more potent but more expensive second-generation TKIs, it remains a preferred first-line option for most CML patients. In the coming decade, patent expirations for all current TKIs should, in theory, yield better affordability for patients requiring these drugs. In the US, however, additional policy changes are required to ensure CML patients have adequate and timely access to treatment.

Acknowledgements Jasmine Johnson, BS. Dominique Seo.

Disclosures BM received research support from the Hematology/Oncology Pharmacy Association. JN received funding for doctoral studies through a fellowship sponsored by Bristol Myers Squibb. AC is a paid employee and stockholder of GSK. RAL acted as a consultant or advisor to Novartis, Amgen, Ariad/Takeda, Astellas, Celgene/BMS, CVS/Caremark, Epizyme, and MorphoSys and has received clinical research support from Novartis, Astellas, Celgene, Cellectis, Daiichi Sankyo, Forty Seven, Rafael Pharmaceuticals, and royalties from UpToDate. SBD reports grants from the

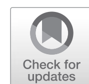
Commonwealth Fund, the Leukemia & Lymphoma Society, Arnold Ventures, and the Robert Wood Johnson Foundation. She also receives honoraria for her participation as an expert advisory panel member for West Health, the Institute for Clinical and Economic Review, and consulting payments from the National Academy of State Health Policy.

References

1. Wilkes JJ, Lyman GH, Doody DR, et al. Health care costs associated with contemporary chronic myelogenous Leukemia (CML) therapy compared to other hematologic malignancies (HEM). Blood. 2019;134(Supplement_1):4753. https://doi.org/10.1182/blood-2019-124106.
2. Dusetzina SB, Jazowski S, Cole A, Nguyen J. Sending the wrong price signal: why do some brand-name drugs cost medicare beneficiaries less than generics? Health Aff (Millwood). 2019;38(7):1188–94. https://doi.org/10.1377/hlthaff.2018.05476.
3. Cole AL, Wood WA, Muluneh B, Lund JL, Elston Lafata J, Dusetzina SB. Comparative safety and health care expenditures among patients with chronic myeloid leukemia initiating first-line imatinib, dasatinib, or nilotinib. JCO Oncol Pract. 2020;16(5):e443–55. https://doi.org/10.1200/JOP.19.00301.
4. 1 Recommendations | Dasatinib, nilotinib and high-dose imatinib for treating imatinib-resistant or intolerant chronic myeloid leukaemia | Guidance | NICE. https://www.nice.org.uk/guidance/ta425/chapter/1-Recommendations. Accessed August 18, 2020.
5. Overview | Bosutinib for untreated chronic myeloid leukaemia (terminated appraisal) | Guidance | NICE. https://www.nice.org.uk/guidance/ta576. Accessed August 18, 2020.
6. 1 Recommendations | Ponatinib for treating chronic myeloid leukaemia and acute lymphoblastic leukaemia | Guidance | NICE. https://www.nice.org.uk/guidance/ta451/chapter/1-Recommendations. Accessed August 18, 2020.
7. Padula WV, Larson RA, Dusetzina SB, et al. Cost-effectiveness of tyrosine kinase inhibitor treatment strategies for chronic myeloid leukemia in chronic phase after generic entry of imatinib in the United States. J Natl Cancer Inst. 2016;108:7. https://doi.org/10.1093/jnci/djw003.
8. Wouters OJ, Kanavos PG, McKEE M. Comparing generic drug markets in Europe and the United States: prices, volumes, and spending. Milbank Q. 2017;95(3):554–601. https://doi.org/10.1111/1468-0009.12279.
9. Venkatesan S, Lamfers M, Leenstra S, Vulto AG. Overview of the patent expiry of (non-)tyrosine kinase inhibitors approved for clinical use in the EU and the US. GaBi J. 2017;6(2):89–96. https://doi.org/10.5639/gabij.2017.0602.016.
10. Generics applications under review by EMA—July 2019 / General / Generics / Home - GaBI Online—Generics and Biosimilars Initiative. http://www.gabionline.net/layout/set/print/Generics/General/Generics-applications-under-review-by-EMA-July-2019. Accessed August 18, 2020.
11. CML Advocates Network—CML TKI Register. https://www.cmladvocates.net/generics/cml-drugs-register. Accessed August 18, 2020.
12. Abruzzese E, Bosi A, Breccia M, et al. Treatment patterns in patients with chronic-phase chronic myeloid leukaemia in routine clinical practice: the SIMPLICITY Italian population. Mediterr J Hematol Infect Dis. 2019;11(1):e2019025. https://doi.org/10.4084/MJHID.2019.025.
13. Geelen IGP, Thielen N, Janssen JJWM, et al. Treatment outcome in a population-based, "real-world" cohort of patients with chronic myeloid leukemia. Haematologica. 2017;102(11):1842–9. https://doi.org/10.3324/haematol.2017.174953.
14. Martinez de Pinillos A, Nasuti P, Anger C, Ricote I. Pcn251—descriptive analysis of the use of tyrosine kinase inhibitors in chronic myeloid leukaemia in Europe, a real-world perspective. Value Health. 2018;21:S57. https://doi.org/10.1016/j.jval.2018.09.333.
15. Experts in Chronic Myeloid Leukemia. The price of drugs for chronic myeloid leukemia (CML) is a reflection of the unsustainable prices of cancer drugs: from the perspective of a large group of CML experts. Blood. 2013;121(22):4439–42. https://doi.org/10.1182/blood-2013-03-490003.
16. Saussele S, Kohlbrenner K, Kaworski T, Vogelmann T, Schubert T. Real-world treatment patterns, health-care costs and predictors for TKI changes in CML: results from a population representative German claims data analysis. Blood. 2019;134(Supplement_1):1645. https://doi.org/10.1182/blood-2019-122649.
17. Rochau U, Sroczynski G, Wolf D, et al. Cost-effectiveness of the sequential application of tyrosine kinase inhibitors for the treatment of chronic myeloid leukemia. Leuk Lymphoma. 2015;56(8):2315–25. https://doi.org/10.3109/10428194.2014.982635.
18. Lejniece S, Udre I, Rivkina A. Generic imatinib in the treatment of chronic myeloid leukemia: two years' experience in Latvia. Exp Oncol. 2017;39(2):151–4.
19. Shih Y-CT, Cortes JE, Kantarjian HM. Treatment value of second-generation BCR-ABL1 tyrosine kinase inhibitors compared with imatinib to achieve treatment-free remission in patients with chronic myeloid leukaemia: a modelling study. Lancet Haematol. 2019;6(8):e398–408. https://doi.org/10.1016/S2352-3026(19)30087-0.
20. Hochhaus A, Baccarani M, Silver RT, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020;34(4):966–84. https://doi.org/10.1038/s41375-020-0776-2.
21. Wolters S, Dijkstra J, Arons A, IJzermans A, Postma M. PCN64 cost-effectiveness of Imatinib since its

introduction as first-line treatment in the Netherlands. Value Health. 2019;22:S447–8.
22. House of Commons—Health Committee: Written evidence from Karl Claxton, Steve Martin, Marta Soares, Nigel Rice, Eldon Spackman, Sebastian Hinde, Peter C Smith and Mark Sculpher (NICE 61). https://publications.parliament.uk/pa/cm201213/cmselect/cmhealth/782/782vw55.htm. Accessed September 19, 2020.
23. Cole AL, Dusetzina SB. Generic price competition for specialty drugs: too little, too late? Health Aff (Millwood). 2018;37(5):738–42. https://doi.org/10.1377/hlthaff.2017.1684.
24. Chen CT, Kesselheim AS. Journey of generic imatinib: a case study in oncology drug pricing. J Oncol Pract. 2017;13(6):352–5. https://doi.org/10.1200/JOP.2016.019737.
25. Kenzik KM, Bhatia R, Bhatia S. Expenditures for first- and second-generation tyrosine kinase inhibitors before and after transition of imatinib to generic status. JAMA Oncol. 2020; https://doi.org/10.1001/jamaoncol.2019.6390.
26. Jabbour E, Makenbaeva D, Lingohr-Smith M, Lin J. Impact of genetic mutations and health plan access to therapies on treatment response and drug costs related to tyrosine kinase inhibitor treatment among patients with chronic myelogenous leukemia. Am J Clin Oncol. 2015; https://doi.org/10.1097/COC.0000000000000252.
27. Jabbour EJ, Mendiola MF, Lingohr-Smith M, Lin J, Makenbaeva D. Economic modeling to evaluate the impact of chronic myeloid leukemia therapy management on the oncology care model in the US. J Med Econ. 2019;22(11):1113–8. https://doi.org/10.1080/13696998.2019.1618316.
28. Nguyen JT, Cole AL, Leech AA, Wood WA, Dusetzina SB. Cost-effectiveness of first line tyrosine kinase inhibitor initiation strategies for chronic myeloid leukemia. Value Health. 23:1292.
29. Yamamoto C, Nakashima H, Ikeda T, et al. Analysis of the cost-effectiveness of treatment strategies for CML with incorporation of treatment discontinuation. Blood Adv. 2019;3(21):3266–77. https://doi.org/10.1182/bloodadvances.2019000745.
30. Latremouille-Viau D, Guerin A, Gagnon-Sanschagrin P, Dea K, Cohen BG, Joseph GJ. Health care resource utilization and costs in patients with chronic myeloid leukemia with better adherence to tyrosine kinase inhibitors and increased molecular monitoring frequency. J Manag Care Spec Pharm. 2017;23(2):214–24. https://doi.org/10.18553/jmcp.2017.23.2.214.
31. Shanmuganathan N, Hughes TP. Molecular monitoring in CML: how deep? How often? How should it influence therapy? Hematology Am Soc Hematol Educ Program. 2018;2018(1):168–76. https://doi.org/10.1182/asheducation-2018.1.168.
32. Radich JP, Deininger M, Abboud CN, et al. Chronic myeloid leukemia, version 1.2019, NCCN clinical practice guidelines in oncology. J Natl Compr Cancer Netw. 2018;16(9):1108–35. https://doi.org/10.6004/jnccn.2018.0071.
33. Shah NP. NCCN guidelines updates: discontinuing TKI therapy in the treatment of chronic myeloid leukemia. J Natl Compr Cancer Netw. 2019;17(5.5):611–3. https://doi.org/10.6004/jnccn.2019.5013.
34. Chamoun K, Kantarjian H, Atallah R, et al. Tyrosine kinase inhibitor discontinuation in patients with chronic myeloid leukemia: a single-institution experience. J Hematol Oncol. 2019;12(1):1. https://doi.org/10.1186/s13045-018-0686-1.
35. Rea D, Nicolini FE, Tulliez M, et al. Discontinuation of dasatinib or nilotinib in chronic myeloid leukemia: interim analysis of the STOP 2G-TKI study. Blood. 2017;129(7):846–54. https://doi.org/10.1182/blood-2016-09-742205.
36. Saussele S, Richter J, Guilhot J, et al. Discontinuation of tyrosine kinase inhibitor therapy in chronic myeloid leukaemia (EURO-SKI): a prespecified interim analysis of a prospective, multicentre, non-randomised, trial. Lancet Oncol. 2018;19(6):747–57. https://doi.org/10.1016/S1470-2045(18)30192-X.
37. Hochhaus A, Masszi T, Giles FJ, et al. Treatment-free remission following frontline nilotinib in patients with chronic myeloid leukemia in chronic phase: results from the ENESTfreedom study. Leukemia. 2017;31(7):1525–31. https://doi.org/10.1038/leu.2017.63.
38. Marin D, Bazeos A, Mahon F-X, et al. Adherence is the critical factor for achieving molecular responses in patients with chronic myeloid leukemia who achieve complete cytogenetic responses on imatinib. J Clin Oncol. 2010;28(14):2381–8. https://doi.org/10.1200/JCO.2009.26.3087.
39. Dusetzina SB, Winn AN, Abel GA, Huskamp HA, Keating NL. Cost sharing and adherence to tyrosine kinase inhibitors for patients with chronic myeloid leukemia. J Clin Oncol. 2014;32(4):306–11. https://doi.org/10.1200/JCO.2013.52.9123.
40. Shen C, Zhao B, Liu L, Shih Y-CT. Adherence to tyrosine kinase inhibitors among Medicare part D beneficiaries with chronic myeloid leukemia. Cancer. 2018;124(2):364–73. https://doi.org/10.1002/cncr.31050.
41. Streeter SB, Schwartzberg L, Husain N, Johnsrud M. Patient and plan characteristics affecting abandonment of oral oncolytic prescriptions. J Oncol Pract. 2011;7(3 Suppl):46s–51s. https://doi.org/10.1200/JOP.2011.000316.
42. Doshi JA, Li P, Huo H, Pettit AR, Armstrong KA. Association of patient out-of-pocket costs with prescription abandonment and delay in fills of novel oral anticancer agents. J Clin Oncol. 2018;36(5):476–82. https://doi.org/10.1200/JCO.2017.74.5091.
43. Kenzik K, Bhatia R, Williams GR, Bhatia S. Health care and out-of-pocket (OOP) costs among medicare beneficiaries diagnosed with chronic myeloid leukemia (CML). Blood. 2018;132(Supplement 1):4730. https://doi.org/10.1182/blood-2018-99-110742.

44. Winn AN, Keating NL, Dusetzina SB. Factors associated with tyrosine kinase inhibitor initiation and adherence among Medicare beneficiaries with chronic myeloid leukemia. J Clin Oncol. 2016;34(36):4323–8. https://doi.org/10.1200/JCO.2016.67.4184.
45. Perry AM, Brunner AM, Zou T, et al. Association between insurance status at diagnosis and overall survival in chronic myeloid leukemia: a population-based study. Cancer. 2017;123(13):2561–9. https://doi.org/10.1002/cncr.30639.
46. The Price They Pay—The New York Times. https://www.nytimes.com/2018/03/05/health/drug-prices.html. Accessed August 7, 2020.
47. Phuar HL, Begley CE, Chan W, Krause TM. Tyrosine kinase inhibitors initiation, cost sharing, and health care utilization in patients with newly diagnosed chronic myeloid leukemia: a retrospective claims-based study. J Manag Care Spec Pharm. 2019;25(10):1140–50. https://doi.org/10.18553/jmcp.2019.25.10.1140.
48. Cole AL, Jazowski SA, Dusetzina SB. Initiation of generic imatinib may improve medication adherence for patients with chronic myeloid leukemia. Pharmacoepidemiol Drug Saf. 2019;28(11):1529–33. https://doi.org/10.1002/pds.4893.
49. Kim R, Malhotra D, Bell T, An F, Mardekian J. Out-of-Pocket (OOP) Costs Associated with Tyrosine Kinase Inhibitors (TKIs) Used for the Treatment of US Patients Diagnosed with Chronic Myeloid Leukemia (CML): A US Retrospective Claims Analysis. Blood. December 2017.
50. NeedyMeds. https://www.needymeds.org/company_list.taf?_function=name&program_id=92af93f73faf3cefc129b6bc55a748a9. Accessed August 7, 2020.
51. Imatinib Mesylate Tablets, 100MG, 90 TABLET (BOTTLE). https://www1.apotex.com/products/us/detail.asp?m=63479. Accessed August 7, 2020.
52. Das L, Gitlin M, Siegartel LR, Makenbaeva D. The value of open access and a patient centric approach to oral oncolytic utilization in the treatment of chronic myelogenous leukemia: a U.S. perspective. Expert Rev Pharmacoecon Outcomes Res. 2017;17(2):133–40. https://doi.org/10.1080/14737167.2017.1305892.
53. Dusetzina S, Muluneh B, Keating N, Huskamp H. Broken promises—how medicare part D has failed to deliver savings to older adults. N Engl J Med. 383:2299.
54. Lin J, Makenbaeva D, Lingohr-Smith M, Bilmes R. Healthcare and economic burden of adverse events among patients with chronic myelogenous leukemia treated with BCR-ABL1 tyrosine kinase inhibitors. J Med Econ. 2017;20(7):687–91. https://doi.org/10.1080/13696998.2017.1302947.
55. Stephens J, Carpiuc KT, Botteman M. The burden of managing pleural effusions in patients with chronic myelogenous leukemia post-imatinib failure: a literature-based economic analysis. Int J Gen Med. 2010;3:31–6.
56. Editor GBIJ. Patent expiry dates for biologicals: 2018 update. GaBi J. 2019;8(1):24–31. https://doi.org/10.5639/gabij.2019.0801.003.
57. www.sec.gov/Archives/edgar/data/1114448/000104746915000433/a2222787z20-f.htm. https://www.sec.gov/Archives/edgar/data/1114448/000104746915000433/a2222787z20-f.htm. Accessed September 20, 2020.
58. BMY-2014.12.31–10-K. https://www.sec.gov/Archives/edgar/data/14272/000001427215000055/bmy-20141231x10xk.htm. Accessed September 20, 2020.



8 Standardization of Molecular Monitoring for Chronic Myeloid Leukemia: 2021 Update

Matthew Salmon, Helen E. White,
Nicholas C. P. Cross, and Andreas Hochhaus

8.1 Background

Studies to explore the possibility of molecular monitoring of chronic myeloid leukemia (CML) patients by reverse transcriptase polymerase chain reaction (RT-PCR) were initiated more than 30 years ago, when the principal clinical challenge was to develop a methodology to detect early relapse after bone marrow transplantation (BMT). The first studies were qualitative, using two-step, nested RT-PCR and standard agarose gel electrophoresis to determine whether *BCR-ABL1* mRNA was detectable or undetectable in patient samples, with a control for adequate cDNA quality being provided by single step amplification of a housekeeping control gene. Standardization at this time focused mainly on the need to eliminate false-positive results arising from contamination of amplification reactions with previously amplified products [1].

Although some of these early studies were able to identify groups of patients that were more or less likely to relapse, the predictive value for individual cases was very limited [2–6], and thus there was a need to develop quantitative RT-PCR approaches that might be able to give an indication of the level of disease in specimens that tested positive for *BCR-ABL1*, and also the kinetics of any changes in the size of the malignant clone over time. Initial quantitative procedures were based on the use of competitive PCR, which relies on the addition of known numbers of molecules of a competitor plasmid to a series of amplification reactions, with the number of *BCR-ABL1* targets in the sample being estimated by determining the point at which the competitor and *BCR-ABL1* amplicons are of equivalent fluorescent intensity on an agarose gel. Using competitive PCR it was shown that rising *BCR-ABL1* levels on sequential analysis predicted relapse after BMT and provided prognostically useful information for patients in complete cytogenetic remission (CCyR) on interferon alpha [7–12]. Competitive PCR was thus effective but extremely labor intensive and was only performed on a research basis in a small number of transplant centers. The development and subsequent commercialization of reverse-transcription real-time quantitative PCR (RT-qPCR) in the late 1990s [13] along with the introduction of highly effective targeted therapy for CML provided the means and the need for widespread adoption of molecular monitoring. However, there was no standard approach as to how the assay should be performed and different methodologies

M. Salmon · H. E. White · N. C. P. Cross (✉)
Faculty of Medicine, University of Southampton, Southampton, UK

Wessex Regional Genetics Laboratory, Salisbury, UK
e-mail: ncpc@soton.ac.uk

A. Hochhaus
Abteilung Hämatologie/Onkologie, Klinik für Innere Medizin II, Universitätsklinikum Jena, Jena, Germany

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
R. Hehlmann (ed.), *Chronic Myeloid Leukemia*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-71913-5_8

proliferated, resulting in results that were difficult or impossible to compare between centers.

8.2 Measurement of Residual Disease in the Laboratory

To understand the problem of standardization it is first necessary to understand how the test is performed. Anticoagulated peripheral blood or bone marrow samples are received in the testing laboratory, ideally within 24 hours of collection. Generally peripheral blood is preferred as this is less invasive, and results are comparable to bone marrow provided that total leukocytes are recovered by lysis of red cells [14]; in contrast to the analysis of residual disease in acute leukemia, mononuclear cells isolated by density gradients such as Lymphoprep® or Ficoll should not be used for CML. Leucocytes are lysed in a chaotropic agent that inactivates pervasive RNA-degrading enzymes, and RNA is extracted and reverse transcribed to cDNA, typically using random hexamer primers (Fig. 8.1). Differences in the amount of RNA extracted, the integrity of that RNA, and the efficiency with which it is reverse transcribed may vary widely between samples, even in established laboratories. This means that the sensitivity with which *BCR-ABL1* can be detected or excluded is also highly variable. It is generally agreed that the best way to take this variation into account is to relate the number of copies of *BCR-ABL1* to those of an housekeeping reference gene, which serves as an internal control for both the quantity and quality of the cDNA for each sample [15].

Two measurements are made by RT-qPCR for all samples: an estimate of the number of *BCR-ABL1* transcripts and an estimate of the number of transcripts of the housekeeping reference gene. Different laboratories use various processes to derive these estimates; for example, some measure *BCR-ABL1* and the reference gene singly, in duplicate or in triplicate from an identical cDNA specimen; others make a single measurement from independent cDNA preparations. In addition, different criteria are used to define whether a result is considered detectable or undetectable based on replicate results and technical parameters.

Results for specimens that are positive for *BCR-ABL1* are expressed as the ratio of *BCR-ABL1* transcript numbers divided by the number of reference gene transcripts in the same volume of cDNA. For samples that test negative for *BCR-ABL1*, the number of reference gene transcripts

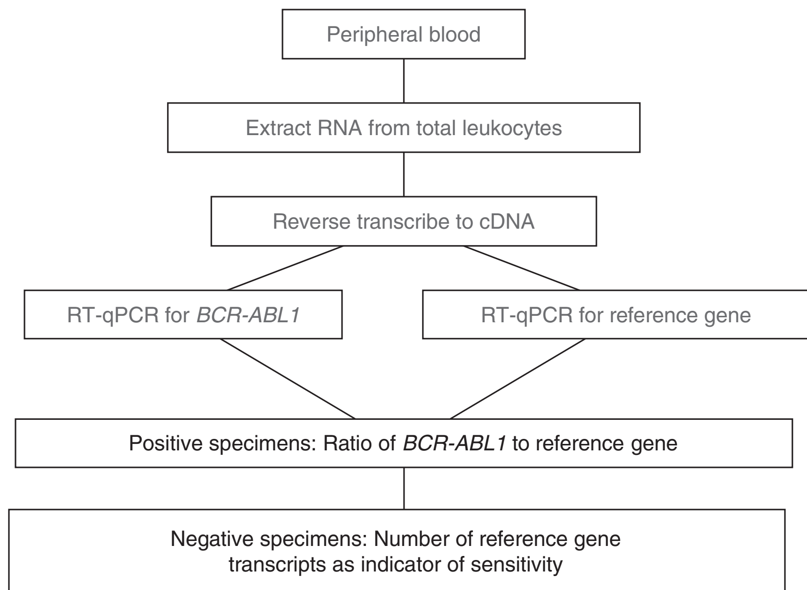


Fig. 8.1 Schematic outline of *BCR-ABL1* RT-qPCR analysis. Positive specimens mean those in which *BCR-ABL1* mRNA is detected; negative specimens are those in which *BCR-ABL1* mRNA is not detected

gives an indication of the sensitivity with which residual disease can be excluded for that particular specimen. It is very important that testing laboratories monitor closely the variability of their assay and reject runs that are considered as outliers, for example, by regularly measuring high and low standards [16, 17]. Establishment of RT-qPCR requires extensive validation, for example, using the methodology described by the Molecular Oncology Resource Committee of the College of American Pathologists [18].

8.3 Choice of Reference Gene

One of the critical variables between centers has been the choice of reference gene. An ideal reference gene would be expressed uniformly in different cell types regardless of their proliferative status, unaffected by therapeutic regimens, invariant between individuals, and expressed at a level similar to *BCR-ABL1*. Unfortunately such a perfect reference gene does not appear to exist and instead several alternatives have emerged. The most widely used is *ABL1;* this is partly a historical accident as the plasmid constructs that were used for competitive PCR quantification could also be used to quantify normal *ABL1* expression [7]. Subsequently, however, the Europe Against Cancer (EAC) group undertook an extensive analysis of candidate reference genes and concluded that *ABL1,* beta-2-microglobulin (*B2M*), and beta-glucuronidase (*GUSB*) were suitable for normalization of RT-qPCR results [15]. *BCR* is also widely used as an internal control for CML, based on the rationale that both normal *BCR* and *BCR-ABL1* are driven by the same promoter, and thus they are likely to be transcribed at similar rates in different cell types [16]. The great majority of testing laboratories worldwide use *ABL1* as an internal reference but *BCR* or *GUSB* is also used in many centers. The use of other reference genes is not recommended. This means that there are at least three distinct units of measurement in widespread use for the estimation of residual disease in CML: *BCR-ABL1*/total *ABL1* (i.e., *ABL1* + *BCR-ABL1*), *BCR-ABL1*/*BCR*, and *BCR-ABL1*/*GUSB*.

Although the use of different reference genes used to be the principal reason for limited comparability of results between centers, there are other important factors that are particularly relevant for laboratory-developed tests. Laboratories using the same reference gene may use different probe/primer combinations, partly as a result of concerns about infringements of intellectual property rights. In addition, laboratories may differ in their approach to the setting of user-defined parameters such as the threshold, what constitutes an acceptable result in terms of slope of the standard curve, minimum number of points to construct a standard curve, what cycle threshold (C_t) value is accepted as a positive result, and the reproducibility between duplicate or triplicate replicates. Finally, in order to achieve sensitive detection of residual disease, it is essential to analyze a sufficiently large sample. Clearly it is impossible to achieve a sensitivity of 1 in 10^5 if only the equivalent of 10^4 cells or fewer are analyzed. Some of these issues have been addressed by the EAC and consensus guidelines published [19, 20]; in addition many commercially available kits provide detailed guidance for RT-qPCR set up and analysis.

8.4 The International Scale for *BCR-ABL1* Measurement

The International Randomized Study of Interferon and STI571 (IRIS) study demonstrated the dramatic superiority of imatinib over interferon-based regimens. In this trial, RT-qPCR analysis was centralized in three centers (Adelaide, London, and Seattle) that used different laboratory procedures and two different control genes [21]. Large, differences in median *BCR-ABL1* values at specific timepoints between the three centers were noted, which prompted the need for an urgent alignment of their respective results. In the absence of any independent reference or calibration materials, an essentially arbitrary decision was made that each center would measure the level of disease in a common set of 30 pretreatment CML patient samples using *BCR* as a control gene, and that results would be normalized to

this standardized baseline. Reanalysis of the data showed improved comparability of results between the three laboratories and the standardized baseline was used to normalize all subsequent trial results [21]. Thus, major molecular response (MMR), for example, was defined as a three log reduction from the IRIS-standardized baseline and not a three log reduction from pre-treatment material for each individual case.

The scale used in the IRIS trial subsequently formed the basis of the international scale (IS) for *BCR-ABL1* measurement, which was proposed as a means to enable laboratories to continue to use their own methods but produce results that are more comparable between centers [22]. Although the samples used to define the IRIS standardized baseline were very limited in quantity and therefore quickly exhausted, excellent traceability was provided by the detailed internal quality control data accrued by the Adelaide laboratory [16], thus enabling the IS to be linked directly to measurements made for IRIS. The IS expresses detectable disease as a percentage, with 100% BCR-ABL^{IS} defined as the IRIS standardized baseline and 0.1% BCR-ABL^{IS} corresponding to MMR (also known as MR^3). A level of 1% BCR-ABL corresponds roughly to the limit at which Ph-positive metaphases can be detected by standard cytogenetics, and thus levels of disease <1% are consistent with complete cytogenetic remission (Fig. 8.2) [23].

The initial focus of the IS was on detectable residual disease and in particular whether a patient had or had not achieved defined milestones, for example, 10% or 0.1% BCR-ABL^{IS}. Second-generation TKIs produce faster and deeper responses compared to imatinib and the need arose for robust, standardized, and workable definitions of DMR [24]. Such definitions are particularly important for the selection of patients who may achieve treatment-free remission (TFR).

Definitions were proposed [24] and accepted by the European LeukaemiaNet (ELN) in their 2013 recommendations for the management of CML patients [25]. These definitions have been elaborated by the European Treatment and Outcome Study (EUTOS) group to enable testing laboratories to score DMR in a comparable fashion [26] and remained unchanged in the 2020 update to the ELN recommendations [27]. The definitions are:

- MR^4 (≥4-log reduction from IRIS baseline) = either (i) detectable disease ≤0.01% BCR-ABL^{IS} or (ii) undetectable disease in cDNA with 10,000–31,999 *ABL1* transcripts or 24,000–76,999 *GUSB* transcripts*.

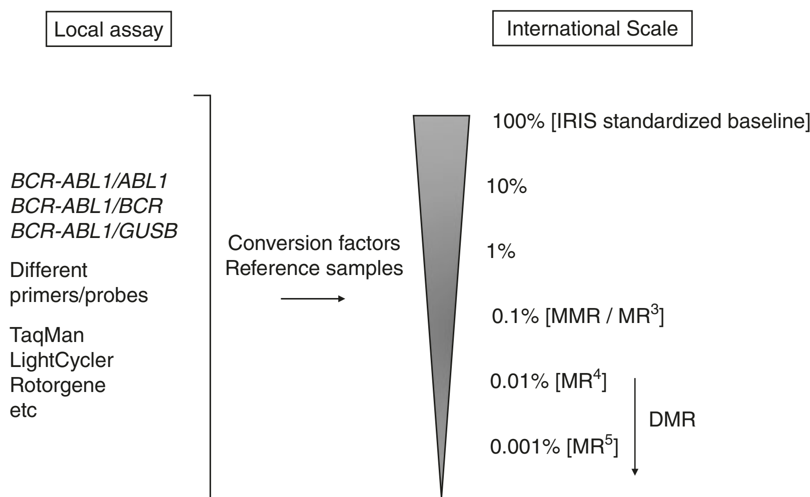


Fig. 8.2 The International Scale for *BCR-ABL1* RT-qPCR measurement. Centers continue to use their established assays for *BCR-ABL1* and convert results to the international scale (IS) using CFs or calibrated reference reagents. MMR = major molecular response; MR^3, MR^4, and MR^5 are 3, 4, and 5 log reductions, respectively, from the IRIS standardized baseline. DMR = deep molecular response is MR^4 or lower

- $MR^{4.5}$ (≥4.5-log reduction from IRIS baseline) = either (i) detectable disease ≤0.0032% BCR-ABL^{IS} or (ii) undetectable disease in cDNA with 32,000–99,999 *ABL1* transcripts or 77,000–239,999 *GUSB* transcripts*.
- MR^{5} (≥5-log reduction from IRIS baseline) = either (i) detectable disease ≤0.001% BCR-ABL^{IS} or (ii) undetectable disease in cDNA with ≥100,000 *ABL1* transcripts ≥240,000 *GUSB* transcripts*.

*Numbers of *ABL1* or *GUSB* transcripts in the same volume of cDNA used to test for *BCR-ABL1*. Equivalent numbers of *BCR* reference gene transcripts for each level of MR have not been defined.

In addition, essential quality criteria with respect to reference gene transcripts numbers must be met and are given in Table 8.1. The definitions depend critically on the ability of testing laboratories to measure absolute numbers of reference gene transcripts in a comparable manner (see below).

Although the terms "complete molecular response" or "complete molecular remission" have been used in the past, it is difficult to define these terms in any meaningful way. Instead, definitions of deep response need to be qualified with the level of sensitivity achieved for that sample, particularly for specimens where *BCR-ABL1* is not detected which should be referred to as "molecularly undetectable leukemia" and specifying the number of reference gene transcripts and/or the level of response [27].

Table 8.1 Summary of reference gene numbers required for scoring deep molecular response

	MR^4	$MR^{4.5}$	MR^5
Minimum sum of reference gene transcripts[a]	10,000 *ABL1* 24,000 *GUSB*	32,000 *ABL1* 77,000 *GUSB*	100,000 *ABL1* 240,000 *GUSB*
BCR-ABL^{IS} level for positive samples[b]	≤0.01%	≤0.0032%	≤0.001%

[a]Irrespective of whether *BCR-ABL1* is detected or not. Numbers of reference gene transcripts in the same volume of cDNA that is tested for *BCR-ABL1*. The minimum number in any individual replicate should be 10,000 *ABL1* or 24,000 *GUSB*

[b]Provided that the minimum reference gene copy numbers in the row above are fulfilled

8.5 Implementing the International Scale

Although the concept of the IS is very attractive, international implementation has proven to be challenging. Initially, the only mechanism for laboratories to adopt the IS was to establish a laboratory-specific conversion factor (CF) using a process initiated by the Adelaide laboratory [28]. For a testing laboratory to establish a CF, a series of samples (typically 20–30) are exchanged with a reference laboratory that span at least three logs of detectable disease but do not exceed an IS value of 10%. Samples are analyzed by both centers over a period of 2–3 months to take into account common intralaboratory variables, e.g., different operators and different batches of reagents. The results for the reference and test laboratories (using the IS and local units, respectively) are compared and the CF for the testing laboratory derived by a straightforward mathematical calculation. To validate the CF, a further set of samples are exchanged which are again analyzed in a similar manner, i.e., in both centers over a period of time. If the converted values for the test laboratory show a bias of within ±1.2-fold compared to the reference laboratory, then the CF is considered validated and suitable for conversion of the test laboratory results to the IS. Of 38 test laboratories which undertook this process (using 19 different methods and 5 different control genes), 22 (58%) successfully established validated CFs, testifying to the success of the process [28]. The reason that the validation process failed in the remaining test laboratories is unclear, but presumably indicates that their assays are nonlinear or unstable over time.

Since it is impossible for a single reference laboratory to standardize all other testing laboratories in the world, the concept of regional or national reference laboratories has been developed, for example, in Europe through EUTOS. Following derivation of a CF with Adelaide, the laboratory in Mannheim has performed sample exchanges and derived further CFs with more than 50 testing centers that can then serve in turn as reference centers for their countries or regions [29]. Although this process worked well, at least for laboratories with stable assays, it is arguably intrinsically flawed as any errors will be propagated along the line. Furthermore there are other obvious issues, for example, (a) derivation of CFs is time consuming and expensive; (b) due to the requirement to involve an established reference laboratory, the process is only open to a limited number of testing laboratories at any given time; (c) many centers struggle to accrue sufficient numbers of suitable samples; (d) it is unclear how often CFs need to be revalidated; (e) it is unclear what happens to the 50% of laboratories who fail to achieve the defined performance criteria; (f) it is unclear what constitutes a stable or unstable CF and how testing laboratories should accommodate CFs that change over time.

8.6 Development of Reference Reagents and Calibrated Kits

While the development of CFs was a major step forward and provided an important proof of principle, it is obvious that this approach is not sustainable in the long term. Ideally, any testing laboratory should be able to access reference standards or use a kit that enables them to convert patient results directly to the IS. The development of standards and kits initially required the development of a process by which these tools could be calibrated to the IS. An important milestone in this process was the establishment in 2010 of the First World Health Organization International Genetic Reference Panel for quantitation of *BCR-ABL1* mRNA [30]. The reference panel comprises four different dilution levels of freeze-dried preparations of K562 cells diluted in HL60 cells that were assigned fixed % *BCR-ABL1*/reference gene values on the IS following an international calibration process. Due to the scale of molecular monitoring, it was not physically possible to manufacture and validate a sufficiently large quantity of reference material to satisfy worldwide demand, and thus the principal function of these primary reagents was limited to the calibration of secondary reference reagents. These secondary reference reagents may be manufactured and calibrated by companies, reference laboratories, or other agencies and made available to testing laboratories either on a commercial basis or as part of specific national or regional standardization initiatives (Fig. 8.3).

An international evaluation of a panel of such secondary reference material demonstrated these reagents can be used to derive laboratory-specific CFs for a wide range of *BCR-ABL1* testing protocols while mitigating some of the logistical challenges of the sample exchange method [31]. This study also highlighted that many local RT-qPCR assays showed signs of poor optimization, and that individual laboratories need to robustly determine the optimal conditions for their protocols, a process for which a panel of calibrated reference material is ideally suited. Local secondary panels have also been produced to harmonize molecular monitoring results in Latin America [32] and China [33]. Recently, the AcroMetrix™ *BCR-ABL* Panel (ThermoFisher) has become the first commercially available set of secondary reference reagents in the form of lyophilized cellular material. These will hopefully enable laboratories to undertake analytical validation and performance monitoring of *BCR-ABL1* assays from RNA extraction through to generation of results of the IS as well as enabling on-demand derivation of CFs.

As indicated above, standardization of DMR requires testing laboratories to be able to estimate absolute numbers of reference gene transcripts in a comparable manner as an indication of the quality of the sample. Determination of the number of *BCR-ABL1* and reference gene transcripts is typically performed by using an external plasmid calibrator; however, different calibrators

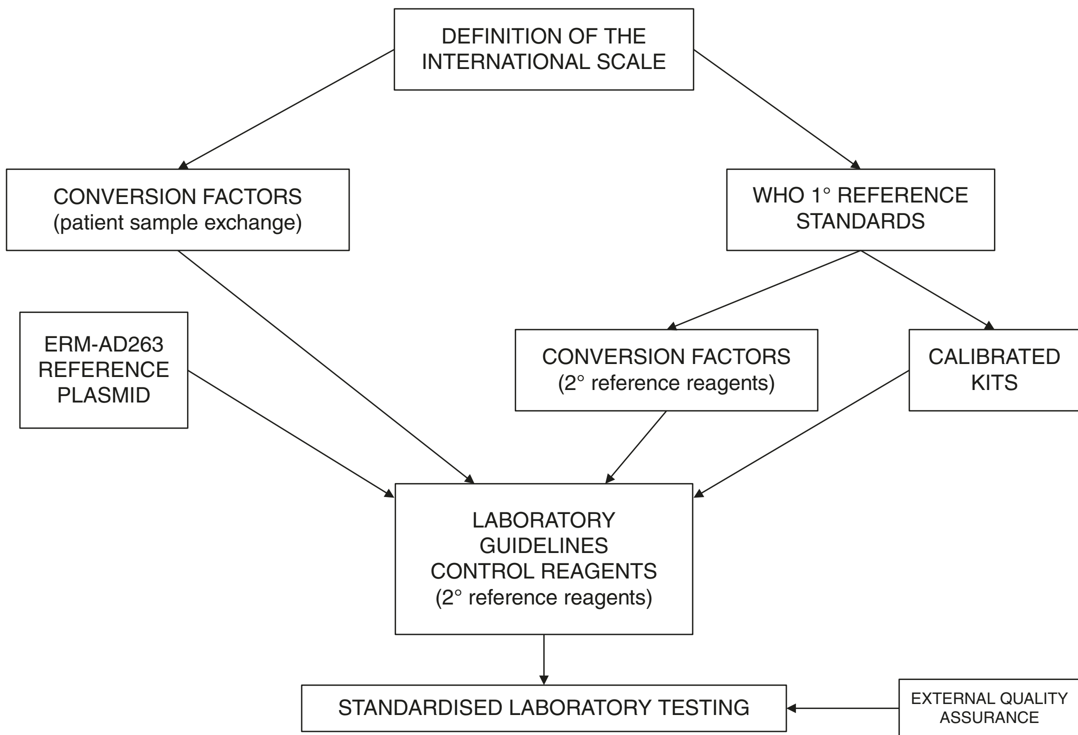


Fig. 8.3 Components of the standardization process for molecular monitoring of CML

(developed in house or commercially available) are in use worldwide and until 2015, no common reference material existed to which they could be aligned. In response to this, an internationally accepted certified reference plasmid, ERM-AD623, was developed that includes *BCR-ABL1* and the three most commonly used reference genes (*ABL1*, *BCR*, and *GUSB*) [34]. The direct or indirect use of this plasmid helps to improve the accuracy of results prior to conversion (Fig. 8.4) as well as the accuracy of reference gene copy number estimates for samples where *BCR-ABL1* is not detected.

A number of different kits, systems, and secondary reagents are available that enable testing laboratories to derive patient results on the IS [35–37]. Comparative data from a large EQA scheme involving laboratories using a diversity of methods showed that, in general, the performance of each IS conversion method in use was acceptable, but significant systematic intermethod differences were apparent [37]. It is not clear which of these methods provides the "correct" result.

8.7 What Is Achievable by Standardization?

The combination of CFs, calibrated reagents, and ERM-AD623 should help testing laboratories to generate more standardized results. Indeed, widespread adoption of the IS seems to have reduced the interlab variability [37]. However, as noted above, some lack of agreement between different laboratories using diverse methodologies and control genes remains, particularly at lower levels of *BCR-ABL1* [37]. Whether this remaining disagreement is acceptable depends on the effect it has on clinical interpretation. When evaluating the performance characteristics of a method, two factors should be considered: trueness (i.e., the degree of closeness of mean measured quantity value and the true quantity value) and the precision (i.e., the degree to which repeated measurements under unchanged conditions show the same results). The trueness of a method can be estimated comparing the average value obtained from several replicate

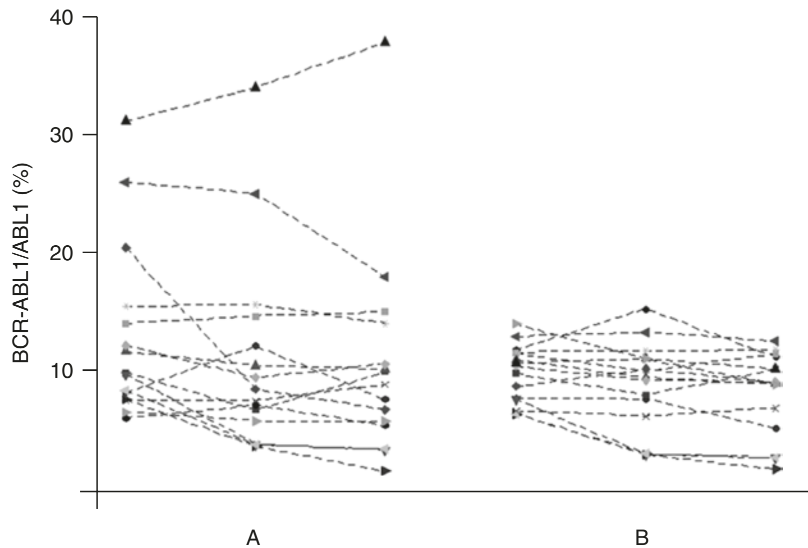


Fig. 8.4 Identical samples were measured in triplicate in 14 different laboratories using either local plasmid calibrators (**a**) or a common plasmid calibrator ERM-AD623 (**b**). It is apparent that the distribution of results is tighter when a common calibrator is used

measurements on a reference material with an established IS value. The precision of a method can be estimated from the 95% limit of agreement of all the individual measurement results obtained for the reference material. Existing experience with the set-up and validation of CFs has shown that an average difference within ±1.2-fold of the established value and 95% limits of agreement within ±five fold of the established value were achieved by the best performing methods [28]. This led to an MMR concordance rate of 91%, a level of agreement which probably represents the maximum that can be achieved using current RT-qPCR technology. However this figure of 91% critically depends on the set of samples that are used and would be expected to be substantially lower if the sample set was restricted to samples that were close to MMR. It is important, therefore, to consider intrinsic assay variation when assessing the response of a patient against specific milestones such as those recommended by the ELN [25]. In addition, at very low levels of disease, variation between replicates is inevitably greater than that seen at higher levels due to the fact that small numbers of molecules are being sampled. This should be taken into account when interpreting changes in levels of disease on sequential analysis, for example, a four-fold increase from 0.002% IS to 0.008% IS might be considered as a prompt to perform repeat analysis at the next scheduled visit, whereas an increase from 0.07% to 0.28% would be considered sufficient for rapid repeat analysis and possibly mutation testing [38].

Standardization of molecular monitoring is an ongoing process and critically requires testing laboratories to implement robust internal quality control to monitor assay drift and reproducibility [16]. In principle, if a laboratory can demonstrate assay stability over time then they only need to derive a CF once, although a new CF will have to be derived either internally or externally if processes or equipment are changed [28]. Laboratories using calibrated kits need to validate or verify that the kit is working correctly in their hands and that all preanalytical steps are optimized.

Of note, approximately 1–2% of CML patients harbor atypical *BCR-ABL1* mRNA fusion transcripts that cannot be monitored by standard *BCR-ABL1* RT-qPCR tests. It is important to recognize these fusions early in the disease course to avoid false-negative MRD assessments, and they can be monitored using bespoke RT-qPCR tests. However, such results cannot be expressed on the IS, and thus the common molecular milestones and triggers for treatment discontinuation are difficult to apply.

8.8 Standardization of Deep Molecular Response

Currently, a major focus of investigation in CML is the concept of TFR; around half of patients who have a prolonged and sustained DMR remain in sustained remission after stopping treatment. A general requirement to consider discontinuing treatment is DMR (MR^4 or better) for at least 2 years, and ideally at least 2 years in $MR^{4.5}$ or 3 years in MR^4 [27, 39, 40]. Standardization of molecular monitoring at this level of deep response is, therefore, particularly important, not only to meet the recommended criteria for attempting TFR, but also to detect patients who relapse as early as possible, as DMR is usually reachieved upon prompt resumption of treatment [41].

A program of QC rounds within the EUTOS consortium has been addressing the standardization of DMR in laboratories throughout Europe by repeated auditing of routine local results as well as distribution and testing of evaluation samples, including an IS-calibrated secondary reference panel. An in-depth analysis of this data is ongoing, but encouragingly, almost all participants could reliably detect *BCR-ABL1* at $MR^{4.5}$. This work has also enabled the monitoring of laboratory CFs over time, which for most labs were generally stable.

Given the increasing technical sensitivity required, a better understanding of the limits of a given assay's performance is crucial, and establishing or verifying the limit of detection (LoD), limit of quantitation (LoQ), and limit of blank (LoB) of an assay is important [42]. The LoD/LoQ values are dependent on the background signal (the LoB), which in an ideal *BCR-ABL1* assay is zero (i.e., there is a $\leq 5\%$ probability of a false-positive result from a true-negative sample). However, an analysis by the EUTOS group found that some testing laboratories had LoBs above acceptable levels, thus potentially compromising their ability to accurately report DMR [43].

On the other hand, an assay with a poorly optimized LoD may not be able to detect very low levels of *BCR-ABL1* transcripts, potentially leading to overestimation of the depth of response, and/or the generation of false-negative results. For example, a hypothetical laboratory with a CF of 0.8 may test a sample in duplicate, detecting a total of 34,500 *ABL1* copies. If the LoD was well optimized, it may also detect 2 copies of *BCR-ABL1* in one replicate and 1 copy of *BCR-ABL1* in the second replicate. As per the guidelines for scoring DMR [26], the result for this sample would be: (sum *BCR-ABL1* = 6)/(sum *ABL1* = 34,500) $\times$ 0.8 $\times$ 100 = 0.014% = MMR. However, if the laboratory had a poorly optimized LoD, then it may not detect *BCR-ABL1* in either replicate of the sample, leading to: undetectable *BCR-ABL1* in 34,500 *ABL1* = $MR^{4.5}$. In the latter case, the inability to reliably detect very low levels of *BCR-ABL1* results in a false-negative result and misclassification of the molecular response from MMR to $MR^{4.5}$.

8.9 Effects of *BCR-ABL1* Transcript Type

An emerging issue is the fact that multiple studies have described an inferior molecular response to treatment for patients carrying the e13a2 *BCR-ABL1* transcript, compared to those with e14a2 [44–46]. There is some evidence that this difference may be at least partially explained by an amplification bias toward the e13a2 transcript when using the EAC RT-qPCR assay [47, 48] which, if confirmed, would necessitate careful evaluation of patient transcript type and would be an additional factor to consider in the standardization of molecular monitoring. Indeed, our preliminary analysis shows that accounting for the amplification efficiency of each transcript may reduce differences in relative amplification of *BCR-ABL1* and the reference gene. Furthermore, an individualized approach to molecular monitoring (i.e., measuring the reduction of *BCR-ABL1* relative to a baseline of the patient's *BCR-ABL1* level at diagnosis or start of treatment) also appears to negate the differential response to treatment [49].

8.10 Droplet Digital PCR

Droplet digital PCR (ddPCR) has been proposed as a solution to some of the challenges faced by RT-qPCR. The main advantage of ddPCR is that measurement of target copy number does not rely on an exogenous calibration curve, making inter-laboratory comparison of results potentially more straightforward. Several studies have now shown that ddPCR produces comparable results to RT-qPCR, and that it may improve the precision of measurement [50–52]. ddPCR also brings improvements in sensitivity (largely through the ability to more effectively test multiple replicates), which might allow for a more granular stratification of patients at or below MR^4, potentially identifying patients that may be at greater risk of relapse after stopping treatment [53, 54]. Additionally, a CE-IVD marked ddPCR assay for monitoring *BCR-ABL1* on the IS is now commercially available (QxDX BCR-ABL %IS Kit, BioRad), which seems to show improved sensitivity and precision compared to RT-qPCR [51], although a reduction in the variability of results may be a natural consequence of comparing a single ddPCR method against a diverse set of RT-qPCR protocols. Further work on developing a standardized ddPCR approach will likely be required before its widespread adoption in routine *BCR-ABL1* monitoring, and this technique remains cost-prohibitive for many.

8.11 Other Approaches

There has been considerable interest in the fact that *BCR-ABL1* mRNA levels vary between CML patients at diagnosis and that measurement of reductions in disease levels from pretreatment levels of individual patients may provide additional prognostic information [55, 56]. Hanfstein et al. determined the level of *BCR-ABL1* transcripts for each patient at diagnosis and compared this to the level after 3 months of treatment, using *GUSB* as the reference gene, and found a median 1.4-log reduction in *BCR-ABL1*. Those patients achieving a 0.46-log reduction of *BCR-ABL1* transcripts at 3 months had significantly better overall and progression-free survival, compared with patients not achieving a 0.46-log reduction [55]. In a slightly different approach (and using *BCR* as the reference gene), Branford et al. noted that despite some patients failing to reach the milestone of 10% $BCR\text{-}ABL1^{IS}$ after 3 months of treatment, there exists a subgroup of these patients that go on to achieve a good response to treatment. The authors calculated the time taken for *BCR-ABL1* levels to reach that of half the diagnostic level for this subgroup and found that patients whose *BCR-ABL1* had reduced by at least half within 76 days had significantly better outcomes than those whose halving time was >76 days, identifying an additional risk factor for patients that fail to reach the 10% milestone at 3 months [56]. Several further studies have shown the halving time to be prognostic of response when using *ABL1* as a control gene [57, 58] and with the use of second-generation TKIs [59]. However, it should be noted that this is an as yet completely unstandardized metric and is not included in current guidelines for routine monitoring of CML.

Alternative approaches such as amplification of patient-specific genomic DNA *BCR-ABL1* fusions might provide greater insights into the dynamics of the malignant clone [60–62]. Recent work has shown levels of genomic *BCR-ABL1* relative to the diagnostic sample after 3 months of treatment may be predictive of optimal response [63]. There also appears to be good agreement between the reduction of levels of *BCR-ABL1* gDNA and mRNA relative to the pretreatment baseline, and, interestingly, the presence of gDNA in mRNA negative samples may be predictive of a loss of DMR during TFR attempts [64], pointing to the presence of a population of CML stem cells that are not actively expressing *BCR-ABL1* mRNA.

The cartridge-based GeneXpert system (Cepheid) offers a more automated approach to *BCR-ABL1* monitoring. The system is RT-qPCR based but does not require the use of a standard curve. Instead, each production-lot of reagents are supplied precalibrated, allowing the delta-Ct between *ABL1* and *BCR-ABL1* to be measured and then used to calculate the ratio of *BCR-ABL1*:*ABL1*

[65]. The latest generation of this assay (Xpert *BCR-ABL* Ultra) is calibrated with secondary reference material aligned to the WHO *BCR-ABL1* genetic reference panel, allowing results to be reported directly on the IS and is sensitive enough to allow monitoring of DMR [66].

The application of single-cell sequencing to CML is also beginning to be investigated. Initial studies have demonstrated the heterogeneity of CML stem cells and revealed distinct subpopulations that persist through TKI treatment [67, 68]. The single-cell approach has been used to enhance molecular monitoring in acute myeloid leukemia [69] and may have the potential to provide similar benefits in CML.

Currently it is not clear if the measurement of *BCR-ABL1* mRNA levels pretreatment and/or using DNA-based approaches are really going to become routine practice, but both will require further standardization since the IS breaks down above levels of 10% when different reference genes are used [6], and it is unclear how to relate DNA-based results to the IS. It seems likely that RT-qPCR on the IS will continue to be the method of choice for monitoring CML patients in most centers for the foreseeable future although digital PCR could have a major impact if it was cheaper.

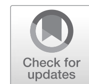
Acknowledgement The authors received research support from the European LeukemiaNet via the European Treatment and Outcome Study (EUTOS). Matthew Salmon was supported by the Salisbury District Hospital Stars Appeal.

References

1. Hughes T, et al. False-positive results with PCR to detect leukaemia-specific transcript. Lancet. 1990;335(8696):1037–8.
2. Sawyers CL, et al. Molecular relapse in chronic myelogenous leukemia patients after bone marrow transplantation detected by polymerase chain reaction. Proc Natl Acad Sci U S A. 1990;87(2):563–7.
3. Gabert J, et al. Detection of residual bcr/abl translocation by polymerase chain reaction in chronic myeloid leukaemia patients after bone-marrow transplantation. Lancet. 1989;2(8672):1125–8.
4. Hughes TP, Goldman JM. Biological importance of residual leukaemic cells after BMT for CML: does the polymerase chain reaction help? Bone Marrow Transplant. 1990;5(1):3–6.
5. Cross NC, et al. Minimal residual disease after allogeneic bone marrow transplantation for chronic myeloid leukaemia in first chronic phase: correlations with acute graft-versus-host disease and relapse. Br J Haematol. 1993;84(1):67–74.
6. Cross NC. Standardisation of molecular monitoring for chronic myeloid leukaemia. Best Pract Res Clin Haematol. 2009;22(3):355–65.
7. Cross NC, et al. Competitive polymerase chain reaction to estimate the number of BCR-ABL transcripts in chronic myeloid leukemia patients after bone marrow transplantation. Blood. 1993;82(6):1929–36.
8. Lion T, et al. Early detection of relapse after bone marrow transplantation in patients with chronic myelogenous leukaemia. Lancet. 1993;341(8840):275–6.
9. Malinge MC, et al. Quantitative determination of the hybrid Bcr-Abl RNA in patients with chronic myelogenous leukaemia under interferon therapy. Br J Haematol. 1992;82(4):701–7.
10. Hochhaus A, et al. Variable numbers of BCR-ABL transcripts persist in CML patients who achieve complete cytogenetic remission with interferon-alpha. Br J Haematol. 1995;91(1):126–31.
11. Hochhaus A, et al. Quantification of residual disease in chronic myelogenous leukemia patients on interferon-alpha therapy by competitive polymerase chain reaction. Blood. 1996;87(4):1549–55.
12. Aguiar RC, et al. Abnormalities of chromosome band 8p11 in leukemia: two clinical syndromes can be distinguished on the basis of MOZ involvement. Blood. 1997;90(8):3130–5.
13. Heid CA, et al. Real time quantitative PCR. Genome Res. 1996;6(10):986–94.
14. Lin F, et al. Correlation between the proportion of Philadelphia chromosome-positive metaphase cells and levels of BCR-ABL mRNA in chronic myeloid leukaemia. Genes Chromosomes Cancer. 1995;13(2):110–4.
15. Beillard E, et al. Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR)—a Europe against cancer program. Leukemia. 2003;17(12):2474–86.
16. Branford S, Hughes T. Diagnosis and monitoring of chronic myeloid leukemia by qualitative and quantitative RT-PCR. Methods Mol Med. 2006;125:69–92.
17. Branford S, et al. Rationale for the recommendations for harmonizing current methodology for detecting BCR-ABL transcripts in patients with chronic myeloid leukaemia. Leukemia. 2006;20(11):1925–30.
18. Jennings LJ, et al. Design and analytic validation of BCR-ABL1 quantitative reverse transcription polymerase chain reaction assay for monitoring minimal residual disease. Arch Pathol Lab Med. 2012;136(1):33–40.
19. Gabert J, et al. Standardization and quality control studies of 'real-time' quantitative reverse

transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia—a Europe Against Cancer program. Leukemia. 2003;17(12):2318–57.
20. Foroni L, et al. Guidelines for the measurement of BCR-ABL1 transcripts in chronic myeloid leukaemia. Br J Haematol. 2011;153(2):179–90.
21. Hughes TP, et al. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. N Engl J Med. 2003;349(15):1423–32.
22. Hughes T, et al. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. Blood. 2006;108(1):28–37.
23. Lauseker M, et al. Equivalence of BCR-ABL transcript levels with complete cytogenetic remission in patients with chronic myeloid leukemia in chronic phase. J Cancer Res Clin Oncol. 2014;140: 1965.
24. Cross NC, et al. Standardized definitions of molecular response in chronic myeloid leukemia. Leukemia. 2012;26(10):2172–5.
25. Baccarani M, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood. 2013;122(6):872–84.
26. Cross NC, et al. Laboratory recommendations for scoring deep molecular responses following treatment for chronic myeloid leukemia. Leukemia. 2015;29(5):999–1003.
27. Hochhaus A, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020;34(4):966–84.
28. Branford S, et al. Desirable performance characteristics for BCR-ABL measurement on an international reporting scale to allow consistent interpretation of individual patient response and comparison of response rates between clinical trials. Blood. 2008;112(8):3330–8.
29. Muller MC, et al. Harmonization of molecular monitoring of CML therapy in Europe. Leukemia. 2009;23(11):1957–63.
30. White HE, et al. Establishment of the first World Health Organization International Genetic Reference Panel for quantitation of BCR-ABL mRNA. Blood. 2010;116(22):e111–7.
31. Cross NCP, et al. Development and evaluation of a secondary reference panel for BCR-ABL1 quantification on the international scale. Leukemia. 2016;30(9):1844–52.
32. Ruiz MS, et al. Programme for Harmonization to the International Scale in Latin America for BCR-ABL1 quantification in CML patients: findings and recommendations. Clin Chem Lab Med (CCLM). 2020:20191283.
33. Zhang J-W, et al. Standardization of BCR-ABL1 quantification on the international scale in China using locally developed secondary reference panels. Exp Hematol. 2020;81:42–9.e3
34. White H, et al. A certified plasmid reference material for the standardisation of BCR-ABL1 mRNA quantification by real-time quantitative PCR. Leukemia. 2015;29(2):369–76.
35. White HE, et al. Establishment and validation of analytical reference panels for the standardization of quantitative BCR-ABL1 measurements on the international scale. Clin Chem. 2013;59(6):938–48.
36. Cayuela JM, et al. Cartridge-based automated BCR-ABL1 mRNA quantification: solving the issues of standardization, at what cost? Haematologica. 2011;96(5):664–71.
37. Scott S, et al. Measurement of BCR-ABL1 by RT-qPCR in chronic myeloid leukaemia: findings from an International EQA Programme. Br J Haematol. 2017;177(3):414–22.
38. Soverini S, et al. BCR-ABL kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: recommendations from an expert panel on behalf of European LeukemiaNet. Blood. 2011;118(5):1208–15.
39. Smith G, et al. A British Society for Haematology Guideline on the diagnosis and management of chronic myeloid leukaemia. Br J Haematol. 2020;191:171.
40. National Comprehensive Cancer Network Guidelines in Oncology: Chronic Myeloid Leukemia. Version 3.2020. 2020.
41. Rea D, Cayuela J-M. Treatment-free remission in patients with chronic myeloid leukemia. Int J Hematol. 2018;108(4):355–64.
42. CLSI, *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition. CLSI document EP17-A2*. 2012: Clinical and Laboratory Standards Institute.
43. White HE, et al. Variation in limit of blank for BCR-ABL1 detection between laboratories impacts on scoring of deep molecular response. HemaSphere. 2018;2(S1):140.
44. Hanfstein B, et al. Distinct characteristics of e13a2 versus e14a2 BCR-ABL1 driven chronic myeloid leukemia under first-line therapy with imatinib. Haematologica. 2014;99(9):1441–7.
45. Castagnetti F, et al. The BCR-ABL1 transcript type influences response and outcome in Philadelphia chromosome-positive chronic myeloid leukemia patients treated frontline with imatinib. Am J Hematol. 2017;92(8):797–805.
46. Jain P, et al. Impact of BCR-ABL transcript type on outcome in patients with chronic-phase CML treated with tyrosine kinase inhibitors. Blood. 2016;127(10):1269–75.
47. Kjaer L, et al. Variant-specific discrepancy when quantitating BCR-ABL1 e13a2 and e14a2 transcripts using the Europe Against Cancer qPCR assay. Eur J Haematol. 2019;103(1):26–34.
48. Bernardi S, et al. "Variant-specific discrepancy when quantitating BCR-ABL1 e13a2 and e14a2 transcripts using the Europe Against Cancer qPCR assay."

is dPCR the key? Eur J Haematol. 2019;103(3): 272–3.
49. Polakova, K.M., et al., *Individual molecular response evaluation on both DNA and mRNA BCR-ABL1 level diminished differences in time to molecular response achievement between CML patients with e13a2 vs e14a2 transcript type.* 25th Congress of EHA, 2020. EP742.
50. Fava C, et al. A comparison of droplet digital PCR and RT-qPCR for BCR-ABL1 monitoring in chronic myeloid leukemia. Blood. 2019;134(Supplement_1):2092.
51. Scott S, et al., *Digital PCR for the measurement of BCR-ABL1 in CML: A new dawn?*, in *25th Congress of EHA*. 2020.
52. Franke G-N, et al. Comparison of real-time quantitative PCR and digital droplet PCR for BCR-ABL1 monitoring in patients with chronic myeloid leukemia. J Mol Diagn: JMD. 2020;22(1):81–9.
53. Nicolini FE, et al. Evaluation of residual disease and TKI duration are critical predictive factors for molecular recurrence after stopping Imatinib first-line in chronic phase CML patients. Clin Cancer Res. 2019;25(22):6606.
54. Bernardi S, et al. Digital PCR improves the quantitation of DMR and the selection of CML candidates to TKIs discontinuation. Cancer Med. 2019;8(5):2041–55.
55. Hanfstein B, et al. Velocity of early BCR-ABL transcript elimination as an optimized predictor of outcome in chronic myeloid leukemia (CML) patients in chronic phase on treatment with imatinib. Leukemia. 2014;28(10):1988–92.
56. Branford S, et al. Prognosis for patients with CML and >10% BCR-ABL1 after 3 months of imatinib depends on the rate of BCR-ABL1 decline. Blood. 2014;124(4):511–8.
57. Fava C, et al. Early BCR-ABL1 reduction is predictive of better event-free survival in patients with newly diagnosed chronic myeloid leukemia treated with any tyrosine kinase inhibitor. Clin Lymphoma Myeloma Leuk. 2016;16:S96–S100.
58. Huet S, et al. Major molecular response achievement in CML patients can be predicted by BCR-ABL1/ABL1 or BCR-ABL1/GUS ratio at an earlier time point of follow-up than currently recommended. PLoS One. 2014;9(9):e106250.
59. Iriyama N, et al. Shorter halving time of BCR-ABL1 transcripts is a novel predictor for achievement of molecular responses in newly diagnosed chronic-phase chronic myeloid leukemia treated with dasatinib: results of the D-first study of Kanto CML study group. Am J Hematol. 2015;90(4): 282–7.
60. Ross DM, et al. Patients with chronic myeloid leukemia who maintain a complete molecular response after stopping imatinib treatment have evidence of persistent leukemia by DNA PCR. Leukemia. 2010;24(10):1719–24.
61. Bartley PA, et al. Sensitive detection and quantification of minimal residual disease in chronic myeloid leukaemia using nested quantitative PCR for BCR-ABL DNA. Int J Lab Hematol. 2010;32(6 Pt 1):e222–8.
62. Sobrinho-Simoes M, et al. In search of the original leukemic clone in chronic myeloid leukemia patients in complete molecular remission after stem cell transplantation or imatinib. Blood. 2010;116(8):1329–35.
63. Pagani IS, et al. BCR-ABL1 genomic DNA PCR response kinetics during first-line imatinib treatment of chronic myeloid leukemia. Haematologica. 2018;103(12):2026.
64. Machova Polakova K, et al. Analysis of chronic myeloid leukaemia during deep molecular response by genomic PCR: a traffic light stratification model with impact on treatment-free remission. Leukemia. 2020;34(8):2113–24.
65. Winn-Deen ES, et al. Development of an integrated assay for detection of BCR-ABL RNA. Clin Chem. 2007;53(9):1593–600.
66. Day G-J, et al. Development of Xpert® BCR-ABL ultra, an automated and standardized multiplex assay with required performance characteristics for BCR-ABL1 quantitative measurement on an international reporting scale. Blood. 2015;126(23):2793.
67. Giustacchini A, et al. Single-cell transcriptomics uncovers distinct molecular signatures of stem cells in chronic myeloid leukemia. Nat Med. 2017;23(6):692–702.
68. Warfvinge R, et al. Single-cell molecular analysis defines therapy response and immunophenotype of stem cell subpopulations in CML. Blood. 2017;129(17):2384–94.
69. Ediriwickrema A, et al. Single-cell mutational profiling enhances the clinical evaluation of AML MRD. Blood Adv. 2020;4(5):943–52.



9 Prognostic Scores for Patients with Chronic Myeloid Leukemia under Particular Consideration of Disease-Specific Death

Markus Pfirrmann, Michael Lauseker, Verena S. Hoffmann, and Joerg Hasford

9.1 What Are Prognostic Scores?

In medicine, patient characteristics showing a statistically significant and clinically relevant association with a well-defined future outcome of a disease are denoted as prognostic factors. Several prognostic factors can be combined in a prognostic score. This combination consists typically of a mathematical formula where a certain weight and, possibly, a certain transformation are attributed to each factor. At last, by a precise rule, the prognostic score is categorized into two or more prognostic groups. To define meaningful prognostic groups, their prediction of outcome should display clinically relevant differences between all of them. The combination of prognostic score and classification rule could be summarized under the term "prognostic model." However, in the field of chronic myeloid leukemia (CML), the use of "score" is more common, and we will use "model" and "score" equivalently [1].

Usually, prognostic scores are developed with respect to a single outcome, which was prospectively determined as the primary end-point, and in a well-defined sample of patients who had received similar treatments. Treatments are regarded similar if no clinically relevant outcome differences have been observed between them and if their mode of action is comparable. Derived from the marker definition of Sargent et al. [2], a score actually can be called "prognostic" for one or more treatments if it categorizes patients into groups with different outcomes when receiving similar treatments. If within the same prognostic group outcome differs between treatments, a score could be called "predictive" for this group [2].

9.2 Relevance of Prognostic Scores

Prognostic scores serve a variety of important tasks in modern medicine. Among the most important ones are predicting the outcome for individual patients, selection of the optimal treatment, development of risk-adjusted treatments, adjustment of imbalances between treatment groups in clinical trials, and comparative assessment of outcomes of different studies [3, 4]. For instance, to prevent possible imbalances between treatment groups, design and analysis of both trials testing the second-generation tyrosine kinase inhibitors (TKIs) nilotinib and dasatinib for treatment of CML comprised stratification according to a prognostic score [5, 6]. Consequently,

M. Pfirrmann (✉) · M. Lauseker · V. S. Hoffmann
J. Hasford
Institut für Medizinische Informationsverarbeitung, Biometrie und Epidemiologie – IBE, Ludwig-Maximilians-Universität, Munich, Germany
e-mail: pfi@ibe.med.uni-muenchen.de

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
R. Hehlmann (ed.), *Chronic Myeloid Leukemia*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-71913-5_9

prognostic scores continue to be part of the European LeukemiaNet (ELN) recommendations for the treatment of CML [7].

9.3 Baseline Prognostic Scores

The Sokal [8], the Euro [4], the EUTOS [9], and the EUTOS long-term survival (ELTS) [10] scores are the baseline scores mentioned in the ELN treatment recommendations [7]. All four scores refer to patient characteristics at diagnosis as "baseline" (Table 9.1). Calculated with baseline characteristics before any therapy is initiated, the scores were meant for predicting treatment outcome of Philadelphia chromosome-positive (Ph+) patients in non-blastic [8] or chronic phases (CP) [4, 9, 10]. While CP stands for stable disease, accelerated (AP) and blastic phases (BC) define progression of CML [11].

9.3.1 Sokal Score

Published in 1984, the development of the Sokal score was based on the data of patients who were

Table 9.1 Established baseline prognostic scores in chronic myeloid leukemia

	Patient population[a]	Formula	Risk groups
Sokal score[b]	678 patients treated with chemotherapy; diagnosed from 1962–1981	Sokal score = 16 × (age [in years] − 43.4) +0.0345 × (spleen size [cm below costal margin] − 7.51) +0.1880 × ((platelet count [in 10^9/L]/700)^2–0.563) +0.0887 × (blasts [% in peripheral blood] − 2.10))	Low risk: <0.80 Intermediate risk: ≥ 0.80 and ≤ 1.20 High risk: >1.20
Euro score[c]	908 patients treated with interferon-alpha; diagnosed from 1983–1994	Euro score = (0.6666 × age [0 when age < 50 years; 1, otherwise] + 0.0420 × spleen size [cm below costal margin] + 0.0584 × blasts [% in peripheral blood] + 0.0413 × eosinophils [% in peripheral blood] + 0.2039 × basophils [0 when basophils [% in peripheral blood] < 3; 1, otherwise] + 1.0956 × platelet count [0 when platelets count [in 10^9/L] < 1500; 1, otherwise]) × 1000	Low risk: ≤780 Intermediate risk: > 780 and ≤ 1480 High risk: >1480
EUTOS score[d]	926 patients treated with imatinib; diagnosed from 2002–2006	EUTOS score = 7 × basophils [% in peripheral blood] +4 × spleen size [cm below costal margin]	Low risk: ≤87 High risk: >87
ELTS score[e]	2205 patients treated with imatinib; diagnosed from 2002–2006	ELTS score = 0.0025 × (age [in completed years]/10)^3 + 0.0615 × spleen size [in cm below costal margin] + 0.1052 × blasts [% in peripheral blood] + 0.4104 × (platelet count [in 10^9/L]/1000)$^{-0,5}$	Low risk: ≤1.5680 Intermediate risk: >1.5680 and ≤2.2185 High risk: >2.2185

[a]Number of patients finally used for estimation of regression coefficients
[b]Ref. Sokal, Cox, Baccarani, Tura, Gomez, Robertson, Tso, Braun, Clarkson, Cervantes, Rozman, and the Italian Cooperative CML Study Group [8]
[c]Ref. Hasford, Pfirrmann, Hehlmann, Allan, Baccarani, Kluin-Nelemans, Alimena, Steegmann, Ansari [4]
[d]Ref. Hasford, Baccarani, Hoffmann, Guilhot, Saussele, Rosti, Guilhot, Porkka, Ossenkoppele, Lindoerfer, Simonsson, Pfirrmann, Hehlmann [9]
[e]Ref. Pfirrmann, Baccarani, Saussele, Guilhot, Cervantes, Ossenkoppele, Hoffmann, Castagnetti, Hasford, Hehlmann, Simonsson [10]

treated with chemotherapy (Table 9.1). The score allocates patients into three prognostic groups predicting different overall survival (OS) probabilities for chemotherapy-treated patients [8]. The score calculation is supported by https://www.leukemia-net.org/content/leukemias/cml/euro__and_sokal_score/.

9.3.2 Euro Score

Applied to a large sample of 1201 patients treated with interferon alpha (IFN), the Sokal score was not able to differentiate OS probabilities between the intermediate- and the high-risk groups in a clinically satisfactory manner [4]. Thus, in 1998, Hasford et al. suggested a new prognostic score for patients treated with IFN (Table 9.1) [4]. In an independent validation sample of 493 patients, the ability of the three resulting risk groups to significantly differentiate OS probabilities was confirmed [12]. The Euro score was prognostic also for hydroxyurea (HU)-treated patients and predictive as, within the low- and the intermediate-risk groups, interferon-treated patients had significantly higher OS probabilities than HU-treated patients [12]. The score calculation is supported by https://www.leukemia-net.org/content/leukemias/cml/euro__and_sokal_score/.

9.3.3 EUTOS Score

With publication of the IRIS trial [13], due to the remarkable survival results with imatinib, it became obvious that for a long time, a significant discrimination of OS probabilities between patient groups treated with imatinib and future, potentially even more improved treatments was unlikely. However, investigators wanted to identify advantages of new treatment approaches earlier on. Consequently, the phase III trials leading to the approval of nilotinib and dasatinib substituted OS by remission probabilities as primary endpoint [5, 6]. Accordingly, Hasford et al. introduced the EUTOS score [9]. Complete cytogenetic remission (0% Ph + marrow cell metaphases, CCyR) after 18 months of therapy was chosen as the primary outcome. The abilities of the EUTOS score to discriminate two risk groups with significantly different CCyR probabilities at 18 months and with significantly different progression-free survival probabilities over time were confirmed by an independent validation based on 616 and 1190 patients, respectively [14]. The score calculation is supported by https://www.leukemia-net.org/content/leukemias/cml/eutos_score/.

9.3.4 EUTOS Long-Term Survival (ELTS) Score

The shift from IFN to TKIs improved OS probabilities from a median survival of about 6 years [4] to a 10-year survival probability of about 80% – at least within clinical trials [15–17]. The highly improved OS probabilities induced by TKIs are a consequence of the shrinking proportion of deaths due to CML. While the 10-year probabilities of dying of CML were 6% and 8% according to Hehlmann et al. [15] and Molica et al. [17], the 10-year probabilities of dying of another cause amounted to 12 and 16%, respectively. With two-thirds of deaths not directly related to CML, investigators wonder in what respect OS probabilities are still interpretable in dependence on CML treatment. Reducing the probabilities of dying of leukemia is the focus of CML therapy. Consequently, in differentiating prognostic risk groups with respect to TKI treatment, the ELTS score was developed for the primary endpoint "death due to CML" while considering all other causes of death as competing risks. In 2205 patients with first-line imatinib treatment, the ELTS score identified three risk groups with significantly different probabilities of CML-specific death. In a validation sample comprising 1120 patients, the significant discrimination of the three groups was confirmed [10]. The score calculation is supported by https://www.leukemia-net.org/content/leukemias/cml/elts_score/ and, like all previous scores, via the Hematology app.

9.4 Methodological Challenges in the Presence of Competing Risks

Depending on the aim of research, different methodological approaches to analyze the influence of a certain cause of death on survival probabilities may be pursued.

9.4.1 Relative Survival Probabilities

Through access to population data which can be downloaded from the Human Mortality Database [18] (www.mortality.org), it is possible to extract survival probabilities of a population matched to a patient sample of a study of interest. The matching criteria are country of study origin and sex as well as age and calendar year when CML treatment was started. Thus adjusting for all causes of death (which are considered in population data), the then estimated relative survival probabilities are only affected by the "excess mortality" which is attributable to CML in the patient sample investigated. In accordance with the method of Pohar-Perme et al. [19], relative survival probabilities are based on the estimation of "net survival," which can be calculated from the excess hazard of dying of CML. Technically, the hazard of dying of CML is also part of the population-based hazard. However, in the general population, the number of cases where people die of CML is negligible in comparison to all other causes of death. This is in contrast to the excess hazard which is to be expected in a sample of patients suffering from CML. In 2290 patients of the in-study section of the EUTOS registry, 8-year relative survival probability was 96% [95% confidence interval (CI), 93–97%] [10].

9.4.2 Probabilities of Dying of CML

Without involvement of external population data, probabilities of dying may be calculated from the patient sample alone. In this case, causes of death have to be differentiated by the investigators themselves. With the ELTS score, as "death due to CML," only death after recorded disease progression was regarded [10]. Progression was given by the observation of AP or BC, both phases defined in accordance with the recommendations of the ELN [11]. To consider only death after recorded disease progression as "death due to CML" surely underestimates the number of events actually attributable to CML. Other causes of death without prior progression, like infection or possibly treatment-related toxicities, might well be attributable to CML. However, using the "progression prerequisite," one can almost rule out ambiguous cases regarding cause of death, and, as with the ELTS score, regression modeling for cumulative incidence probabilities (CIPs) will be based on "real" cases of death due to CML. The underestimation of the CIPs for death due to CML and the reduction of statistical power remain obvious drawbacks.

To estimate the probabilities of dying of CML, causes of death unrelated to CML have to be considered as competing risks because they prevent the potential observation of a death due to CML. In the presence of competing risks, the use of the Kaplan-Meier method would lead to biased estimates. Instead, CIPs are estimated with the cumulative incidence function (CIF) [20, 21]. In the field of CML, an easy-to-follow example to explain the differences between the wrong use of the Kaplan-Meier method and the correct application of the CIF has been published earlier [22]. For the calculation of confidence intervals, the method of Choudhury can be used [23].

9.4.3 Prognostic Modelling under Consideration of Competing Risks

In the presence of competing risks, two different types of hazards should be considered for all competing events: the cause-specific hazard (CSH) and the subdistribution hazard (SH) [24, 25].

In the case of proportional CSHs, the hazard ratio (HR) for the particular event of interest, like death due to CML, can be estimated with the well-known standard Cox proportional hazards

model [26]. Technically, the times of all other kinds of death are censored, just like the cases which are censored due to the end of follow-up. For the investigated cause of interest, at time t, the CSH gives the rate of failure per time unit (e.g., per day) *for individuals still alive just before t* [24]. Accordingly, the CSH can be interpreted as the epidemiological rate of an event of interest, ruling out the possibility that the patient is at risk of any competing event. However, the cumulative incidence probabilities of death due to CML also depend on the CSHs of all competing events [24]. As a result, the assumption of proportional CSHs precludes proportional CIPs. Thus, a simple relationship between the CSH of a certain event and its cumulative incidences does not exist, and unlike standard survival analysis without any competing events present, the cause-specific hazards model can be interpreted as a hazards model but does not provide a simple relationship between covariates and the easier interpretable CIPs [24].

In contrast, the subdistribution hazards model keeps individuals who have already experienced a competing event in the risk set. Thus, for a specific event of interest, at time t, the SH provides the rate of failure per time unit *for individuals who are either still alive or have already died from other causes before t* [24]. Assuming proportionality, the SH ratio (SHR) can be estimated by the Fine–Gray model [27]. Proportional SHs imply proportional CIFs, and, therefore, the Fine–Gray model provides a direct interpretation of the influence of explanatory factors on the event probabilities (CIPs) [27]. However, while the CSH of an event "A" is influenced only by effects that operate directly on this event, the SH is also influenced by effects that operate on the competing event "B". That means, even without a direct effect on event "A", a strong positive predictor of event "B" will be found influential on the SH for event "A" as well (with a negative coefficient) but not influential on the CSH for event "A" [24, 28]. As patients who have experienced a competing event remain in the risk set for the event of interest, in contrast to the CSH, the SH is not a rate in the standard epidemiological sense [24]. To understand the meaning of explanatory factors, the use of both the CSH and the SH model is encouraged [24, 25].

With preference for a direct interpretation of prognostic factors on event probabilities, the Fine–Gray model was used for the development of the ELTS score. For exemplary interpretation of results, $(\text{age in years}/10)^3$ and spleen size enlargement (cm below costal margin) are considered. When "death due to CML" was the event of interest, the subdistribution hazard ratio of spleen size was 1.063 [95% CI, 1.029–1.099] and for age 1.003 [95% CI, 1.001–1.005] (Table 3, [10]). Thus for a spleen size increase by 1 cm, the hazard for an event was estimated to increase by 6.3%. Since modeling of age is not linear, increases of risks are not constant when the differences between two patients' age values are the same. For example, the expression "$(\text{age in years}/10)^3$" results in "27" for a 30-year old, in "64" for a 40-year old, in "216" for a 60-year old, and in "343" for a 70-year old. With otherwise the same values in the rest of the score variables, the SHR of a 40-year old to a 30-year old is calculated by $(1.003)^{(64-27)} = 1.003^{37} = 1.117$. Hence, the risk of dying of CML increases from 30 to 40 years of age by 11.7%. However, with an SHR of $(1.003)^{(343-216)} = 1.463$, the risk of dying of CML increases from 60 to 70 years by 46.3%.

With application of the Fine–Gray model, the individuals with causes of death unrelated to CML remained in the risk set. Applying the CSH model, death due to causes unrelated to CML was censored. However, for spleen size (1.065, [95% CI, 1.029–1.104]) and age (1.003, [95% CI, 1.001–1.005]), the resulting CSH ratios were similar to the SH ratios (second part of Supplementary Table 3, [10]).

Apart from other factors, the event "death unrelated to CML" was also significantly influenced by age (SHR: 1.008 [95% CI, 1.006–1.009]) but not by spleen size (Supplementary Table 4, [10]). Again, the cause-specific hazards model provided results which were largely alike to the ones of the Fine–Gray model; for age the CSH was even identical (second part of Supplementary Table 4, [10]).

Since the two hazard ratios estimated by the CSH and the SH model were hardly different,

spleen size can be regarded to have an actual effect on the CIPs of death due to CML, and its significance is not due to an indirect effect on the competing event "death unrelated to CML." The equivalent interpretation holds for the effect of age on death due to CML and also on death unrelated to CML. Both events "death due to CML" and "death due to causes unrelated to CML" seem to be independent. In this case, one event could be interpreted as an independent censoring of the other one. Statistically, it is not possible to verify this independence. In general, this concordance between the estimates and the reasonable fulfillment of the proportionality assumption for both models does not apply. Instead, investigators should be aware that (usually) only one of the two proportionality assumptions (either proportional CSH or proportional SH) is met [24, 25]. The observation of apparent independence was likely boosted by analyzing data of the in-study section of the registry [10]. The in-study section consisted of patients from randomized clinical trials. Here, in- and exclusion criteria prevented inclusion of patients with quite a few serious concomitant diseases. Thus, for many patients with high-risk at diagnosis, CML was the primary danger to life when treatment was started. Typical prognostic factors for CML, like spleen size and blasts, were identified as relevant for death due to CML but not for death due to other causes. In the course of CML treatment, further diseases were acquired "independently" (?) of CML.

The cause-specific hazards model may be preferable when the main interest is disease etiology, since it is interpretable as an epidemiological rate among the individuals who are actually at risk [24]. The subdistribution hazards model has a direct relation to the cumulative incidence probabilities. These probabilities are easier to interpret. Thus, subdistribution hazards are better suited when the focus is on prognostic modeling [24]. However, the choice of the model should also take the validity of the proportional hazards assumption into account.

9.5 Validation of Prognostic Scores

9.5.1 Prerequisites for Patient Samples and Endpoints

When considering application and validation of a prognostic score, it is essential to keep the patient sample in mind that was used for the original development of the score. The ELTS score was developed in Ph + or BCR-ABL1-positive adult patients diagnosed in chronic phase receiving imatinib as first-line treatment [10]. Accordingly, a satisfactory prognostic performance of the score should primarily be expected and evaluated in similar patient samples.

Furthermore, validation should primarily be attempted for the endpoint(s) the authors of the score claimed that their prognostic model would work for. Since the ELTS score was developed to discriminate probabilities of "death due to CML", statistically significant and clinically relevant differences between the score risk groups should be identified for this endpoint in an appropriate validation sample. In addition, though not optimized for it, the creators of the ELTS score stated that it would also perform with respect to OS probabilities considering any kind of death. For any other endpoint, reasonable risk group discrimination was not claimed nor can it be guaranteed.

Finally, the term "appropriate validation sample" comprises not only an adequate definition by in- and exclusion criteria but, in general, also the number of cases. For time-to-event endpoints like probabilities of dying or of survival, rather the number of events matters. Obviously, small patient samples and short follow-up times are opposed to the probability to observe a sufficient number of events. It is inappropriate to report the failure of a prognostic model if in the validation attempt, the number of events in the risk groups was too low. Hazard rates and hazard ratios may be retrieved from the original publication of the score. Without the presence of competing risks, sample size estimation for the validation sample could then be performed using the freely available program "PS: Power and Sample Size

Calculation." The program can be found on http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize. Sample size estimation is based on the method of Schoenfeld and Richter [29]. In case of the presence of competing risks, the ratio of the subdistribution hazards is of relevance. Here, Latouche and Porcher [30] provided an approach to estimate the necessary sample size.

For sake of the quality of any study, it is highly recommended to work with 95% CIs when presenting new estimates in relation to the endpoints of interest. Confidence intervals help readers to assess the power of a study to provide a significant finding if the supposed differences (between risk groups) were actually true.

Of note, the prognostic performance of a score might be regarded as "adequate" even if it was assessed in a patient sample or for an endpoint the prognostic model was not originally intended for. Significant discrimination may also be discovered in samples with rather few events. Examples are provided in the section on the validation of the ELTS score. However, using the correct methodological approach when assessing the prognostic performance with respect to a certain endpoint is inevitable. The presence of competing risks may not be ignored.

9.5.2 The Importance of Validation

Validation of a prognostic model is indispensable. The development of a prognostic model in a learning sample, like the data on the 2205 patients used for the ELTS score, is an explorative proceeding and could be regarded as the suggestion of the hypothesis that the score should also work for other patient samples alike. Accordingly, the prognostic performance has to be assessed in data different from the learning sample and independent of the score's development. It adds to the likely usefulness of a score if it has already been published together with its successful validation in an independent patient sample. However, it is more convincing and absolutely desirable that a prognostic model can later be validated by independent investigators, ideally from different countries and ideally also in validation samples which were as representative as possible for all CML patients. Patient samples coming from population-based registries with hardly any exclusion criteria are more representative for all CML patients than patient samples from randomized clinical trials where many CML patients, e.g., with serious comorbidities, were excluded.

The chance of a successful score validation depends on the development process of the model in the first place. Wyatt and Altman provided criteria to examine the use of a prognostic model in order to support clinical decisions [31]. Main criteria are clinical credibility, evidence of accuracy, and evidence of generality. From a methodological point of view, above comments on sample size and the report of 95% CIs for all relevant estimates (like hazard ratios) also apply in the development phase. It is important to choose the correct statistical model when analyzing the influence of potential prognostic factors. In multiple regression models, as a rule of thumb, it was recommended that for each prognostic candidate variable included, at least 10 events should have been observed for the dependent variable of interest [32]. Dichotomizing continuous variables at an early modelling stage is rather discouraged [33]. It is not unlikely that another learning sample would lead to another cutoff. As an alternative to linear modelling of a continuous variable, fractional polynomials could be considered [34]. Variable selection and the assessment of the stability of the multiple model may be supported by bootstrap resampling [35]. The common prognostic influence of all finally selected variables is summarized in the actual prognostic score. To arrive at a clinical decision, in dependence on their (continuous) prognostic score values, patients could be allocated to different risk groups (e.g., low-, intermediate-, and high risk). The definition of clinically useful risk groups is boosted when the smallest risk group still comprises around 10% of all patients. It is important to adjust for multiple testing when searching for the statistically "optimal" cutoff between risk groups [36]. Without adjustment, the probability

of finding *some* cutoff is relatively high but the chance for reproducibility of *the same* cutoff as statistically meaningful in another patient sample is rather low. A prognostic model with a prognostic performance that cannot be satisfactorily reproduced in and generalized to independent patient data is useless.

It is recommended to abstain from hardly interpretable, frequently biased composite endpoints combining outcome of heterogeneous severity and providing results with limited reproducibility in another patient sample [22]. Lack of their reproducibility is usually a mixture of differences in definition, calculation, and measurement bias of endpoints.

In attempting validation, the prerequisites outlined in the previous section should be considered. For a successful validation of a prognostic model that was meant to discriminate a survival endpoint, various testing is possible: the (subdistribution) hazard ratios between the risk groups should point in the same direction as in the learning sample, e.g., with higher hazard rates for higher risk groups (see Table 2; [10], or Fig. 1, [37]). Ideally, with a sufficient sample size, the 95% CIs around the (S)HR estimates exclude "1," i.e., reject "no significant difference" between the risk groups. Alternatively, log-rank or the Gray test [38] provide significantly different survival or cumulative incidence probabilities. Especially for a comparative assessment of the discrimination abilities between different prognostic models, a method like the truncated concordance index suggested by Wolbers et al. [39] is useful. A higher concordance index hints at a better discrimination of the survival outcome. With indices greater than 50, a prognostic model provides clinically useful information different from chance; the closer to 100, the more supportive the model is [37].

9.5.3 Validation of the ELTS Score in 2949 Patients of the EUTOS Registry Patients

In 2007, a registry of CML patients was established by the European LeukemiaNet (ELN) and maintained within the EUTOS framework. The first two sections of the registry comprise individual data on adult chronic-phase patients prospectively enrolled between 2002 and 2006, either within or outside a clinical trial (in-study and out-study sections, respectively). Within the population-based section of the registry, data on adult patients, diagnosed with Ph + and/or BCR-ABL1-positive CML between 2008 and 2013, were collected, aiming to include all new CML patients within well-defined European regions at the time [40]. Adding 1831 patients of the population-based section to 1118 patients of the out-study section (in an update, two cases were identified as double entries) a sample of 2949 patients with data entirely independent of any score development was formed [37]. Median age of the 2949 patients was 52 years (range: 18–91 years); 52% were male. Survival time was measured from the date of start of TKI treatment to death or to the latest follow-up date.

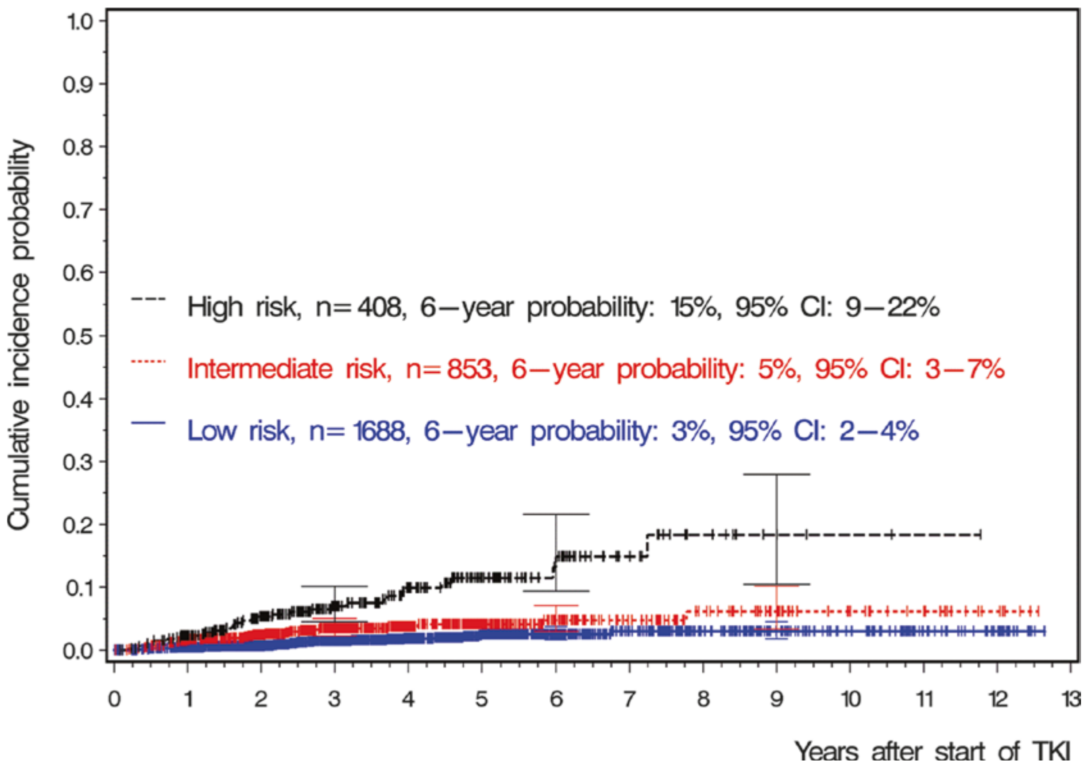
9.5.3.1 Calculation of Survival Probabilities

Survival was censored at the time of allogeneic hematopoietic stem cell transplantation in first CP. Again, progression was defined by the observation of AC or BC, with both phases determined according to the ELN criteria [11]. CP was defined by the absence of progression. Only death after recorded disease progression was regarded as "death due to CML". Death without prior disease progression was rated as "death unrelated to CML" [37]. OS probabilities were estimated by the Kaplan-Meier method and the hazard ratios (HRs) for dying from any cause were calculated by the Cox regression model [41]. When differentiating competing causes of death, cumulative incidence probabilities of dying of CML were obtained using the Aalen–Johansen estimator [21, 23] and the subdistribution hazard ratios (SHRs) for dying of CML were estimated using the Fine–Gray model [27]. Median follow-up was 3.3 years (range: 0.01–12.6 years). With a recorded progression as prerequisite, causes of death were due to CML in 89 of 236 cases (38%).

Ten-year OS probability in the 2949 patients was 82% (95% CI: 79–85%), and 10-year probability of death due to CML was 6% (95% CI: 4–7%). To describe survival probabilities at a time when also in the subgroups a sufficiently large number of patients was still under observation, in the following, the focus was on 6-year survival probabilities. At 6 years, OS probability was 88% (95% CI: 86–89%), and 6-year probability of death due to CML was 5% (95% CI: 4–6%).

9.5.3.2 Successful Validation for Probabilities of Dying of CML

Cumulative incidences were compared by the SHRs of the Fine–Gray model. The significance level of the two-sided p-values was 0.05. The ELTS score was able to discriminate the cumulative incidence probabilities of dying of CML (Fig. 9.1). The intermediate-risk group ($n = 853$, 29%; $p = 0.0031$) and the high-risk group ($n = 408$, 14%; $p < 0.0001$) had significantly



Number of patients still at risk (n) at different years of observation

Year	*0*	*3*	*6*	*9*
Low risk, n	1688	935	302	86
Intermediate risk, n	853	427	115	34
High risk, n	408	177	43	6

Fig. 9.1 Cumulative incidence probabilities of death due to CML treating death due to causes not related to CML as competing risk in 2949 patients from the combined out-study and population-based registry sections, considering risk group stratification according to the ELTS score. At 3, 6, and 9 years, horizontal crossbars indicate the upper and lower limit of the 95% confidence interval for the estimated cumulative incidence probability of death due to CML.

higher incidence probabilities than the low-risk group ($n = 1688$, 57%). The corresponding SHRs were 2.203 (95% CI: 1.306–3.718) and 5.646 (95% CI: 3.397–9.387). The concordance indices at 1, 5, and 10 years were 68.0, 66.0, and 68.1 [37]. Differences between intermediate- and high-risk patients were also significant ($p = 0.0002$).

Lauseker and Zu Eulenburg [42] elucidated that the use of the competing risk model led to biased cumulative incidence probability estimates when the censoring mechanism differs between status, e.g., between patients in chronic- or progressive phases. In the case of a status-dependent censoring mechanism, they showed that the progressive illness-death model should be preferred over the competing risk model. In the combined out-study/population-based sample, of 2949 patients, 153 patients (5%) experienced progression. The cumulative hazard of censoring was significantly higher for patients in the progressive phase ($p < 0.0001$). This led to slightly biased cumulative incidence probabilities for death after progression when compared with the gold standard of the progressive illness-death model. In the 2949 patients, after 8 years, the probability of death after progression was 7.3% with the progressive illness death model and 5.7% with the competing risk model. Applying the illness death model, the significantly different hazards for both the transitions from CP into progression and from CP into death in CP confirmed a satisfactory discrimination between the risk groups of the ELTS score (Supplementary Table 2, [37]).

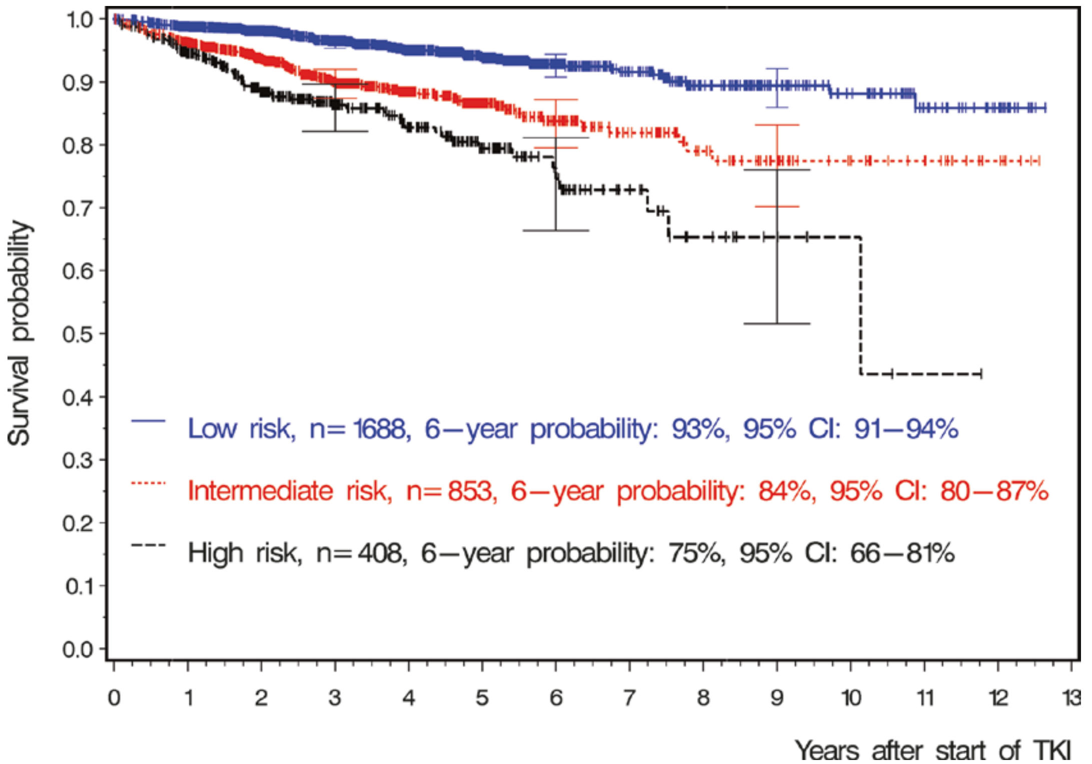
9.5.3.3 Successful Validation for Overall Survival Probabilities

The intermediate-risk group ($p < 0.0001$) and the high-risk group ($p < 0.0001$) of the ELTS score had significantly lower OS probabilities than the low-risk group (Fig. 9.2). The corresponding HRs were 2.479 (95% CI: 1.836–3.345) and 4.012 (95% CI: 2.884–5.582). The concordance indices at 1, 5, and 10 years were 65.6, 64.0, and 64.0 [37], respectively. Differences between intermediate- and high-risk patients were also significant ($p = 0.0031$).

The ELTS score has already several times provided a significant discrimination of risk groups with respect to OS probabilities [37, 43–45]. However, it is important to keep in mind that the ELTS score was developed to discriminate probabilities of dying of CML. Accordingly, the ELTS score was not "optimized" to discriminate OS probabilities. Regarding OS probabilities, for sure, a superior score could have been identified in the 2205 patients of the learning sample. As seen in Sect. 9.4.3., in the best multiple model for the cumulative event probabilities of "death unrelated to CML," with 1.008, age (in years/10)^3 had a higher SHR than in the ELTS model for "death due to CML" (SHR = 1.003). With 1.003, the SHR for dying of CML of a 70-year old in relation to a 60-year old was $(1.003)^{(343-216)} = 1.463$, an increase of 46.3%. In contrast, for the same ages, the SHR for death unrelated to CML was $(1.008)^{(343-216)} = 2.751$, a hazard ratio almost twice as high. Both calculations are based on the assumption that the patients differed only in age and otherwise had the same values in the remaining variables of the respective model. Although the two models are not directly comparable, the essential message is that at higher ages, a stronger increase of the hazard of dying of a cause unrelated to CML than of the hazard of dying of CML is to be expected. With OS probabilities built on both kinds of death, in an "optimized" model for OS, age should play a more prominent role than with the ELTS score.

9.5.3.4 Successful Validation by Independent Study Groups

Successful validation of the ELTS score was also reported by several independent investigators. In some cases, validation was successful despite a rather low number of events in the risk groups, patients' samples quite different from the original learning sample of the ELTS score, or using endpoints the score was not intended for. Results are presented below. However, results of validation attempts where the statistical methods were not correct or complex composite endpoints were used are not referred to.



Number of patients still at risk (n) at different years of observation

Year	*0*	*3*	*6*	*9*
Low risk, n	1688	935	302	86
Intermediate risk, n	853	427	115	34
High risk, n	408	177	43	6

Fig. 9.2 Overall survival probabilities with death due to any cause in 2949 patients from the combined out-study and population-based registry sections, considering risk group stratification according to the ELTS score. At 3, 6, and 9 years, horizontal crossbars indicate the upper and lower limit of the 95% confidence interval for the estimated survival probability.

Geelen et al. [44] applied the ELTS score to 709 patients with first-line imatinib treatment. The score was able to identify three pairwise significantly different risk groups with respect to OS and to achievement of a first major molecular response. With only 23 deaths after progression, between low- and high-risk patients, the ELTS score also provided satisfactory differences in cumulative incidences of death due to CML, but numbers were too low to allow a reliable assessment of prognostic performance.

Yang et al. [45] observed significant risk group discriminations of OS probabilities between the high-risk group and each of the two more favorable risk groups when the ELTS score was applied to 462 imatinib-treated Chinese patients. Median follow-up was 69 months; the total number of events was not given.

Millot et al. [46] found that the ELTS risk groups differed significantly from each other with respect to progression-free survival in 350 children with imatinib as first-line treat-

ment—despite only 23 events (progression or death).

In 202 Italian patients ≥65 years treated with imatinib or nilotinib, the ELTS score provided a significant discrimination of the three risk groups regarding major (MMR, BCR-ABL1 ≤ 0.1%, international scale, IS) and deep molecular remission (MR^4, BCR-ABL1 ≤ 0.01%, IS), regarding OS probabilities and the probabilities of leukemia-related deaths [43]. Median follow-up of the patients was 77 months.

The ELTS score was also applied to 258 patients diagnosed in advanced phase and significantly distinguished OS probabilities between high-risk patients and each of the more favorable risk groups but not between low- and intermediate-risk patients [47].

Of note, publication bias could be present if unsuccessful validation attempts are not reported. However, this is only of relevance in attempts where the prerequisites for patient samples and endpoints had been met.

9.6 Comparative Assessments of the Four Prognostic Scores

9.6.1 Comparative Assessment of the Scores in 2949 Independent Patients of the EUTOS Registry

Not only the prognostic performance of the ELTS score but also of the three other scores was investigated within the 2949 patients of the combined out-study and population-based sections of the registry.

9.6.1.1 Comparative Assessment of Probabilities of Dying of CML

In contrast to the ELTS score, the Sokal score failed to identify an intermediate-risk group with significantly different probabilities of dying because of CML than the low-risk group (Fig. 9.3). However, the high-risk group of the Sokal score (n = 698, 24%) had significantly higher probabilities of dying because of CML than the intermediate-risk group (n = 1177, 40%; p = 0.0014) and the low-risk group (n = 1074, 36%; $p < 0.0001$). The SHR of high- to low-risk patients was 3.559 (95% CI: 2.030–6.240) and of intermediate- to low-risk patients 1.668 (95% CI: 0.934–2.978). The concordance indices at 1, 5, and 10 years were 59.7, 62.4, and 63.3, respectively [37].

With the Euro and the EUTOS scores, discrimination abilities were also inferior to the ones of the ELTS score. The Euro score was not able to find a significant discrimination between the intermediate- and the low-risk group, and the EUTOS score was not able to find a significant discrimination between the low- and the high-risk group (Supplementary Fig. 2a–b, [37]).

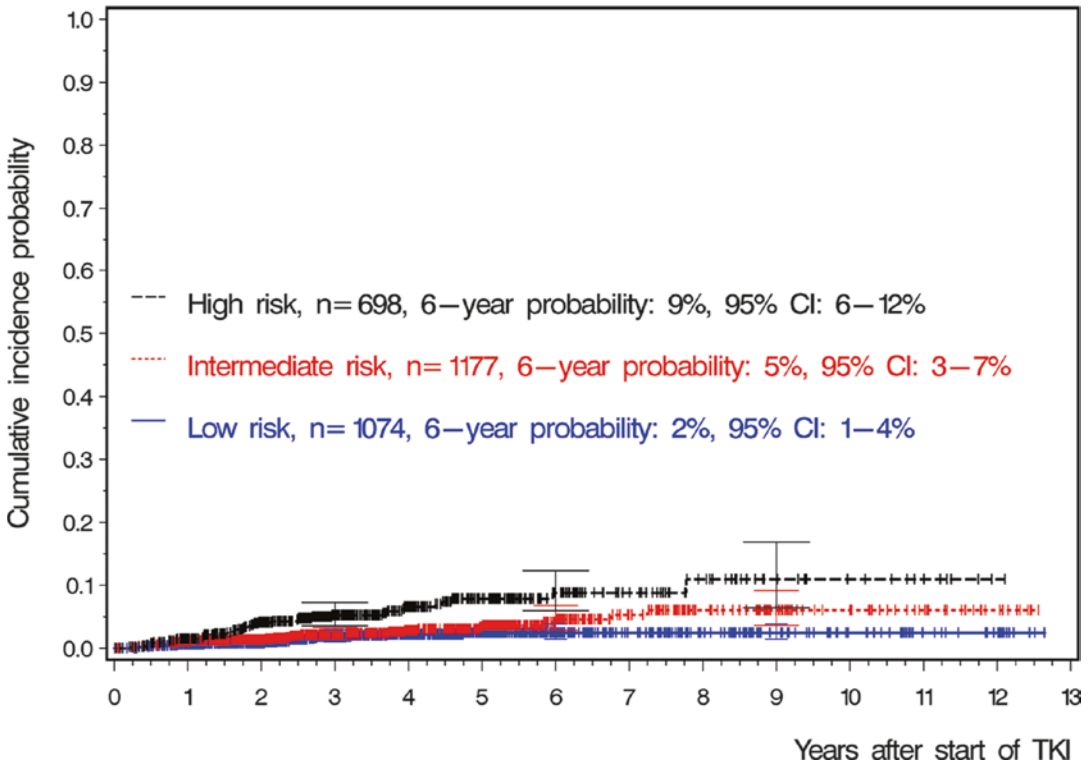
When the progressive illness death model was applied, compared with the ELTS score, no other score displayed a better discrimination of transition probabilities (Supplementary Table 2, [37]).

9.6.1.2 Comparative Assessment of Overall Survival Probabilities

Like the ELTS score, the intermediate-risk group ($p < 0.0001$) and the high-risk group ($p < 0.0001$) of the Sokal score had significantly lower OS probabilities than the low-risk group (Fig. 9.4). The corresponding HRs were 2.256 (95% CI: 1.590–3.201) and 3.384 (95% CI: 2.359–4.852). The concordance indices at 1, 5, and 10 years were 62.9, 62.0, and 61.3 [37]. Differences between intermediate- and high-risk patients were also significant (p = 0.0053). With slightly higher hazard ratios and concordance indices, the ELTS score was just superior to the Sokal score and also to the Euro score (Supplementary Fig. 4a, [37]) with regard to the discrimination of OS probabilities. The EUTOS score failed to discriminate risk groups (Supplementary Fig. 4b, [37]).

9.6.2 Comparative Assessment of the Scores in 5154 Patients from all Three Combined Registry Sections

In the 2949 patients of the combined out-study and population-based sections the 10-year



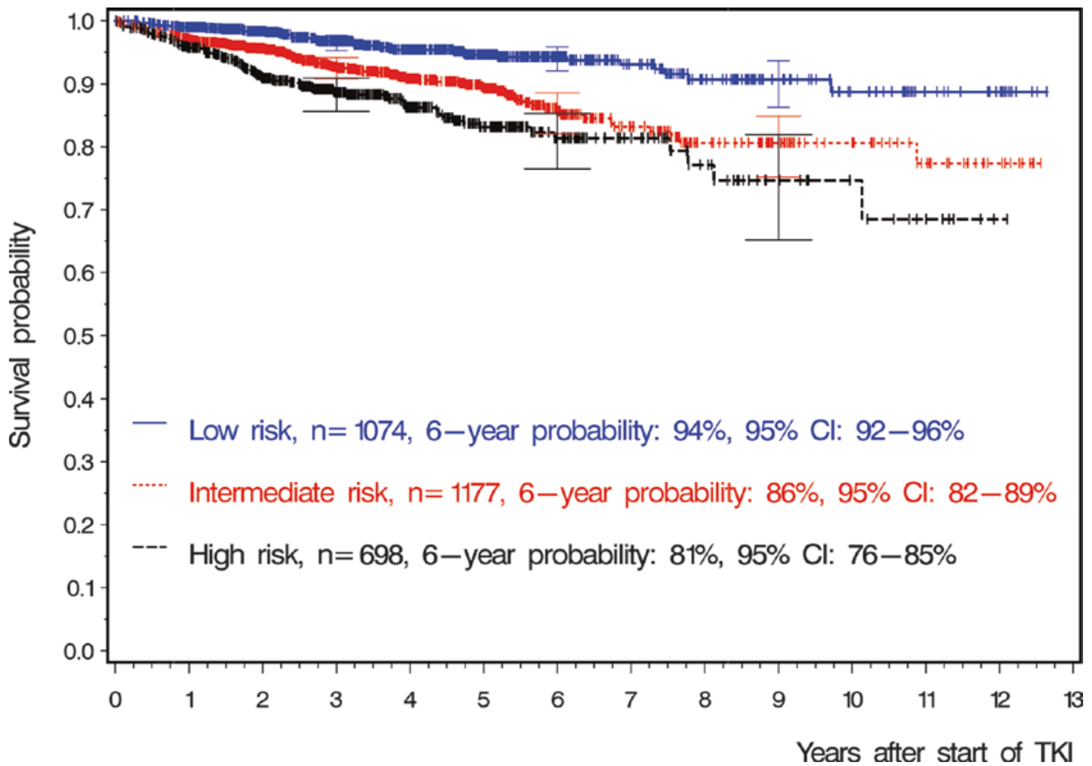
Number of patients still at risk (n) at different years of observation

Year	*0*	*3*	*6*	*9*
Low risk, n	1074	600	203	57
Intermediate risk, n	1177	598	175	50
High risk, n	698	341	82	19

Fig. 9.3 Cumulative incidence probabilities of death due to CML treating death due to causes not related to CML as competing risk in 2949 patients from the combined out-study and population-based registry sections, considering risk group stratification according to the Sokal score. At 3, 6, and 9 years, horizontal crossbars indicate the upper and lower limit of the 95% confidence interval for the estimated cumulative incidence probability of death due to CML.

probability of dying of CML was 6%. The result indicates that nowadays, the number of deaths after progression is rather low. This is excellent news. Only for the assessment of prognostic performance it means that it is not easy to identify statistically significant differences between risk groups, in particular not in smaller patient samples. To assess different risk group categorizations between two prognostic scores is even more difficult. This was only possible after addition of the 2205 patients of the learning sample.

Median age of the 5154 patients from all three combined registry sections remained 52 years (range: 18–91 years) and again, 52% were male. With a median follow-up of now 5.3 years (range: 0.01–12.6 years), 175 patients had died of CML (41% of 429 deaths). Ten-year OS probability in the 5154 patients was 85% (95% CI: 82–87%), and 10-year probability of death due to CML was 5% (95% CI: 4–6%). At 6 years, OS probability was 90% (95% CI: 89–91%), and 6-year probability of death due to CML was 4% (95% CI: 4–5%; [37]).



Number of patients still at risk (n) at different years of observation

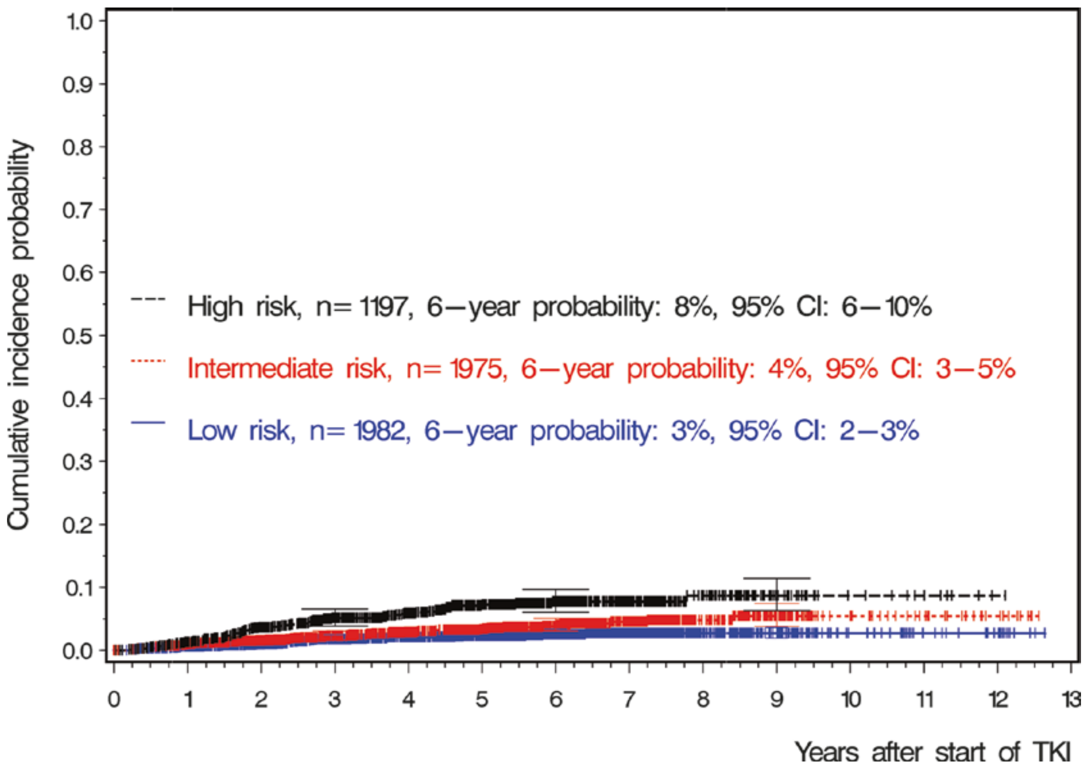
Year	*0*	*3*	*6*	*9*
Low risk, n	1074	600	203	57
Intermediate risk, n	1177	598	175	50
High risk, n	698	341	82	19

Fig. 9.4 Overall survival probabilities with death due to any cause in 2949 patients from the combined out-study and population-based registry sections, considering risk group stratification according to the Sokal score. At 3, 6, and 9 years, horizontal crossbars indicate the upper and lower limit of the 95% confidence interval for the estimated survival probability.

9.6.2.1 Rather Too Many Patients Classified as High-Risk by the Sokal Score

The Sokal identified an intermediate-risk ($n = 1975$, 38%) and a high-risk group ($n = 1197$, 23%) with significantly higher probabilities of dying because of CML than the low-risk group ($n = 1982$, 38%; Fig. 9.5), $p = 0.0088$ and $p < 0.0001$, respectively. The SHR of intermediate- to low-risk patients was 1.695 (95% CI: 1.142–2.515) and of high- to low-risk patients 3.161 (95% CI: 2.146–4.655). The concordance indices at 1, 5, and 10 years were 58.8, 62.1, and 62.2, respectively [37].

However, of the 1197 Sokal high-risk patients, 671 (56%) were non-high-risk according to the ELTS score and had a significantly more favorable long-term survival prognosis than the 526 high-risk patients according to both scores ($p = 0.0003$, Fig. 9.6). The SHR of the 671 ELTS non-high-risk patients to the 526 categorized as high-risk by both prognostic



Number of patients still at risk (n) at different years of observation

Year	*0*	*3*	*6*	*9*
Low risk, n	1982	1432	741	79
Intermediate risk, n	1975	1328	621	74
High risk, n	1197	768	320	32

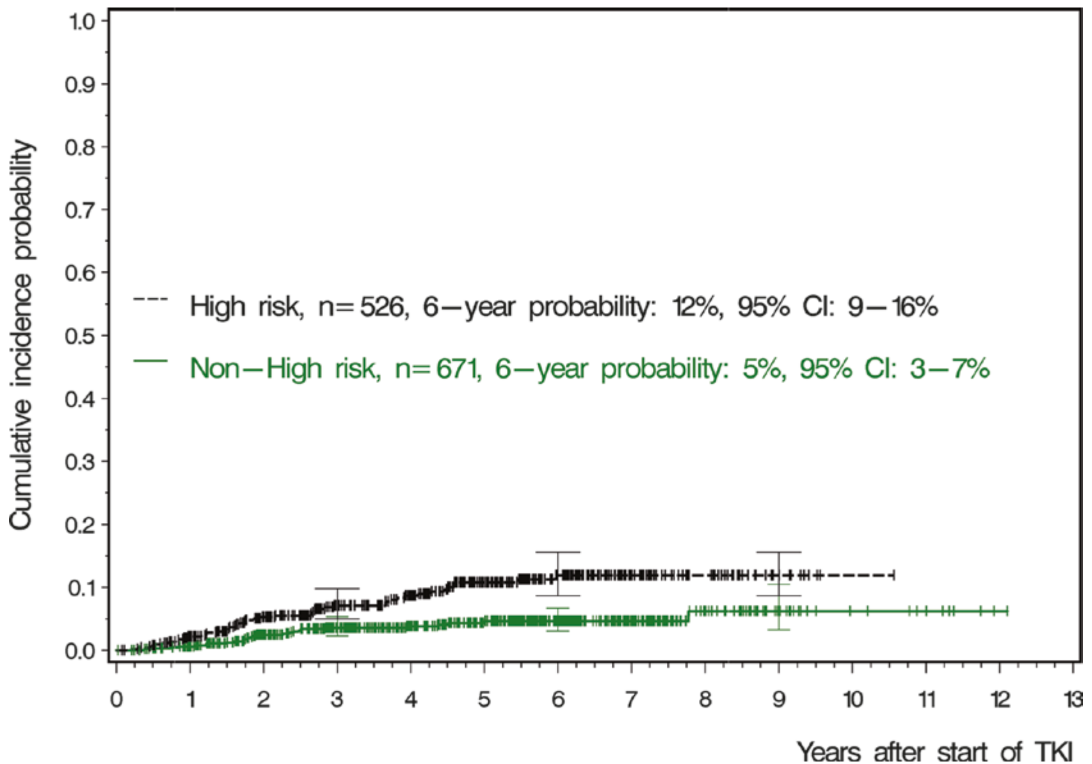
Fig. 9.5 Cumulative incidence probabilities of death due to CML treating death due to causes not related to CML as competing risk in 5154 patients from all three combined registry sections, considering risk group stratification according to the Sokal score. At 3, 6, and 9 years, horizontal crossbars indicate the upper and lower limit of the 95% confidence interval for the estimated cumulative incidence probability of death due to CML.

models was 0.415 (95% CI: 0.256–0.671). Concordance indices at 1, 5, and 10 years were 63.3, 60.8, and 59.9, respectively. For 56% of 1197, the allocation of high-risk by the Sokal score was inappropriate [37].

9.6.2.2 A Justifiable Higher Proportion of Low-Risk Patients with the ELTS Score

Using the ELTS score, both the intermediate-risk (n = 1449, 28%, $p < 0.0001$) and the high-risk group (n = 668, 13%, $p < 0.0001$) had also significantly higher probabilities of dying due to CML than the low-risk group (n = 3037, 59%; Fig. 9.7). With SHRs of 2.584 (95% CI: 1.795–3.721) and 5.667 (95% CI: 3.912–8.209), respectively, the concordance indices at 1, 5, and 10 years were 69.6, 66.8, and 67.3 [37].

Of the 3037 low-risk patients according to the ELTS score, the Sokal score classified 1200 (40%) as non-low-risk. Cumulative incidence probabilities of death because of CML were hardly different from the ones of the 1837 patients allocated to low-risk by both scores, though



Number of patients still at risk (n) at different years of observation

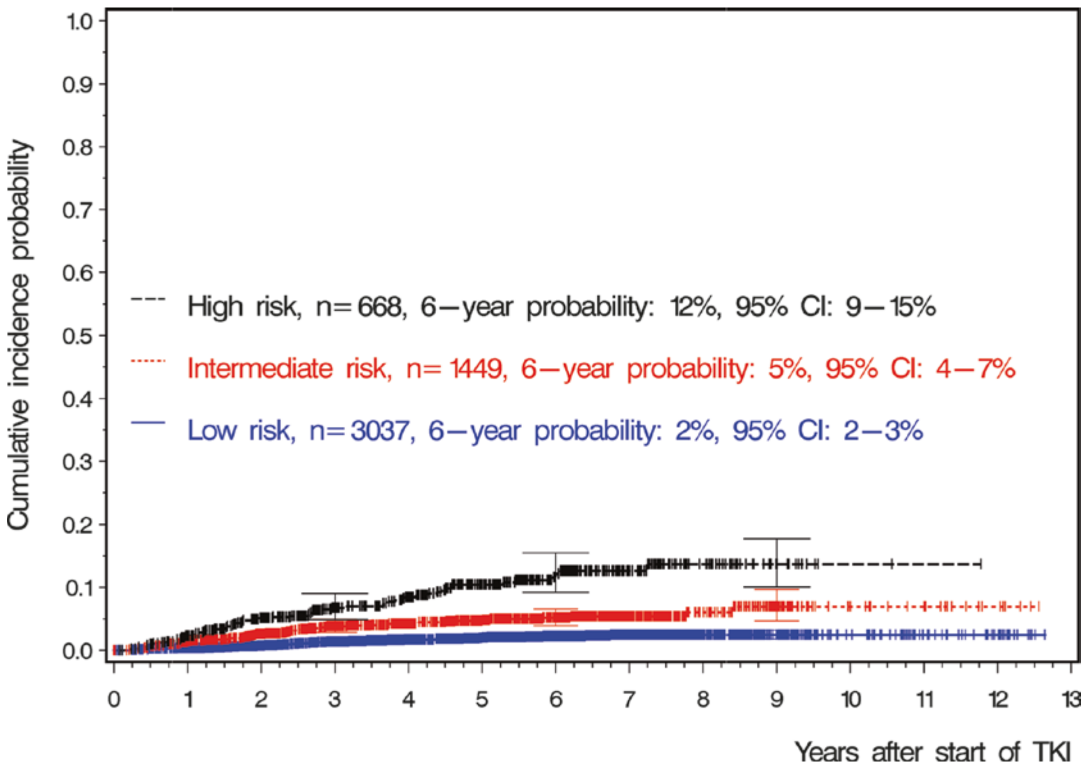
Year	*0*	*3*	*6*	*9*
Non -high risk, n	671	452	192	20
High risk, n	526	316	128	12

Fig. 9.6 Cumulative incidence probabilities of death due to CML treating death due to causes not related to CML as competing risk in 1197 high-risk patients according to the Sokal score, considering risk group stratification according to the ELTS score. At 3, 6, and 9 years, horizontal crossbars indicate the upper and lower limit of the 95% confidence interval for the estimated cumulative incidence probability of death due to CML.

(SHR of non-low- to low-risk: 1.129 (95% CI: 0.653–1.951, $p = 0.6635$, Fig. 9.8). This observation hinted at another inappropriate risk group classification by the Sokal score.

In the 5154 patients from all three combined registry sections, no status-dependent censoring mechanism and, consequently, no biased cumulative incidence probabilities were observed (Supplementary Fig. 6, [37]). However, it has to be acknowledged that the inclusion of the 2205 in-study patients used for its development meant some advantage for the ELTS score compared with other scores. While the extent of this limitation cannot be quantified, the very distinctive results suggest that the risk of inappropriate classification is decidedly higher with the Sokal score [37].

Pfirrmann et al. [37] performed an according comparative examination of the prognostic scores with respect to OS probabilities. Here, as for the cumulative incidence probabilities of dying of CML, the ELTS score provided the best prognostic discrimination in comparison with the three other scores (details see [37]).

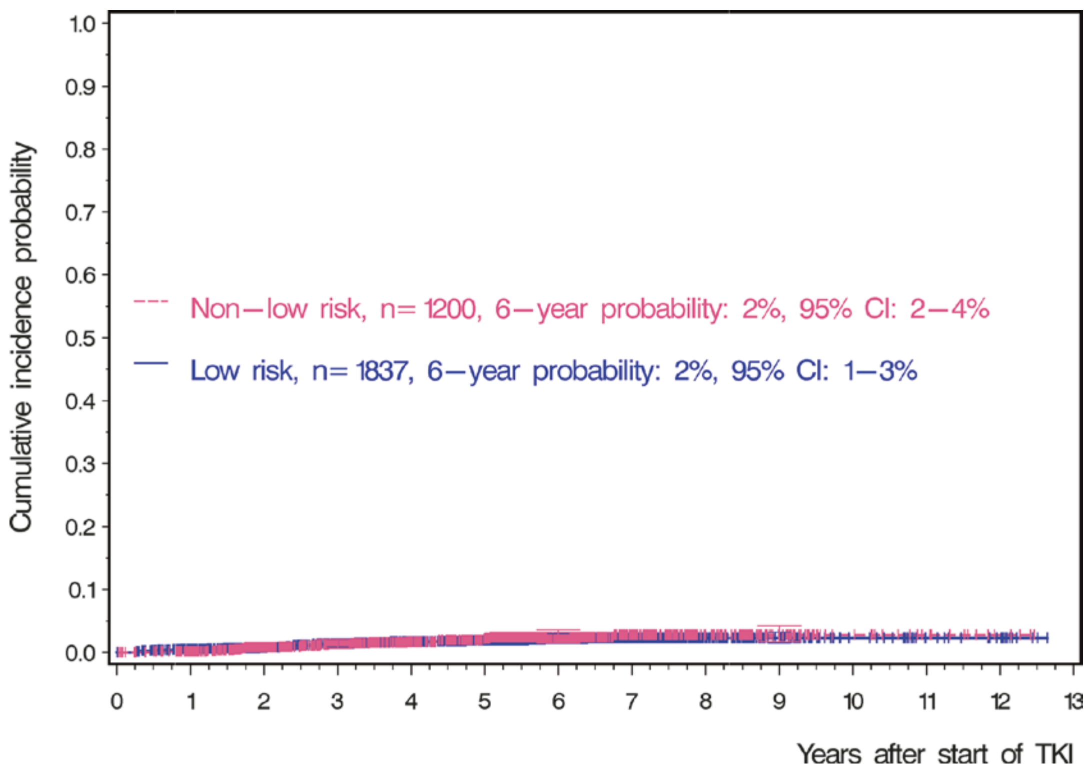


Number of patients still at risk (n) at different years of observation

Year	*0*	*3*	*6*	*9*
Low risk, n	3037	2180	1084	120
Intermediate risk, n	1449	955	431	51
High risk, n	668	393	167	14

Fig. 9.7 Cumulative incidence probabilities of death due to CML treating death due to causes not related to CML as competing risk in 5154 patients from all three combined registry sections, considering risk group stratification according to the ELTS score. At 3, 6, and 9 years, horizontal crossbars indicate the upper and lower limit of the 95% confidence interval for the estimated cumulative incidence probability of death due to CML.

In abovementioned analyses, the ELTS score proved to be superior to the three other scores in the discrimination of cumulative incidence probabilities of dying of CML and of OS probabilities. While the ELTS score was developed to discriminate risk groups with respect to the former probabilities, the Sokal and the Euro score were developed to discriminate risk groups with respect to the latter ones. The EUTOS score, however, was developed to distinguish CCyR probabilities at 18 months. Thus, it is not fair to expect an according prognostic performance in the discrimination of long-term survival as for the three other scores. With respect to response outcome (CCyR or major molecular response), the EUTOS score could be validated by several independent study groups [14]. Nevertheless, since response outcome is only a potential surrogate for survival outcome and in addition, its measurement accuracy is less precise (e.g., interval censoring), here the focus was on long-term survival.



Number of patients still at risk (n) at different years of observation

Year	*0*	*3*	*6*	*9*
Low risk, n	1837	1338	694	75
Non -low risk, n	1200	842	390	45

Fig. 9.8 Cumulative incidence probabilities of death due to CML treating death due to causes not related to CML as competing risk in 3037 low-risk patients according to the ELTS score, considering risk group stratification according to the Sokal score. At 3, 6, and 9 years, horizontal crossbars indicate the upper and lower limit of the 95% confidence interval for the estimated cumulative incidence probability of death due to CML.

9.6.3 Comparative Assessments of the Scores in Other Study Groups

Together with its successful validation, superiority of the ELTS score in comparison with other scores was reported.

In the imatinib-treated Swedish and Dutch patients investigated by Geelen et al. [44], only the ELTS score was able to find three pairwise significantly different risk groups with respect to OS and to achievement of a first major molecular response. And while the ELTS score identified significant differences in cumulative incidences of death due to CML between low- and high-risk patients, no significant differences were discovered by any of the other three scores. The authors concluded that the ELTS score “outperformed the Sokal, the Hasford (Euro), and the EUTOS score” and judged the ELTS score as “an excellent risk stratification tool for contemporary CML patients treated with imatinib” [44].

In contrast to the ELTS score identifying significant risk group discriminations of OS probabilities between the high-risk group and each of the two more favorable ones, the Sokal and the

Euro score could not distinguish OS probabilities between any of the risk groups in the 462 Chinese patients. However, OS probabilities were significantly different between the low- and the high-risk groups of the EUTOS score [45].

In the 350 children considered by Millot et al. [46], neither the Euro nor the Sokal score were able to significantly discriminate risk groups with respect to progression-free survival. However, instead of the conventional Sokal score [8], Millot et al. considered the Sokal score for younger patients [48]. Both, the ELTS and the EUTOS score found significant PFS differences. Between the low- and the high-risk groups, the differences in 5-year PFS probabilities were 29% with the ELTS (96% vs. 67%) and 12% with the EUTOS score (93% vs. 81%). This might have led to the conclusion that the ELTS score "showed better differentiation of PFS" than any of the other scores, including the EUTOS score [46].

In the 202 Italian patients ≥65 years, in contrast to the ELTS score, the Sokal score was not able to find any significant difference with respect to major or deep molecular response or the probabilities of leukemia-related deaths [43].

In the 258 patients diagnosed in progressive disease, of the four scores, the ELTS score was the only one to significantly distinguish OS probabilities between high- and low-risk patients. Due to significant differences also between the high- and intermediate-risk groups but not between low- and intermediate risk groups, it was suggested to apply the ELTS score to discriminate long-term survival between high-risk and non-high-risk patients until a better model developed in patients with accelerated phase and/or blast crisis is introduced [47].

9.7 ELTS Score and Age

There is considerable evidence that TKIs provide substantial treatment success at any age. In the German CML study IV, Kalmanti et al. [49] observed no differences in MMR and MR^4 probabilities or in cumulative incidences of disease progression between more than 400 patients with an age of at least 60 years and more than 800 patients of younger age groups. In a study on 263 imatinib-treated patients with a median age of 79 years (range: 75–94 years), Crugnola et al. [50] reported MMR in 56% and MR^4 in 24% of the cases. After a median follow-up of 45 months, five-year OS probability was 71%. Of 93 deaths, 84 were not related to CML. Developed for the primary endpoint "death due to CML," regarding the ELTS score, it was hence to be expected that age would play a less dominant role than in scores designed to differentiate OS probabilities.

Table 9.2 displays the distribution of the 5154 patients of the EUTOS registry into four age groups. With 45%, the age group 40–59 years

Table 9.2 Distribution of 5154 patients into age and risk groups of the ELTS and the Sokal scores

Age group	Risk group according to ELTS score		Risk group according to Sokal score	
Years *n* % of total		*n*, % of age group		*n*, % of age group
18–39 1250 24.3%	Low	888, 71%	Low	716, 57%
	Intermediate	241, 19%	Intermediate	307, 25%
	High	121, 10%	High	227, 18%
40–59 2340 45.4%	Low	1561, 67%	Low	1028, 44%
	Intermediate	541, 23%	Intermediate	769, 33%
	High	238, 10%	High	543, 23%
60–74 1290 25.0%	Low	580, 45%	Low	238, 18%
	Intermediate	498, 39%	Intermediate	718, 56%
	High	212, 16%	High	334, 26%
≥75 274 5.3%	Low	8, 3%	Low	0
	Intermediate	169, 62%	Intermediate	181, 66%
	High	97, 35%	High	93, 34%

comprised almost half of the patients. It is noticeable that most patients of the age group 60–74 years (n = 580, 45%) were allocated to low risk with the ELTS score. In comparison with the ELTS score, the Sokal score allocated less patients to low risk across all age groups. It were only 18% Sokal low-risk patients in the age group 60–74 years. Of course, differences in risk assessment between the scores also depend on the distributions of blasts, spleen size, and platelet count in the particular sample under consideration but the reduced influence of age with the ELTS score is evident.

As previously illustrated, the non-linear modeling of age with the ELTS score modulates that at younger ages, an age increase induces a less strong rise of the event hazard than at older ages (see Sect. 9.4.3). With otherwise the same values in the rest of the score variables, the age increase from 36 to 60 years causes the same rise of 66% in the SHR as the age increase from 70 to 80 years.

To view it from a different angle, a patient with median values is considered. In the sample of 5154 patients, median spleen size enlargement was 1 cm, median percentage in peripheral blasts was 1%, and median platelet count was 385 × 10^9/L. Calculating the ELTS score for the "median patient" in accordance with the formula in Table 9.1, score values remain below the cutoff 1.5680 for low-risk for ages up to 66 years; patients are allocated to intermediate-risk between 67 and 82, and enter the high-risk group from ages of 83 years. Of note, this does not imply any treatment suggestion. Most high-risk patients, young or old, profit from TKI treatment. It rather illustrates that up to a certain age limit, higher age is not necessarily associated with high-risk group with the ELTS score.

In none of the three risk groups of the ELTS score, a significant difference of the SHRs between the four age groups, hinting at some additional interaction of age, was observed.

By the way, sex did not show any relevance for the cumulative incidence probabilities of dying of CML. For female patients, the 10-year probability was 4.9% (95% CI: 3.8–6.2%) and for male patients 5.1% (95% CI: 3.9–6.6%).

9.8 Software

Most analyses were undertaken with SAS (version 9.4). Additional analyses were based on the programming software R: The truncated concordance index was calculated using the function *pec*. The function *etm* supports the calculation of competing risks and of the progressive illness death model. Association between risk groups and transition probabilities were estimated using the R function *mstate*. Scrucca et al. [51] provided a detailed tutorial on how to calculate and compare CIPs [51] and also on the estimation of SHs with the Fine–Gray model [52]. To assess the assumption of proportional hazards, for both regression models, Schoenfeld residuals were investigated [41, 52].

9.9 Summary and Conclusions

The meaning of a prognostic model depends on its clinical usefulness. Nowadays with more than half of the patients dying of causes not directly related to CML, the association between CML treatment and OS probabilities has considerably shrunken. CML therapy aims at the reduction of the probabilities of dying of leukemia. The ELTS score was the first prognostic system which was developed in patients treated with a TKI and with respect to the most appropriate long-term endpoint "death due to CML" (i.e., after progression).

The ELTS score has successfully been validated in 2949 independent patients of the EUTOS registry. The score was able to distinguish three pairwise significantly different risk groups with respect to the cumulative incidences of dying of CML and OS probabilities (Figs. 9.1 and 9.2). Meanwhile, successful validation of the ELTS score was reported by a couple of independent study groups, even in patient samples quite different from the original sample where the score was developed and also for endpoints the use of the score not intended for [43–47].

Relatively high survival probabilities and the restriction on CML-specific death aggravate the identification of a statistically significant

long-term outcome discrimination between risk groups in general. To arrive at a proper assessment on the prognostic quality of a score, a validation sample should have a sufficient size and a sufficient number of events. Smaller samples might lack the power for an adequate assessment, and, thus, investigators might come to a misleading conclusion about the quality of the score examined.

In a comparative assessment of risk group discrimination, the ELTS score outperformed the Sokal, the Euro, and the EUTOS scores in the 2949 independent patients as well as in the 5154 patients from all three combined registry sections. Superiority comprised the cumulative incidence probabilities of dying of CML and OS probabilities. In relation to the low-risk groups, the SHRs and HRs were consistently higher with the ELTS score than with the three other scores.

The ELTS score provided truncated concordance indices between 64 and 70 [37] and, thus, clinically useful information. It supports the decision on (imatinib) treatment. Higher indices than with the other scores hint at the better prognostic discrimination with the ELTS score. Absolute index sizes have to be interpreted in the context of the disease. Patients diagnosed with chronic phase CML have a 10-year probability of dying of CML of less than 10% [15, 17]. It is thus unlikely to identify risk groups with >30% difference in 10-year probabilities of dying of CML and to elaborate a score with concordance indices of 80 or even higher.

Together with its successful validation, also superiority of the ELTS score in comparison with other scores was reported by independent study groups [43–47].

Of 1197 patients classified as high-risk by the Sokal score (Fig. 9.5), the ELTS score allocated the majority to low risk ($n = 671$, 56%). Compared with the 526 high-risk patients according to both scores, the cumulative incidences of dying of CML were significantly lower for the 671 ELTS non-high-risk patients (Fig. 9.6). For 56% of 1197 patients the classification of high-risk by the Sokal score was rather inappropriate [37].

Originally, the cutoffs of the Sokal score were defined to provide three risk groups of approximately the same size [8]. In contrast to this, the cutoffs of the Euro, the EUTOS, and the ELTSs score were defined to provide risk groups that were statistically most different from each other regarding their primary endpoints [4, 9, 10]. This led to much smaller high-risk groups, mostly comprising between 10 and 15% of the patients. Already during the era of IFN treatment, it was shown that the number of patients attributed to high-risk by the Sokal score is too large [12, 53]. With IFN and, in particular, with TKIs, much less patients actually are at high risk. Ten-year probabilities of dying of CML of less than 10% rather speak in favor of 10–15% high-risk patients than in favor of more than 20% as suggested by the Sokal score.

In small patient samples, just due to their size, small high-risk groups have a disadvantage in providing statistically significant differences to the other risk groups. Sometimes, this drawback can be compensated by the fact that their outcome is statistically more different from other risk groups than the outcome of a larger high-risk group comprising actual intermediate- or low-risk patients. With risk-adapted treatment, it is the correct risk classification of a patient that is most important and not a definition of risk groups of approximately the same size.

Of the 3037 low-risk patients according to the ELTS score (Fig. 9.7), the Sokal score identified 1200 (40%) as non-low-risk. However, their cumulative incidences of dying of CML were only slightly different from those of the remaining 1837 patients allocated to low-risk by both scores (Fig. 9.8). This pointed to another inappropriate risk group classification by the Sokal score [37]. With similar probabilities of dying of CML, the ELTS score was able to identify an absolute proportion of 20% more low-risk patients than the Sokal and the Euro scores [37].

The mechanism behind the superiority of the ELTS score is its development in imatinib-treated patients and for probabilities of death due to CML (after disease progression) rather than for OS probabilities based on death due to any cause. This resulted in a different weighting of the four prognostic factors and in a more adequate patient distribution into risk groups of about

60%/30%/10% (low- /intermediate- /high-risk groups) instead of about 40%/40%/20% as with the Sokal score – in times when patients have much better survival prospects due to TKIs. There is considerable evidence that TKIs provide substantial treatment success at any age [49, 50]. This is met by the reduced influence of age with the ELTS score.

The ELTS score has been validated several times for its ability to significantly discriminate risk groups regarding long-term survival outcome but mainly in patients first-line treated with imatinib [37, 43–47]. Despite significantly faster achievement of molecular responses with second-generation TKIs [5, 6, 54–57], first-line treatment with imatinib and its generics is still widespread. Most physicians continue to see room for first-line treatment with imatinib depending on age, comorbidities, kinase domain mutations, treatment goal, costs, and availability of generic imatinib [7, 16, 54, 58–60].

In prognostic support of first-line treatment selection, the ELTS score offers the most appropriate risk group classification. Starting first-line treatment with imatinib, for about 60% low-risk patients, the ELTS score showed a very favorable long-term outcome which could be hardly improved by any other TKI [10]. This is also of interest as imatinib has fewer side effects than second-generation TKIs, and it is perceived that a statistically significant overall superiority in long-term efficacy over imatinib has not yet been shown for another TKI [7, 54, 58, 59].

There is indication that the ELTS score would also discriminate risk groups with respect to long-term survival if a second-generation TKI were chosen as first-line treatment [44]. More evidence is needed. Regarding high- and intermediate-risk patients, an upfront comparison between different TKIs would be desirable. However, a very large patient sample would be necessary to recognize significant differences in long-term survival between TKIs within a certain risk group.

The ELTS score supports the prospective assessment of long-term antileukemic efficacy with first-line imatinib treatment. Its consideration aids and improves risk-stratified planning, analysis and outcome interpretation of clinical trials, and the development of risk-adapted treatments [10]. A valid score and its common application support comparative evaluation of efficacy and safety.

The ELN recommendations published in 2020 advocate the use of the ELTS score as the preferred model to assess baseline CML risk [7]. Most recently, Pfirrmann et al. [37] backed the ELN recommendation with statistical evidence.

Conflict of Interest The authors declare that they have no conflict of interest.

References

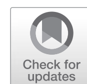
1. Pfirrmann M, Lauseker M, Hoffmann VS, Hasford J. Prognostic scores for patients with chronic myeloid leukemia under particular consideration of competing causes of death. Ann Hematol. 2015;94(Suppl 2):S209–18. https://doi.org/10.1007/s00277-015-2316-0.
2. Sargent DJ, Conley BA, Allegra C, Collette L. Clinical trial designs for predictive marker validation in cancer treatment trials. J Clin Oncol. 2005;23(9):2020–7. 23/9/2020 [pii]. doi: https://doi.org/10.1200/JCO.2005.01.112
3. Byar DP. Identification of prognostic factors. In: Buyse ME, Staquet MJ, Sylvester RJ, editors. Cancer clinical trials—methods and practice. Oxford: Oxford University Press; 1984. p. 423–41.
4. Hasford J, Pfirrmann M, Hehlmann R, Allan NC, Baccarani M, Kluin-Nelemans JC, Alimena G, Steegmann JL, Ansari H. A new prognostic score for survival of patients with chronic myeloid leukemia treated with interferon alfa. Writing Committee for the Collaborative CML Prognostic Factors Project Group. J Natl Cancer Inst. 1998;90(11):850–8.
5. Kantarjian H, Shah NP, Hochhaus A, Cortes J, Shah S, Ayala M, Moiraghi B, Shen Z, Mayer J, Pasquini R, Nakamae H, Huguet F, Boque C, Chuah C, Bleickardt E, Bradley-Garelik MB, Zhu C, Szatrowski T, Shapiro D, Baccarani M. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2010;362(24):2260–70. NEJMoa1002315 [pii]. https://doi.org/10.1056/NEJMoa1002315.
6. Saglio G, Kim DW, Issaragrisil S, le Coutre P, Etienne G, Lobo C, Pasquini R, Clark RE, Hochhaus A, Hughes TP, Gallagher N, Hoenekopp A, Dong M, Haque A, Larson RA, Kantarjian HM. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med. 2010;362(24):2251–9. NEJMoa0912614 [pii]. https://doi.org/10.1056/NEJMoa0912614.

7. Hochhaus A, Baccarani M, Silver RT, Schiffer C, Apperley JF, Cervantes F, Clark RE, Cortes JE, Deininger MW, Guilhot F, Hjorth-Hansen H, Hughes TP, Janssen J, Kantarjian HM, Kim DW, Larson RA, Lipton JH, Mahon FX, Mayer J, Nicolini F, Niederwieser D, Pane F, Radich JP, Rea D, Richter J, Rosti G, Rousselot P, Saglio G, Saussele S, Soverini S, Steegmann JL, Turkina A, Zaritskey A, Hehlmann R. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020;34(4):966–84.
8. Sokal JE, Cox EB, Baccarani M, Tura S, Gomez GA, Robertson JE, Tso CY, Braun TJ, Clarkson BD, Cervantes F, Rozman C, the Italian Cooperative CML Study Group. Prognostic discrimination in "good-risk" chronic granulocytic leukemia. Blood. 1984;63(4):789–99.
9. Hasford J, Baccarani M, Hoffmann V, Guilhot J, Saussele S, Rosti G, Guilhot F, Porkka K, Ossenkoppele G, Lindoerfer D, Simonsson B, Pfirrmann M, Hehlmann R. Predicting complete cytogenetic response and subsequent progression-free survival in 2060 patients with CML on imatinib treatment: the EUTOS score. Blood. 2011;118(3):686–92. https://doi.org/10.1182/blood-2010-12-319038.
10. Pfirrmann M, Baccarani M, Saussele S, Guilhot J, Cervantes F, Ossenkoppele G, Hoffmann VS, Castagnetti F, Hasford J, Hehlmann R, Simonsson B. Prognosis of long-term survival considering disease-specific death in patients with chronic myeloid leukemia. Leukemia. 2016;30(1):48–56.
11. Baccarani M, Deininger MW, Rosti G, Hochhaus A, Soverini S, Apperley JF, Cervantes F, Clark RE, Cortes JE, Guilhot F, Hjorth-Hansen H, Hughes TP, Kantarjian HM, Kim D-W, Larson RA, Lipton JH, Mahon F-X, Martinelli G, Mayer J, Müller MC, Niederwieser D, Pane F, Radich JP, Rousselot P, Saglio G, Saußele S, Schiffer C, Silver R, Simonsson B, Steegmann J-L, Goldman JM, Hehlmann R. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood. 2013;122(6):872–84. https://doi.org/10.1182/blood-2013-05-501569.
12. Hasford J, Pfirrmann M, Hehlmann R, Shepherd P, Guilhot F, Mahon FX, Thaler J, Steegmann JL, Kluin-Nelemans HC, Louwagie A, Ohnishi K, Kloke O. Prognostic factors. In: Carella AM, Daley G, Eaves C, Goldman J, Hehlmann R, editors. Chronic myeloid leukemia—biology and treatment. London: Martin Dunitz; 2001. p. 205–23.
13. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, Cornelissen JJ, Fischer T, Hochhaus A, Hughes T, Lechner K, Nielsen JL, Rousselot P, Reiffers J, Saglio G, Shepherd J, Simonsson B, Gratwohl A, Goldman JM, Kantarjian H, Taylor K, Verhoef G, Bolton AE, Capdeville R, Druker BJ. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2003;348(11):994–1004.
14. Hoffmann VS, Baccarani M, Lindoerfer D, Castagnetti F, Turkina A, Zaritsky A, Hellmann A, Prejzner W, Steegmann JL, Mayer J, Indrak K, Colita A, Rosti G, Pfirrmann M. The EUTOS prognostic score: review and validation in 1288 patients with CML treated frontline with imatinib. Leukemia. 2013;27(10):2016–22. https://doi.org/10.1038/leu.2013.171.
15. Hehlmann R, Lauseker M, Saussele S, Pfirrmann M, Krause S, Kolb HJ, Neubauer A, Hossfeld DK, Nerl C, Gratwohl A, Baerlocher GM, Heim D, Brummendorf TH, Fabarius A, Haferlach C, Schlegelberger B, Muller MC, Jeromin S, Proetel U, Kohlbrenner K, Voskanyan A, Rinaldetti S, Seifarth W, Spiess B, Balleisen L, Goebeler MC, Hanel M, Ho A, Dengler J, Falge C, Kanz L, Kremers S, Burchert A, Kneba M, Stegelmann F, Kohne CA, Lindemann HW, Waller CF, Pfreundschuh M, Spiekermann K, Berdel WE, Muller L, Edinger M, Mayer J, Beelen DW, Bentz M, Link H, Hertenstein B, Fuchs R, Wernli M, Schlegel F, Schlag R, de Wit M, Trumper L, Hebart H, Hahn M, Thomalla J, Scheid C, Schafhausen P, Verbeek W, Eckart MJ, Gassmann W, Pezzutto A, Schenk M, Brossart P, Geer T, Bildat S, Schafer E, Hochhaus A, Hasford J. Assessment of imatinib as first-line treatment of chronic myeloid leukemia: 10-year survival results of the randomized CML study IV and impact of non-CML determinants. Leukemia. 2017;31(11):2398–406.
16. Hochhaus A, Larson RA, Guilhot F, Radich JP, Branford S, Hughes TP, Baccarani M, Deininger MW, Cervantes F, Fujihara S, Ortmann CE, Menssen HD, Kantarjian H, O'Brien SG, Druker BJ. Long-term outcomes of Imatinib treatment for chronic myeloid Leukemia. N Engl J Med. 2017;376(10):917–27.
17. Molica M, Colafigli G, Scalzulli E, Alunni Fegatelli D, Chiatamone Ranieri S, Rizzo L, Diverio D, Efficace F, Latagliata R, Foà R, Breccia M. Ten-year outcome of chronic-phase chronic myeloid leukemia patients treated with imatinib in real life. Ann Hematol. 2019;98(8):1891–904.
18. Human Mortality Database: University of California, Berkeley (USA), and Max Planck Institute of Demographic Research (Germany): the Human Mortality Database provides mortality and population data on various countries. http://www.mortality.org/ or, http://www.humanmortality.de/
19. Pohar-Perme M, Stare J, Estève J. On estimation in relative survival. Biometrics. 2012;68(1):113–20. https://doi.org/10.1111/j.1541-0420.2011.01640.x.
20. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med. 1999;18(6):695–706.
21. Kalbfleisch JD, Prentice RL. The statistical analysis of failure time data. New York: Wiley; 1980.
22. Pfirrmann M, Hochhaus A, Lauseker M, Saussele S, Hehlmann R, Hasford J. Recommendations to meet statistical challenges arising from endpoints beyond overall survival in clinical trials on chronic myeloid leukemia. Leukemia. 2011;25(9):1433–8.
23. Choudhury JB. Non-parametric confidence interval estimation for competing risks analysis: application to contraceptive data. Stat Med. 2002;21:1129–44.

24. Andersen PK, Geskus RB, de Witte T, Putter H. Competing risks in epidemiology: possibilities and pitfalls. Int J Epidemiol. 2012;41(3):861–70. https://doi.org/10.1093/ije/dyr213.
25. Latouche A, Allignol A, Beyersmann J, Labopin M, Fine JP. A competing risks analysis should report results on all cause-specific hazards and cumulative incidence functions. J Clin Epidemiol. 2013;66(6):648–53. https://doi.org/10.1016/j.jclinepi.2012.09.017.
26. Putter H, Fiocco M, Geskus RB. Tutorial in biostatistics: competing risks and multi-state models. Stat Med. 2007;26(11):2389–430.
27. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc. 1999;94(446):496–509.
28. Allignol A, Schumacher M, Wanner C, Drechsler C, Beyersmann J. Understanding competing risks: a simulation point of view. BMC Med Res Methodol. 2011;11:86. https://doi.org/10.1186/1471-2288-11-86.
29. Schoenfeld DA, Richter JR. Nomograms for calculating the number of patients needed for a clinical trial with survival as an endpoint. Biometrics. 1982;38(1):163–70.
30. Latouche A, Porcher R. Sample size calculations in the presence of competing risks. Stat Med. 2007;26(30):5370–80.
31. Wyatt JC, Altman DG. Commentary: prognostic models: clinically useful or quickly forgotten? BMJ. 1995;311(7019):1539–41. https://doi.org/10.1136/bmj.311.7019.1539.
32. Simon R, Altman DG. Statistical aspects of prognostic factor studies in oncology. Br J Cancer. 1994;69(6):979–85.
33. Royston P, Altman DG, Sauerbrei W. Dichotomizing continuous predictors in multiple regression: a bad idea. Stat Med. 2006;25(1):127–41.
34. Royston P, Sauerbrei W. Multivariable model-building: a pragmatic approach to regression analysis based on fractional polynomials for continuous variables. Chichester: John Wiley & Sons; 2008.
35. Davison AC, Hinkley DV. Bootstrap methods and their application: Cambridge Cambridge University Press; 1997.
36. Altman DG, Lausen B, Sauerbrei W, Schumacher M. Dangers of using "optimal" Cutpoints in the evaluation of prognostic factors. J Natl Cancer Inst. 1994;86(11):829–35.
37. Pfirrmann M, Clark RE, Prejzner W, Lauseker M, Baccarani M, Saussele S, Guilhot F, Heibl S, Hehlmann R, Faber E, Turkina A, Ossenkoppele G, Hoglund M, Zaritskey A, Griskevicius L, Olsson-Stromberg U, Everaus H, Koskenvesa P, Labar B, Sacha T, Zackova D, Cervantes F, Colita A, Zupan I, Bogdanovic A, Castagnetti F, Guilhot J, Hasford J, Hochhaus A, Hoffmann VS. The EUTOS long-term survival (ELTS) score is superior to the Sokal score for predicting survival in chronic myeloid leukemia. Leukemia. 2020;34(8):2138–49.
38. Gray RJ. A class of k-sample tests for comparing the cumulative incidence of a competing risk. Ann Stat. 1988;16(3):1141–54.
39. Wolbers M, Blanche P, Koller MT, Witteman JC, Gerds TA. Concordance for prognostic models with competing risks. Biostatistics. 2014;15(3):526–39.
40. Hoffmann VS, Baccarani M, Hasford J, Castagnetti F, Di Raimondo F, Casado LF, Turkina A, Zackova D, Ossenkoppele G, Zaritskey A, Hoglund M, Simonsson B, Indrak K, Sninska Z, Sacha T, Clark R, Bogdanovic A, Hellmann A, Griskevicius L, Schubert-Fritschle G, Sertic D, Guilhot J, Lejniece S, Zupan I, Burgstaller S, Koskenvesa P, Everaus H, Costeas P, Lindoerfer D, Rosti G, Saussele S, Hochhaus A, Hehlmann R. Treatment and outcome of 2904 CML patients from the EUTOS population-based registry. Leukemia. 2017;31(3):593–601. https://doi.org/10.1038/leu.2016.246.
41. Therneau TM, Grambsch PM. Modeling survival data: extending the Cox model. New York: Springer; 2000.
42. Lauseker M, Zu Eulenburg C. Analysis of cause of death: competing risks or progressive illness-death model? Biom J. 2019;61(2):264–74.
43. Castagnetti F, Gugliotta G, Breccia M, Stagno F, Specchia G, Levato L, Martino B, D'Adda M, Abruzzese E, Pregno P, Tiribelli M, Iurlo A, Intermesoli T, Scortechini AR, Cavazzini F, Fava C, Bonifacio M, Salvucci M, Bocchia M, Caocci G, Gozzini A, Bergamaschi M, Soverini S, Foà R, Martinelli G, Cavo M, Saglio G, Pane F, Baccarani M, Rosti G. The use of EUTOS long-term survival score instead of Sokal score is strongly advised in elderly chronic myeloid Leukemia patients. Blood. 2018;132(Supplement 1):44.
44. Geelen IGP, Sandin F, Thielen N, Janssen J, Hoogendoorn M, Visser O, Cornelissen JJ, Hoglund M, Westerweel PE. Validation of the EUTOS long-term survival score in a recent independent cohort of "real world" CML patients. Leukemia. 2018;32(10):2299–303.
45. Yang X, Bai Y, Shi M, Zhang W, Niu J, Wu C, Zhang L, Xu Z, Liu X, Chen Y, Sun K. Validation of the EUTOS long-term survival score in Chinese chronic myeloid leukemia patients treated with Imatinib: a Multicenter Real-World Study. Cancer Manag Res. 2020;12:1293–301.
46. Millot F, Guilhot J, Suttorp M, Gunes AM, Sedlacek P, De Bont E, Li CK, Kalwak K, Lausen B, Culic S, Dworzak M, Kaiserova E, De Moerloose B, Roula F, Biondi A, Baruchel A. Prognostic discrimination based on the EUTOS long-term survival score within the international registry for chronic myeloid Leukemia in children and adolescents. Haematologica. 2017;102(10):1704–8.
47. Lauseker M, Bachl K, Turkina A, Faber E, Prejzner W, Olsson-Stromberg U, Baccarani M, Lomaia E, Zackova D, Ossenkoppele G, Griskevicius L, Schubert-Fritschle G, Sacha T, Heibl S, Koskenvesa P, Bogdanovic A, Clark RE, Guilhot J, Hoffmann VS,

Hasford J, Hochhaus A, Pfirrmann M. Prognosis of patients with chronic myeloid leukemia presenting in advanced phase is defined mainly by blast count, but also by age, chromosomal aberrations and hemoglobin. Am J Hematol. 2019;94(11):1236–43.

48. Sokal JE, Baccarani M, Tura S, Fiacchini M, Cervantes F, Rozman C, Gomez GA, Galton DA, Canellos GP, Braun TJ, et al. Prognostic discrimination among younger patients with chronic granulocytic leukemia: relevance to bone marrow transplantation. Blood. 1985;66(6):1352–7.
49. Kalmanti L, Saussele S, Lauseker M, Proetel U, Muller MC, Hanfstein B, Schreiber A, Fabarius A, Pfirrmann M, Schnittger S, Dengler J, Falge C, Kanz L, Neubauer A, Stegelmann F, Pfreundschuh M, Waller CF, Spiekermann K, Krause SW, Heim D, Nerl C, Hossfeld DK, Kolb HJ, Hochhaus A, Hasford J, Hehlmann R, German Chronic Myeloid Leukemia Study Group, Schweizerische Arbeitsgemeinschaft für Klinische Krebsforschung (SAKK). Younger patients with chronic myeloid leukemia do well in spite of poor prognostic indicators: results from the randomized CML study IV. Ann Hematol. 2014;93(1):71–80.
50. Crugnola M, Castagnetti F, Breccia M, Ferrero D, Trawinska MM, Abruzzese E, Annunziata M, Stagno F, Tiribelli M, Binotto G, Bonifacio M, Fava C, Iurlo A, Bucelli C, Mansueto G, Gozzini A, Falzetti F, Montefusco E, Crisa E, Gugliotta G, Russo S, Cedrone M, RussoRossi A, Pregno P, Isidori A, Mauro E, Atelda R, Giglio G, Celesti F, Sora F, Storti S, D'Addosio A, Galimberti S, Orlandi E, Calistri E, Bocchia M, Cavazzini F, Rege Cambrin G, Orofino N, Luciano L, Sgherza N, Rosti G, Latagliata R, Capodanno I. Outcome of very elderly chronic myeloid leukaemia patients treated with imatinib frontline. Ann Hematol. 2019;98(10):2329–38.
51. Scrucca L, Santucci A, Aversa F. Competing risk analysis using R: an easy guide for clinicians. Bone Marrow Transpl. 2007;40(4):381–7. 1705727 [pii]. https://doi.org/10.1038/sj.bmt.1705727.
52. Scrucca L, Santucci A, Aversa F. Regression modeling of competing risk using R: an in depth guide for clinicians. Bone Marrow Transpl. 2010;45(9):1388–95.
53. Pfirrmann M, Hasford J. Testing Sokal's and the new prognostic score for chronic myeloid leukaemia treated with alpha-interferon: comments. Br J Haematol. 2001;114(1):241–3. bjh2873 [pii]
54. Baccarani M, Abruzzese E, Accurso V, Albano F, Annunziata M, Barulli S, Beltrami G, Bergamaschi M, Binotto G, Bocchia M, Caocci G, Capodanno I, Cavazzini F, Cedrone M, Cerrano M, Crugnola M, D'Adda M, Elena C, Fava C, Fazi P, Fozza C, Galimberti S, Giai V, Gozzini A, Gugliotta G, Iurlo A, La Barba G, Levato L, Lucchesi A, Luciano L, Lunghi F, Lunghi M, Malagola M, Marasca R, Martino B, Melpignano A, Miggiano MC, Montefusco E, Musolino C, Palmieri F, Pregno P, Rapezzi D, Rege-Cambrin G, Rupoli S, Salvucci M, Sancetta R, Sica S, Spadano R, Stagno F, Tiribelli M, Tomassetti S, Trabacchi E, Bonifacio M, Breccia M, Castagnetti F, Pane F, Russo D, Saglio G, Soverini S, Vigneri P, Rosti G. Managing chronic myeloid leukemia for treatment-free remission: a proposal from the GIMEMA CML WP. Blood Adv. 2019;3(24):4280–90.
55. Cortes JE, Saglio G, Kantarjian HM, Baccarani M, Mayer J, Boque C, Shah NP, Chuah C, Casanova L, Bradley-Garelik B, Manos G, Hochhaus A. Final 5-year study results of DASISION: the Dasatinib versus Imatinib study in treatment-naive chronic myeloid Leukemia patients trial. J Clin Oncol. 2016;34(20):2333–40.
56. Cortes JE, Gambacorti-Passerini C, Deininger MW, Mauro MJ, Chuah C, Kim DW, Dyagil I, Glushko N, Milojkovic D, le Coutre P, Garcia-Gutierrez V, Reilly L, Jeynes-Ellis A, Leip E, Bardy-Bouxin N, Hochhaus A, Brummendorf TH. Bosutinib versus Imatinib for newly diagnosed chronic myeloid Leukemia: results from the randomized BFORE trial. J Clin Oncol. 2018;36(3):231–7.
57. Hochhaus A, Saglio G, Hughes TP, Larson RA, Kim DW, Issaragrisil S, le Coutre PD, Etienne G, Dorlhiac-Llacer PE, Clark RE, Flinn IW, Nakamae H, Donohue B, Deng W, Dalal D, Menssen HD, Kantarjian HM. Long-term benefits and risks of frontline nilotinib vs imatinib for chronic myeloid leukemia in chronic phase: 5-year update of the randomized ENESTnd trial. Leukemia. 2016;30(5):1044–54.
58. Claudiani S, Apperley JF. The argument for using imatinib in CML. Hematology Am Soc Hematol Educ Program. 2018;2018(1):161–7.
59. Hantel A, Larson RA. Imatinib is still recommended for frontline therapy for CML. Blood Adv. 2018;2(24):3648–52.
60. Radich JP, Deininger M, Abboud CN, Altman JK, Berman E, Bhatia R, Bhatnagar B, Curtin P, DeAngelo DJ, Gotlib J, Hobbs G, Jagasia M, Kantarjian HM, Maness L, Metheny L, Moore JO, Pallera A, Pancari P, Patnaik M, Purev E, Rose MG, Shah NP, Smith BD, Snyder DS, Sweet KL, Talpaz M, Thompson J, Yang DT, Gregory KM, Sundar H. Chronic myeloid leukemia, version 1.2019, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Cancer Netw. 2018;16(9):1108–35.



10 A Review and an Update of European LeukemiaNet Recommendations for the Management of Chronic Myeloid Leukemia

Gabriele Gugliotta, Fausto Castagnetti, Simona Soverini, Gianantonio Rosti, and Michele Baccarani

10.1 Introduction

The first version of the European LeukemiaNet (ELN) recommendations for the treatment of chronic myeloid leukemia (CML) was published in 2006 [1], the second and the third ones were published in 2009 and in 2013, respectively [2, 3]. Over this period, the ELN recommendations have provided an internationally shared basis for the treatment and monitoring of CML, contributing to the improvement of the management of CML. Over this period, patients with CML have enjoyed a survival that is nearly identical to the survival of the general population [4, 5], with an acceptable quality of life, due to the high efficacy and the low toxicity of the targeted treatment. Other ELN recommendations and reviews concerning the management of BCR-ABL kinase point mutations, the side-effects, and the toxicity of tyrosine kinase inhibitors (TKI) were published in 2011 and in 2016, respectively [6, 7]. Now the recommendations have been updated and published [8]. In this chapter we analyze and discuss the evolution of the ELN recommendations over a 15-year period, and we compare the last version with other recent recommendations and guidelines that have been proposed by the European Society of Medical Oncology (ESMO) [9], the Italian Group for Hematologic Diseases of Adults (GIMEMA) [10], the British Society of Haematology (BSH) [11], and the National Comprehensive Cancer Center Network (NCCN) [12].

G. Gugliotta
IRCCS Azienda Ospedaliero-Universitaria di Bologna, Istituto di Ematologia "Seràgnoli", Bologna, Italy

F. Castagnetti · S. Soverini
IRCCS Azienda Ospedaliero-Universitaria di Bologna, Istituto di Ematologia "Seràgnoli", Bologna, Italy

Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Bologna, Italy

G. Rosti (✉)
IRCCS - IRST "Dino Amadori", Meldola (FC), Italy
e-mail: gianantonio.rosti@unibo.it

M. Baccarani
Department of Hematology and Oncology "L. and A. Seràgnoli", University of Bologna, Bologna, Italy

10.2 The Phases of CML

More than 95% of patients are diagnosed in the chronic phase (CP), 2–3% are diagnosed in the accelerated phase (AP), and 2–3% are diagnosed in the blastic phase (BP) [1–3, 8, 9]. The patients who are diagnosed in AP or in BP require TKI as the patients who are diagnosed in CP, but, in many cases, they require also allogeneic stem cell transplantation (SCT) [8]. In the chemotherapy era, almost all patients were progressing to BP, either directly (blast crisis) or

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
R. Hehlmann (ed.), *Chronic Myeloid Leukemia*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-71913-5_10

through a transient AP. In the TKI era, the rate of progression is much lower (<10%) than in the chemotherapy era [8], but the recognition of the disease phase is still important. It is noticeable that in spite of progress in molecular biology, the boundaries between the three phases are mostly based on the same clinical and hematologic criteria that were selected many years ago [13, 14].

The 2013 version of the ELN recommendations [3] proposed the following definitions: for AP 15–29% blast cells, or blasts plus promyelocytes in blood or marrow >30%, with blasts <30%, or a platelet count <100 × 10^9/L unrelated to treatment, or another clonal chromosome abnormality (ACA) in Ph + cells (ACA/Ph+); for BP, a blast cell percentage ≥ 30% in blood or marrow or blast cell involvement of non-hematopoietic tissues or organs, excluding spleen and liver.

In the latest 2020 ELN version [8], the boundaries between CP and AP are no longer specified. Therefore, one could still rely on the definition of AP given in the 2013 ELN recommendations [3] or rely on another definition, which includes provisional criteria of response to TKI, as proposed in the 2017 WHO classification [15]. This uncertainty reflects doubts on whether the term "AP" should be maintained and used in clinical studies, as it has been for so many years, or if the term "AP" should be removed. As a matter of fact, in the TKI era it is difficult, and it is not very useful to assess the status of the disease based on clinical and hematological findings because the status of the disease can be assessed earlier, based on molecular response. A patient who "fails" (being resistant or intolerant) four TKIs has already entered into a phase of the disease that puts him at a high-risk of dying of leukemia [10], without taking into consideration blood cell counts and differential, particularly without waiting for a progressive increase of blast cells or the development of splenomegaly. In addition, the emergence of high-risk additional chromosome abnormalities in Ph + cells (ACA/Ph+) during TKI treatment is another confirmed signal of progression [16–22], and the finding of other somatic mutations may be also important [23]. For these reasons, the ELN 2020 recommendations use the term "end-phase CML," which comprises "early progression with emerging high-risk ACA and late progression with failing hematopoiesis and blast cell proliferation." BP is a late feature of progression, defined by the ELN only by the blast cells count (≥30%) in blood or marrow. In contrast, in the 2017 WHO classification [15], the definition of BP is still based on a proportion of blast cells ≥20% or on a blast cell involvement of other non-hematopoietic tissues or organs. Importantly, not all patients dying of CML reach the BP-defining blast levels.

10.3 Prognostic Factors

10.3.1 Baseline

Historically, CML was an almost always fatal disease, but it was well recognized that survival could range between few and many years, already in the chemotherapy era. The Sokal score [13], which was proposed as early as 36 years ago, based on the survival of patients treated with conventional chemotherapy, was found to predict response and survival also for patients treated with interferon-alpha and for patients treated with TKIs, particularly with imatinib. By the Sokal score, about 40% of patients are classified low-risk, about 40% are classified intermediate risk, and about 20% are classified high-risk. Other prognostic scores have been developed based on patients treated by interferon-alpha (EURO or Hasford score) [24], and in patients treated with imatinib (EUTOS score) [25]. Both EURO and EUTOS scores predict response and survival also in patients treated with imatinib. However, in the TKI era the causes of death are changed: about 50% of the CML patients who die, die in remission and not of leukemia. Therefore, another prognostic score, the Eutos Long-Term Survival Score (ELTS) [26] has been proposed. It has the merit of distinguishing the patients according to the risk of dying of leukemia: by the ELTS score about 60% of patients fall in the low-risk group, with a probability of dying of leukemia of 1–2%, about 30% in the intermediate-risk group, with a probability of dying of leukemia of 5–10%, and only about 10% in the high-risk group, with a probability of dying of leukemia of 10–20%. The last ELN recommendations [8] and the GIMEMA [10] and the BSH [11] recommendations recommend to use, prospectively, the ELTS score. The ELTS score is calculated using age, spleen size,

platelet count, and blast cell percentage in blood as it was the Sokal score. It is noticeable, and a bit unexpected, that after two decades of molecular studies, the prognosis at baseline is still based on clinical and hematologic findings and that splenomegaly is still assessed by manual palpation of the spleen, expressed as the maximum distance below the costal margin.

The calculation of the Sokal and of the ELTS scores is reported in Table 10.1. Both scores provide valuable information of long-term survival, and both are currently used to plan treatment because it is believed that low-risk patients may have a maximum benefit using imatinib in the first line. This belief is supported by solid evidence. It is also believed that high- and also intermediate-risk patients may benefit more of the earlier, first line, use of the "more potent" second-generation TKIs, but this expectation is not supported by solid evidence [8–11]. As a matter of fact, the strategies of treatment of high-risk patients have never been specifically designed and tested, such as the choice of the TKI, the doses, the role of allogeneic SCT, and the degree of the molecular response, optimal, warning or failure at the conventional cornerstones (3, 6, 12 months, and later).

Sokal and ELTS are not the unique prognostic factors that have been identified and proposed. Several reports have highlighted the importance of ACA/Ph + (including trisomy 8, +Ph, isochromosome 17 [i(17q10], trisomy 19, −7/−7q, 11q23 or 3q26.2 aberrations, and complex abnormalities [16–22, 27]), so that ACA/Ph + have been now recognized as baseline high-risk factors irrespective of Sokal and ELTS [8, 10]. Other factors were proposed [28–41], including the low expression of the organic cation transporter (OCT1) that prevents the influx into the cells of imatinib, the high expression and some polymorphisms of the MDR1 (ABCB1) proteins that increase the efflux of TKI from the cells, a high level of the cancerous inhibitor of PP2A (CIP2A), some polymorphisms or the deletion of BIM more frequently found in Asian countries, the KIR2DS1 genotype associated with resistance to imatinib, a high serum level of tryptase, the fiber content in bone marrow biopsies, and also the immunophenotype showing the simultaneous detection of lymphoid markers in blast cells. Although some of these factors could theoretically be useful to guide the choice beteween imatinib and second-generation TKIs, none of these factors have come into clinical use, and none were recommended so far.

The transcript type of the major BCR-ABL gene may influence to some extent the sensitivity to TKIs, the e13a2 (b2a2) type being less sensitive to TKIs than the e14a2 (b3a2) type and, accordingly, the probability of achieving a deep molecular response and a treatment-free remission [42]. Until now, the BCR-ABL transcript type has not been included as a prognostic parameter in the ELN 2020 recommendations.

Different gene expression profiles (GEP) associated with progression from CP to advanced phases, and with some degree of resistance to imatinib, were reported already years ago [43]. More recently the introduction of new, next-generation, molecular biotechnologies has called attention to the value of additional somatic genomic abnormalities [44], similar to those that have been detected in acute leukemia, in the myelodysplastic syndromes, and also in healthy, elderly people [45]. These studies could pave the way to new targeted therapies.

10.3.2 During TKI Treatment

The response to TKIs, including the time to response and the depth of the response, are more

Table 10.1 The two main risk scoring systems, at diagnosis

Score	Calculation
Sokal	Exp 0.0116 × (age − 43.4) + 0.0345 × (spleen − 7.51) + 0.188 × ((platelets/700)^2 − 0.563) + 0.0887 × (blasts − 2.10)
	Low-risk < 0.80. intermediate 0.81–1.20 high > 1.21
	http://www.leukemia-net.org/content/leukemias/cml/cml_score index_eng.Html.
ELTS	0.0025 × (age/10)^3 + (0.0615 × spleen) + (0.1052 × blasts) + ((0.4104 × (platelets/1000)$^{-0.5}$).
	Low-risk ≤ 1.5680, intermediate risk 1.5680–2.2185, high-risk > 2.2185
	http://www.leukemianet.org/content/leukemias/cml/eutos_score/index_eng.html.

Age in years, spleen in cm below the costal margin; platelets × 1000; blasts in % (peripheral blood)

important than all baseline factors. Cytogenetics is still valuable, but the assessment of the BCR-ABL level by the international standard (IS) is more sensitive and more accurate than the cytogenetic response. The definition of molecular response during treatment, as proposed by the most recent recommendations and guidelines [8, 10–12], is discussed thoroughly in the next section. Still, the definition of the response and its interpretation for guiding the treatment are based on a single value, sometimes on two consecutive tests in case of borderline values. However, the value of the qPCR may be better assessed not by an absolute value, but by the time that is necessary to reach that value [46] and more generally by the dynamics of the decrease of the BCR-ABL transcript level [47]. There is some reluctancy to adopt these dynamic criteria in practice, which is regrettable, because they may help taking several important decisions, concerning both the early and the late switch from one TKI to another, particularly to improve the rate of treatment-free remission.

10.4 Response Definition: The Evolution of Treatment Recommendations

Imatinib (IMA) was approved in the first-line treatment of CML in 2003, and for a short period it was the only TKI that was available [1]. Soon after, due to the development and the approval of second-generation (2G) TKIs, nilotinib (NIL) and dasatinib (DAS), in second line (in the patients resistant to or intolerant of imatinib) [2], as well as in the first-line setting [3], the criteria for the assessment of response definition have been progressively modified, leading to a more flexible definition of the responses, either optimal or failure or suboptimal (warning), at several critical cornerstones. In the case of optimal response, the recommendation is to continue the same TKI at the same dose. In the case of failure, the recommendation is to switch to another TKI. When the response is suboptimal (warning), the recommendation is to consider another TKI, depending on several variables, including the patient's age, health conditions, comorbidities, tolerability, and also on the goal of the treatment, either survival and quality of life or the achievement of a condition of treatment-free remission (TFR).

The evolving scenario of response definition and treatment recommendations can be better appreciated comparing the ELN recommendations that were published from 2006 (first version) [1] to 2020 (fourth and last version) [8] (Table 10.1). In 2006 and in 2009 the early (3, 6, and 12 months) response was based on hematologic and cytogenetic data [1, 2]. In 2013 the response was based on cytogenetic or on molecular data [3]. In 2020 only the molecular data were considered because molecular tests are more sensitive than cytogenetics [8]. Moreover, they are performed on blood cells, so avoiding a marrow aspirate. However,cytogenetics is still recommended in case of molecular failure because the detection of ACA/Ph highlights the danger of progression.

In the last version of the ELN recommendations [8], at 3 months the response is optimal if BCR-ABL is $\leq 10\%$, it is warning if BCR-ABL is >10% in one test, and it is failure, if BCR-ABL is >10%, and the value is confirmed within 1–3 months; at 6 months it is optimal if BCR-ABL is ≤1%, it is warning if BCR-ABL is >1–10%, and it is failure if BCR-ABL is >10%; at 12 months the response is optimal if BCR-ABL is ≤0.1% (MMR or MR 3.0), it is warning if BCR-ABL is >0.1–1%, and it is failure if BCR-ABL is >1%. The detection of mutations during treatment is always a marker of failure. After 12 months, and later on, if the BCR-ABL transcript level is ≤0.1% (optimal response) ELN 2020 recommends to continue the same TKI, while in the case of BCR-ABL > 0.1–1% (warning) there is a choice, either to change or to continue. If the BCR-ABL transcript level is >1% (failure), ELN recommends changing the TKI. These definitions are the same as in the last but one version of 2013.

In Table 10.2, the last ELN recommendations [8] are compared with the last ESMO (2017) [9] and NCCN (2.2021) guidelines [12], and with the recommendations that were recently proposed by GIMEMA (2019) [10] and by the BSH (2020) [11]. At 3 months, the response is defined as optimal by all recommendations and guidelines if the

Table 10.2 ELN definitions

Optimal response (= continue with the same TKI)					
Year	**3 months**	**6 months**	**12 months**	**18 months**	**Later**
2006	CHR	PCyR	CCyR	BCR-ABL ≤ 0.1%	NS
2009	CHR and Ph + ≤ 65%	Ph + ≤ 35%	CCyR	BCR-ABL ≤ 0.1%	Stable MMR
2013	PCyR or BCR-ABL ≤ 10%	CCyR or BCR-ABL ≤ 1%	BCR-ABL ≤ 0.1%	NS	MMR or better
2020[a]	BCR-ABL ≤ 10%	BCR-ABL ≤ 1%	BCR-ABL ≤ 0.1%	NS	BCR-ABL ≤ 0.1%
Suboptimal response or warning (= consider a switch to another TKI)					
Year	**3 months**	**6 months**	**12 months**	**18 months**	**Later**
2006	CHR	Ph+ > 35%	Ph + 1–35%	BCR-ABL > 0.1%	ACA/Ph+, response loss, mutations
2009	Ph+ > 95%	Ph + 35–95%	Ph + 1–35%	BCR-ABL >0.1–1%	MMR loss, mutations
2013	Ph + 36–95% or BCR-ABL > 10%	Ph + 1–35% or BCR-ABL > 1–10%	Ph + ≥ 1% or BCR-ABL > 0.1–1%	NS	ACA/Ph+
2020	BCR-ABL > 10%	BCR-ABL > 1–10%	BCR-ABL > 0.1–1%	NS	BCR-ABL > 0.1–1%
Failure (= switch to another TKI)					
Year	**3 months**	**6 months**	**12 months**	**18 months**	**Later**
2006	No HR	< CHR or Ph+ > 95%	Ph+ > 35%	Ph + ≥ 1%	Response loss
2009	< CHR	Ph+ > 95%	Ph+ > 35%	Ph + ≥ 1%	Response loss, mutations, ACA/Ph+
2013	< CHR or Ph+ > 95%	Ph+ > 35% or BCR-ABL > 10%	Ph + ≥ 1% or BCR-ABL > 1%	NS	Response loss, ACA/Ph+, mutations
2020	BCR-ABL > 10%, confirmed	BCR-ABL > 10%	BCR-ABL > 1%	NS	BCR-ABL > 1%, HR ACA/ Ph+, resistance mutations

NS not specified, *HR* high-risk, *ELN* European LeukemiaNet. CHR = complete hematologic response (WBC count <10,000/µl, differential without immature granulocytes and with less than 5% basophils, platelet count <450,000/µl, nonpalpable spleen) ACA/Ph+ additional chromosomal abnormalities in Ph+ cells

Cytogenetic response (CyR): Ph+ >95% (no CyR), Ph + 66–95% (minimal CyR), Ph + 36–65% (minor CyR),Ph + 1–35% (Partial CyR), Ph + 0 (Complete CyR). Molecular response (MR): BCR-ABL >10% (no MR), BCR-ABL >1–10% (MR 1.0), BCR-ABL > 0.1–1% (MR 2.0), BCR-ABL ≤ 0.1% (MMR or MR 3.0), BCR-ABL ≤ 0.01% (MR 4.0)

[a]For patients aiming at TFR, the optimal response (at any time) is BCR-ABL1 ≤ 0.01% (MR4)

Table 10.3 ELN, NCCN, BSH, GIMEMA, and ESMO latest definitions

Optimal response (= continue with the same TKI)				
	3 months	**6 months**	**12 months**	**Later**
ESMO 2017	Ph + ≤ 35% or BCR-ABL < 10%	Ph + 0% or BCR-ABL < 1%	BCR-ABL < 0.1%	BCR-ABL < 0.01%[a]
GIMEMA 2019	BCR-ABL ≤ 10%	BCR-ABL ≤ 1%	BCR-ABL ≤ 0.1%	BCR-ABL ≤ 0.01%
BSH 2020	BCR-ABL ≤ 10%	BCR-ABL ≤ 1%	BCR-ABL ≤ 0.1%	BCR-ABL ≤ 0.1%
ELN 2020[b]	BCR-ABL ≤ 10%	BCR-ABL ≤ 1%	BCR-ABL ≤ 0.1%	BCR-ABL ≤ 0.1%
NCCN 2.2021	BCR-ABL ≤ 10%	BCR-ABL ≤ 10%	BCR-ABL ≤ 0.1%[a] BCR-ABL > 0.1–1%[c]	NS
Suboptimal response or warning (= consider a switch to another TKI)				
	3 months	**6 months**	**12 months**	**Later**
ESMO 2017	Ph + 36–95% or BCR-ABL > 10%	Ph + 1–65% or BCR-ABL ≥ 1–10%	Ph + 0% or BCR-ABL ≥ 0.1–1%	BCR-ABL ≥ 0.1–1%
GIMEMA 2019	NS	BCR-ABL > 1–10%	BCR-ABL > 0.1–1%	BCR-ABL > 0.01–0.1%
BSH 2020	BCR-ABL > 10%	BCR-ABL > 1–10%	BCR-ABL > 0.1–1%	BCR-ABL 0.1–1%
ELN 2020	BCR-ABL > 10%	BCR-ABL > 1–10%	BCR-ABL > 0.1–1%	BCR-ABL > 0.1%
NCCN 2.2021	BCR-ABL > 10%	NS	BCR-ABL > 1–10%	NS
Failure (= switch to another TKI)				
	3 months	**6 months**	**12 months**	**Later**
ESMO 2017	<CHR or Ph+ > 95%	Ph+ > 35% or BCR-ABL > 10%	Ph + ≥ 1% or BCR-ABL > 1%	Relapse, loss of MMR
GIMEMA 2019	<CHR or BCR-ABL > 10%, confirmed	BCR-ABL > 10%	BCR-ABL > 1%	BCR-ABL > 0.1%, mutations
BSH 2020	NS	BCR-ABL > 10%	BCR-ABL > 1%	BCR-ABL > 1%
ELN 2020	BCR-ABL > 10%, confirmed	BCR-ABL > 10%	BCR-ABL > 1%	BCR-ABL > 1%, HR ACA/Ph+, resistance mutations
NCCN 2.2021	NS	BCR-ABL > 10%	BCR-ABL > 10%	NS

NS not specified, *HR* high-risk, *ELN* European LeukemiaNet, *ESMO* European Society of Medical Oncology, *NCCN* National Comprehensive Cancer Network, *GIMEMA* Gruppo Italiano Malattie Ematologiche dell' Adulto (Italian Group for Adult Hematologic Diseases), *BSH* British Society of Haematology, *CHR* complete hematologic response (WBC count <10,000/μl, differential without immature granulocytes and with less than 5% basophils, platelet count <450,000/μl, nonpalpable spleen) ACA/Ph+ additional chromosomal abnormalities in Ph+ cells

Cytogenetic response (CyR): Ph+ >95% (no CyR), Ph + 66–95% (minimal CyR), Ph + 36–65% (Minor CyR), Ph + 1–35% (Partial CyR), Ph + 0 (Complete CyR). Molecular response (MR): BCR-ABL >10% (no MR), BCR-ABL >1–10% (MR 1.0), BCR-ABL >0.1–1% (MR 2.0), BCR-ABL >0.01–0.1% (MMR or MR 3.0), BCR-ABL ≤ 0.01% (MR 4.0)

[a]If treatment goal is TFR

[b]For patients aiming at TFR, the optimal response (at any time) is BCR-ABL1 ≤ 0.01% (MR4)

[c]if treatment goal is long-term survival

BCR-ABL transcript level is ≤10%, while it is defined as a failure if BCR-ABL is >10% (confirmed in two consecutive tests) by ESMO, GIMEMA, and ELN 2020. No definition of failure at 3 months is given by the BSH and by NCCN 2.2021. At 6 months, if BCR-ABL is ≤1% the response is optimal by ESMO, GIMEMA, BSH, and ELN 2020, but not by the NCCN (BCR-ABL 1–10%), and the response is failure by all recommendations and guidelines if BCR-ABL is >10%. At 12 months, if BCR-ABL transcript level is ≤0.1% (MMR), the response is optimal in all recommendations, while it is a failure if BCR-ABL is >1%, with the exception of NCCN 2.2021 (> 10%). After the first year of treatment, the response is optimal if BCR-ABL is ≤0.1% by ESMO, BSH, and NCCN 2.2021, but only if BCR-ABL is ≤0.01% by GIMEMA while the response is failure if BCR-ABL is >1% by ELN 2020, but if it is >0.1% by GIMEMA. The definition of later responses was not specified by ESMO, BSH, and NCCN 2.2021.

In conclusion, all recommendations agree on the definition of failure at 6 months. The major difference is in the level of the transcript at 12 months: a value >1% is a failure, by all the European recommendations, but not by GIMEMA that defines a failure even at lower BCR-ABL transcript level (≤ 0.1%, or MMR), and not by the American guidelines that define failure only if that value is much higher at >10%. These are important differences that cannot easily be explained because although it is almost universally recognized that achieving a major molecular response (MMR) is required for a "normal" survival, there is no consensus on the cut-off value of transcript level, and there is no agreement on the time that may take to achieve the MMR.

Among the European recommendations, an important difference is in the level of the transcript after 1 year of treatment. For ELN 2020, ESMO, and the BHS, the response is optimal if BCR-ABL is ≤0.1% (MMR), while for GIMEMA the response is optimal only if BCR-ABL is ≤0.01% (MR 4.0). As already noticed, for ELN 2020 and the BSH the response at 12 months is a failure if BCR-ABL is >1%, while for GIMEMA it is already a failure if BCR-ABL is >0.1% (less than MMR). The reason of these differences is that the ELN 2020 and the BSH recommendations privilege survival, although they highlight that achievement of TFR may be a valid aim of treatment for selected patients, using a shared decision-making policy, while the GIMEMA recommendations privilege always the achievement of a deeper molecular response for treatment-free remission. In any case, the existence of differences at several cornerstones warns that in case of borderline values of the BCR-ABL transcript, a second test should be performed before deciding to continue or to change a TKI.

10.5 Treatment

10.5.1 First-Line

Four TKIs are currently approved as the first-line treatment of newly diagnosed CP CML: imatinib, nilotinib (Tasigna, Novartis Pharma), dasatinib (Sprycel, Bristol-Myers Squibb), and bosutinib (Bosulif, Pfizer). The respective approved doses are 400 mg once daily (OD), 300 mg twice daily (BID), 100 mg OD, and 400 mg OD. The last version of ELN recommendations [8], as well as ESMO, BSH, and NCCN 2021 [9, 11, 12], do not give priority to a TKI over another one as first-line treatment. Dasatinib, nilotinib, and bosutinib have been tested against imatinib in company-sponsored randomized trials [48–56]. The results of these trials have provided the basis for approval of these TKIs in the first-line setting. Dasatinib, nilotinib, and bosutinib have never been evaluated formally in comparative clinical trials. Furthermore, comparisons among different trials, either company sponsored or academic, are quite challenging because the patient selection and the endpoints are different and are differently evaluated. The choice of the first-line treatment, anyway, is mainly based on the final endpoint of the treatment and on patient's comorbidities. Imatinib remains the reference drug because most physicians have a long experience with it and because clinically relevant or life-threatening complications have not been reported so far.Therefore, imatinib remains, probably, the safest drug. During the last 20 years imatinib has been studied

not only in company-sponsored trials but also in important academic trials [57, 58]. Moreover, imatinib is currently less expensive than dasatinib, nilotinib, and bosutinib. Recently, the brand product Gleevec has been substituted in most countries by generic products, which are further less expensive. By comparison with imatinib 400 mg OD, dasatinib and nilotinib induce faster and deeper responses but the 5-year progression-free survival and the OS were reported to give marginal improvement with respect to imatinib [51, 53]. The same considerations apply to bosutinib vs. imatinib but with a much shorter observation period of 2 years [56]. There is a consensus favoring imatinib in elderly patients, in case of comorbidities, and in case of CML low-risk (the 5-year LRS of low-risk patients is higher than 95%) and favoring nilotinib, dasatinib, or bosutinib in case of high-risk. Moreover, the choice between nilotinib, dasatinib and bosutinib is influenced by comorbidities (cardiovascular risk, lung disease) and cost, which differs from country to country. However, the most important guide to the choice is the goal of treatment. If the goal of treatment is OS, imatinib may be sufficient. If the goal of treatment is a condition of treatment-free remission (TFR), it is likely that more patients will achieve that condition if they are treated first line with a second-generation TKI, but this expectation must still be proven.

10.5.2 Second-Line and beyond

In the second-line treatment, imatinib, nilotinib, dasatinib and bosutinib can be used at different doses: up to 400 mg BID for imatinib and nilotinib, up to 140 mg OD for dasatinib, and up to 600 mg OD for bosutinib [3, 59–61]. Finally, ponatinib (Iclusig, Takeda/Incyte) is licensed at a dose of 45 mg OD [62, 63] as second line for patients failing previous TKIs (USA), while in most EU countries the second line use is licensed for patients failing nilotinib or dasatinib first line, or in patients harboring the T315I mutation.

In the second-line treatment, four main scenarios are recognizable. Scenario no. 1 is that of intolerance to first-line treatment (toxicity). In that case, switching to nilotinib, dasatinib, or bosutinib should be prioritized over ponatinib because it is a situation very similar to first line. Scenario no. 2 is that of failure of first-line treatment (resistance). In that case, the choice of the second-line TKI will be guided by BCR-ABL1 mutations (if a mutation is found), by age, comorbidities, the type of side effects of first-line therapy, physician experience, and TKI availability and cost. Regrettably, there are no trials comparing the five available TKIs in second line. Ponatinib will always be the drug of choice in the case of T315I mutation [62, 63]. Scenario no. 3 is that of "warning" at early milestones (3–6 months), particularly in the case of BCR-ABL1 transcript level > $10\%^{IS}$ at 3 months (absence of early molecular response, EMR). EMR predicts the rate and the depth of late molecular response as well as progression-free survival (PFS) and overall survival, and EMR is achieved more frequently with second-generation TKIs than with imatinib [51, 53]. Not achieving EMR with imatinib suggests considering an early switch to a second-generation TKI; the absence of EMR with a second-generation TKIs in first line is a more worrisome situation, in which strict monitoring is mandatory and switching to ponatinib should be considered. Scenario no. 4 is that of the patient who is an optimal responder but never reaches a deep molecular response, so that he or she becomes a candidate for a late switch to another TKI, looking for treatment discontinuation and TFR [64, 65]. A careful patient selection is required to balance the benefit of a possible future TFR versus the potential new toxicities after switching to another TKI. In summary, in the case of scenarios no. 1 and 2, the TKI must be changed; in the case of scenarios no. 3 and 4, the TKI should or may be changed, and prospective studies are needed to assess the benefit and cost of the change.

An important and mostly uncovered issue is that of dose. All five TKIs were approved in second line at a specific dose: imatinib 400 mg OD to 400 mg BID, nilotinib 400 mg BID, dasatinib 100–140 mg OD, bosutinib 500–600 mg OD, and ponatinib 45 mg OD. Regrettably, there are no robust data with different doses, but there is a general consensus that in many patients all these TKIs are overdosed. Today, nilotinib and

dasatinib in second line are mostly used at a dose of 300 mg TD and 100 mg OD, respectively. The dose of bosutinib is likely to be higher than required and unnecessarily toxic. Recent data suggest that in second line a lower starting dose of bosutinib with response-driven dose escalation up to 400 mg OD is effective and well tolerated, at least in elderly patients [66]. Ponatinib is used at 45 mg only in a minority of cases (those bearing a T315I mutation or showing a high level or resistance to previous TKIs); the starting dose of 30 mg OD is preferred in most instances [8]. Ponatinib is currently tested at 30 or 15 mg OD to better balance efficacy versus cardiovascular toxicity [67]. Moreover, once MMR is achieved, ponatinib dose can be reduced to 15 mg OD with careful monitoring of response.

The scenario of third-line treatment is very heterogeneous, including patients who can still be rescued to an optimal response and patients at high-risk of progression and death [68–72]. Third-line treatment has an important impact on survival and may provide an essential bridge to allogeneic stem cell transplantation (allo-SCT). For these reasons, the patients who fail two or more TKIs should be referred to a center specialized in the treatment of CML, and allogeneic stem cell transplantation should be considered [8].

There are no published studies comparing different TKIs in third line. Ponatinib may be the first, or the last choice, because it covers almost all known mutations. Asciminib (Novartis Pharma), a new BCR-ABL allosteric inhibitor, active also against the T315I mutation, is currently in advanced clinical development [73], and a trial of asciminib vs. bosutinib in third line is ongoing; therefore, asciminib may become a valid option in this setting in the near future.

10.6 Treatment Continuation or Discontinuation, Treatment-Free Remission (TFR), Cure

In the 2013 ELN recommendations, continuous treatment with TKIs at the approved dose was referred as the standard of care [3]. Indeed, this approach demonstrated that the life expectancy of newly diagnosed CML patients was similar to that of the age-matched general population [4, 74]. As a matter of fact, TKIs can induce deep and stable responses with up to a 5-log reduction of the BCR-ABL1 transcript levels. Experimental and clinical data strongly suggest that TKIs cannot eliminate all BCR-ABL1+ stem cells [75–89]. However, there is evidence from several clinical studies [90–100] that 40–60% of the patients who discontinue treatment, having been treated with TKI for at least 3–5 years and being in stable deep MR (MR4 or better for at least 2 years), remain in remission. Monitoring of genomic mesurable residual disease may help to identify true cures or very long lasting TFR [101]. The risk of molecular relapse is higher during the first 6–12 months after discontinuation, then the risk decreases progressively. Whether they will relapse much later is not yet known, but it is known that discontinuation does not increase the risk of progression: almost all patients who have a molecular relapse can regain a molecular remission upon reassumption of the same TKI used prior to discontinuation.

Given the several available studies and the vast experience with TFR gained in the recent years, the ELN 2020 recommendation [8] recognized TFR as a new significant goal of CML management and provided for the first time a set of requirements for TKI discontinuation, distinguishing mandatory, minimal (stop allowed) and optimal (stop recommended for consideration) criteria. However, the best treatment strategy, in first or subsequent lines, to drive patients toward a successful TFR is yet to be defined, and the 2020 ELN recommendations could not provide specific recommendations on that. Indeed, only a few trials are currently ongoing with TFR as a primary objective, including the GIMEMA SUSTRENIM trial and the German CML TIGER study, that is, however, a trial of nilotinib versus a combination of nilotinib and interferon-α. Waiting for the results of these trials, the GIMEMA published in 2019 a set of proposals specifically designed to optimize the treatment strategy for TFR.

The biological mechanisms underlying TFR are not well understood, but TFR probably represents an "operational cure" rather than a "true cure" (disease eradication). Indeed, residual BCR-ABL1+ stem cells are quiescent, no longer

BCR-ABL1 addicted and not sensitive to TKIs [76, 80, 81, 83, 85, 88, 89]. Other agents, targeting the stem cells [75, 78], may be necessary to attain CML eradication. Over the last 20 years, many studies have been dedicated to the identification of new targets in the stem cells [102], and many studies have shown that, experimentally, the combination of a TKI with anti-stem-cell agents can eliminate BCR-ABL1+ stem cells [103]. Regrettably, none of these combinations have been tested successfully in vivo, also because there is a legitimate concern of the use of potentially toxic agents in patients with minimal residual disease, with a normal life expectancy and a normal quality of life. For the time being, the search of a cure is theoretical and is addressed toward the immunologic control of minimal residual disease [104–108] which today mainly rely on the addition of IFNs to TKI treatment [109–111].

Certainly, TFR is a clinically relevant endpoint that influences on the well-being and the psychologic behavior of the patients because it limits the long-term toxicity of the treatment and spares the economical resources that are necessary for life-long TKI therapy.

With current treatment approaches it is expected that no more than 20–30% of all newly diagnosed CML patients will be able to achieve a stable TFR. The remaining 70–80% of patients. would need lifelong “standard” dose TKI as per current recommendation and guidelines. For these patients the main objectives are an improvement of quality of life [112] and minimization of long-term toxicities. With this regard, de-escalation of treatment through permanent dose reductions or even intermittent treatment has been investigated and results are promising [113, 114].

10.7 Conclusions

In the last 15 years ELN recommendations contributed to the improvement of the management of CML. Today the survival of CML patients is comparable to that of the general population; the next step is the achievement of a normal survival without continuous treatment. The ELN 2020 recommendations recognized TFR as an important goal of CML therapy and identified a set of requirements for TKI discontinuation. However, it was not possible to recommend specific treatment strategies, both in first or subsequent lines, to increase the proportion of patients reaching TFR. For these reasons, next prospective studies should be designed to consider TFR as the primary endpoint (instead of response rates at a time point). Hopefully, the next version of ELN recommendations will take advantage of the results of such trials to finally propose an evidence-based path to TFR.

References

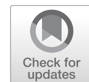
1. Baccarani M, Saglio G, Goldman J, et al. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. Blood. 2006;108(6):1809–20.
2. Baccarani M, Cortes J, Pane F, et al. Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. J Clin Oncol. 2009;27(35):6041–51.
3. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood. 2013;122(6):872–84.
4. Bower H, Bjorkholm M, Dickman PW, Hoglund M, Lambert PC, Andersson TM. Life expectancy of patients with chronic myeloid Leukemia approaches the life expectancy of the general population. J Clin Oncol. 2016;34(24):2851–7.
5. Hehlmann R, Lauseker M, Saussele S, et al. Assessment of imatinib as first-line treatment of chronic myeloid leukemia: 10-year survival results of the randomized CML study IV and impact of non-CML determinants. Leukemia. 2017;31(11):2398–406.
6. Soverini S, Hochhaus A, Nicolini FE, et al. BCR-ABL kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: recommendations from an expert panel on behalf of European LeukemiaNet. Blood. 2011;118(5):1208–15.
7. Steegmann JL, Baccarani M, Breccia M, et al. European LeukemiaNet recommendations for the management and avoidance of adverse events of treatment in chronic myeloid leukaemia. Leukemia. 2016;30(8):1648–71.
8. Hochhaus A, Baccarani M, Silver RT, et al. European LeukemiaNet 2020 recommendations

for treating chronic myeloid leukemia. Leukemia. 2020;34(4):966–84.
9. Hochhaus A, Saussele S, Rosti G, et al. Chronic myeloid leukaemia: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2017;28(Suppl 4):iv41–51.
10. Baccarani M, Abruzzese E, Accurso V, et al. Managing chronic myeloid leukemia for treatment-free remission: a proposal from the GIMEMA CML WP. Blood Adv. 2019;3(24):4280–90.
11. Smith G, Apperley J, Milojkovic D, et al. A British Society for Haematology guideline on the diagnosis and management of chronic myeloid leukaemia. Br J Haematol. 2020;191:171.
12. Deininger MW, Shah NP, Altman JK, et al. Chronic myeloid Leukemia, version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Cancer Netw. 2020;18(10):1385–415.
13. Sokal JE, Cox EB, Baccarani M, et al. Prognostic discrimination in "good-risk" chronic granulocytic leukemia. Blood. 1984;63(4):789–99.
14. Spiers AS. Metamorphosis of chronic granulocytic leukaemia: diagnosis, classification, and management. Br J Haematol. 1979;41(1):1–7.
15. Barbui T, Thiele J, Gisslinger H, Finazzi G, Vannucchi AM, Tefferi A. The 2016 revision of WHO classification of myeloproliferative neoplasms: clinical and molecular advances. Blood Rev. 2016;30(6):453–9.
16. Hehlmann R, Voskanyan A, Lauseker M, et al. High-risk additional chromosomal abnormalities at low blast counts herald death by CML. Leukemia. 2020;34(8):2074–86.
17. Fabarius A, Leitner A, Hochhaus A, et al. Impact of additional cytogenetic aberrations at diagnosis on prognosis of CML: long-term observation of 1151 patients from the randomized CML study IV. Blood. 2011;118(26):6760–8.
18. Luatti S, Castagnetti F, Marzocchi G, et al. Additional chromosomal abnormalities in Philadelphia-positive clone: adverse prognostic influence on frontline imatinib therapy: a GIMEMA Working Party on CML analysis. Blood. 2012;120(4):761–7.
19. Fabarius A, Kalmanti L, Dietz CT, et al. Impact of unbalanced minor route versus major route karyotypes at diagnosis on prognosis of CML. Ann Hematol. 2015;94(12):2015–24.
20. Wang W, Cortes JE, Lin P, et al. Clinical and prognostic significance of 3q26.2 and other chromosome 3 abnormalities in CML in the era of tyrosine kinase inhibitors. Blood. 2015;126(14):1699–706.
21. Wang W, Cortes JE, Tang G, et al. Risk stratification of chromosomal abnormalities in chronic myelogenous leukemia in the era of tyrosine kinase inhibitor therapy. Blood. 2016;127(22):2742–50.
22. Alhuraiji A, Kantarjian H, Boddu P, et al. Prognostic significance of additional chromosomal abnormalities at the time of diagnosis in patients with chronic myeloid leukemia treated with frontline tyrosine kinase inhibitors. Am J Hematol. 2018;93(1):84–90.
23. Branford S, Wang P, Yeung DT, et al. Integrative genomic analysis reveals cancer-associated mutations at diagnosis of CML in patients with high-risk disease. Blood. 2018;132(9):948–61.
24. Hasford J, Pfirrmann M, Hehlmann R, et al. A new prognostic score for survival of patients with chronic myeloid leukemia treated with interferon alfa. Writing Committee for the Collaborative CML Prognostic Factors Project Group. J Natl Cancer Inst. 1998;90(11):850–8.
25. Hasford J, Baccarani M, Hoffmann V, et al. Predicting complete cytogenetic response and subsequent progression-free survival in 2060 patients with CML on imatinib treatment: the EUTOS score. Blood. 2011;118(3):686–92.
26. Pfirrmann M, Baccarani M, Saussele S, et al. Prognosis of long-term survival considering disease-specific death in patients with chronic myeloid leukemia. Leukemia. 2016;30(1):48–56.
27. Wang W, Cortes JE, Lin P, et al. Impact of trisomy 8 on treatment response and survival of patients with chronic myelogenous leukemia in the era of tyrosine kinase inhibitors. Leukemia. 2015;29(11):2263–6.
28. White DL, Saunders VA, Dang P, et al. OCT-1-mediated influx is a key determinant of the intracellular uptake of imatinib but not nilotinib (AMN107): reduced OCT-1 activity is the cause of low in vitro sensitivity to imatinib. Blood. 2006;108(2):697–704.
29. White DL, Saunders VA, Dang P, et al. Most CML patients who have a suboptimal response to imatinib have low OCT-1 activity: higher doses of imatinib may overcome the negative impact of low OCT-1 activity. Blood. 2007;110(12):4064–72.
30. White DL, Dang P, Engler J, et al. Functional activity of the OCT-1 protein is predictive of long-term outcome in patients with chronic-phase chronic myeloid leukemia treated with imatinib. J Clin Oncol. 2010;28(16):2761–7.
31. Engler JR, Frede A, Saunders VA, Zannettino AC, Hughes TP, White DL. Chronic myeloid leukemia CD34+ cells have reduced uptake of imatinib due to low OCT-1 activity. Leukemia. 2010;24(4):765–70.
32. Watkins DB, Hughes TP, White DL. OCT1 and imatinib transport in CML: is it clinically relevant? Leukemia. 2015;29(10):1960–9.
33. Augis V, Airiau K, Josselin M, Turcq B, Mahon FX, Belloc F. A single nucleotide polymorphism in cBIM is associated with a slower achievement of major molecular response in chronic myeloid leukaemia treated with imatinib. PLoS One. 2013;8(11):e78582.
34. Thomas J, Wang L, Clark RE, Pirmohamed M. Active transport of imatinib into and out of cells: implications for drug resistance. Blood. 2004;104(12):3739–45.
35. Katagiri S, Tauchi T, Saito Y, et al. BCL2L11 (BIM) deletion polymorphism is associated with molecular relapse after ABL tyrosine kinase inhibitor discontinuation in patients with chronic myeloid Leukemia with complete molecular response. Blood. 2014;124(21):1797.

36. Sperr WR, Pfeiffer T, Kundi M, Sillaber C, Herndlhofer S, Valent P. Serum Tryptase is a strong predictive biomarker that improves prognostication in Ph+ chronic myeloid Leukemia. Blood. 2012;120(21):2783.
37. Mahon FX, Belloc F, Lagarde V, et al. MDR1 gene overexpression confers resistance to imatinib mesylate in leukemia cell line models. Blood. 2003;101(6):2368–73.
38. Lucas CM, Harris RJ, Giannoudis A, Copland M, Slupsky JR, Clark RE. Cancerous inhibitor of PP2A (CIP2A) at diagnosis of chronic myeloid leukemia is a critical determinant of disease progression. Blood. 2011;117(24):6660–8.
39. Marin D, Gabriel IH, Ahmad S, et al. KIR2DS1 genotype predicts for complete cytogenetic response and survival in newly diagnosed chronic myeloid leukemia patients treated with imatinib. Leukemia. 2012;26(2):296–302.
40. El Rassi F, Bergsagel JD, Arellano M, et al. Predicting early blast transformation in chronic-phase chronic myeloid leukemia: is immunophenotyping the missing link? Cancer. 2015;121(6):872–5.
41. Hidalgo-Lopez JE, Kanagal-Shamanna R, Quesada AE, et al. Bone marrow core biopsy in 508 consecutive patients with chronic myeloid leukemia: assessment of potential value. Cancer. 2018;124(19):3849–55.
42. Baccarani M, Castagnetti F, Gugliotta G, et al. The proportion of different BCR-ABL1 transcript types in chronic myeloid leukemia. An international overview. Leukemia. 2019;33(5):1173–83.
43. Radich JP, Dai H, Mao M, et al. Gene expression changes associated with progression and response in chronic myeloid leukemia. Proc Natl Acad Sci U S A. 2006;103(8):2794–9.
44. Branford S, Kim DDH, Apperley JF, et al. Laying the foundation for genomically-based risk assessment in chronic myeloid leukemia. Leukemia. 2019;33(8):1835–50.
45. Ashley EA. Towards precision medicine. Nat Rev Genet. 2016;17(9):507–22.
46. Branford S, Yeung DT, Parker WT, et al. Prognosis for patients with CML and >10% BCR-ABL1 after 3 months of imatinib depends on the rate of BCR-ABL1 decline. Blood. 2014;124(4):511–8.
47. Hanfstein B, Shlyakhto V, Lauseker M, et al. Velocity of early BCR-ABL transcript elimination as an optimized predictor of outcome in chronic myeloid leukemia (CML) patients in chronic phase on treatment with imatinib. Leukemia. 2014;28(10):1988–92.
48. Kantarjian H, Shah NP, Hochhaus A, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2010;362(24):2260–70.
49. Kantarjian HM, Hochhaus A, Saglio G, et al. Nilotinib versus imatinib for the treatment of patients with newly diagnosed chronic phase, Philadelphia chromosome-positive, chronic myeloid leukaemia: 24-month minimum follow-up of the phase 3 randomised ENESTnd trial. Lancet Oncol. 2011;12(9):841–51.
50. Kantarjian HM, Shah NP, Cortes JE, et al. Dasatinib or imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: 2-year follow-up from a randomized phase 3 trial (DASISION). Blood. 2012;119(5):1123–9.
51. Cortes JE, Saglio G, Kantarjian HM, et al. Final 5-year study results of DASISION: the Dasatinib versus Imatinib study in treatment-naive chronic myeloid Leukemia patients trial. J Clin Oncol. 2016;34(20):2333–40.
52. Saglio G, Kim DW, Issaragrisil S, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med. 2010;362(24):2251–9.
53. Hochhaus A, Saglio G, Hughes TP, et al. Long-term benefits and risks of frontline nilotinib vs imatinib for chronic myeloid leukemia in chronic phase: 5-year update of the randomized ENESTnd trial. Leukemia. 2016;30:1044.
54. Wang J, Shen ZX, Saglio G, et al. Phase 3 study of nilotinib vs imatinib in Chinese patients with newly diagnosed chronic myeloid leukemia in chronic phase: ENESTchina. Blood. 2015;125(18):2771–8.
55. Cortes JE, Kim DW, Kantarjian HM, et al. Bosutinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: results from the BELA trial. J Clin Oncol. 2012;30(28):3486–92.
56. Cortes JE, Gambacorti-Passerini C, Deininger MW, et al. Bosutinib versus Imatinib for newly diagnosed chronic myeloid leukemia: results from the randomized BFORE trial. J Clin Oncol. 2018;36(3):231–7.
57. Castagnetti F, Gugliotta G, Breccia M, et al. Long-term outcome of chronic myeloid leukemia patients treated frontline with imatinib. Leukemia. 2015;29(9):1823–31.
58. Hehlmann R, Muller MC, Lauseker M, et al. Deep molecular response is reached by the majority of patients treated with imatinib, predicts survival, and is achieved more quickly by optimized high-dose imatinib: results from the randomized CML-study IV. J Clin Oncol. 2014;32(5):415–23.
59. Shah NP, Guilhot F, Cortes JE, et al. Long-term outcome with dasatinib after imatinib failure in chronic-phase chronic myeloid leukemia: follow-up of a phase 3 study. Blood. 2014;123(15):2317–24.
60. Giles FJ, le Coutre PD, Pinilla-Ibarz J, et al. Nilotinib in imatinib-resistant or imatinib-intolerant patients with chronic myeloid leukemia in chronic phase: 48-month follow-up results of a phase II study. Leukemia. 2013;27(1):107–12.
61. Kantarjian HM, Cortes JE, Kim DW, et al. Bosutinib safety and management of toxicity in leukemia patients with resistance or intolerance to imatinib and other tyrosine kinase inhibitors. Blood. 2014;123(9):1309–18.
62. Cortes JE, Kantarjian H, Shah NP, et al. Ponatinib in refractory Philadelphia chromosome-positive leukemias. N Engl J Med. 2012;367(22):2075–88.

63. Cortes JE, Kim DW, Pinilla-Ibarz J, et al. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. N Engl J Med. 2013;369(19):1783–96.
64. Hughes TP, Hochhaus A, Kantarjian HM, et al. Safety and efficacy of switching to nilotinib 400 mg twice daily for patients with chronic myeloid leukemia in chronic phase with suboptimal response or failure on front-line imatinib or nilotinib 300 mg twice daily. Haematologica. 2014;99(7):1204–11.
65. Hughes TP, Lipton JH, Spector N, et al. Deep molecular responses achieved in patients with CML-CP who are switched to nilotinib after long-term imatinib. Blood. 2014;124(5):729–36.
66. Castagnetti F, Gugliotta G, Bocchia M, et al. Dose optimization in elderly CML patients treated with Bosutinib after intolerance or failure of first-line tyrosine kinase inhibitors. Blood. 2019;134(Supplement_1):496.
67. Cortes JE, Lomaia E, Turkina A, et al. Interim analysis (IA) of OPTIC: a dose-ranging study of three ponatinib (PON) starting doses. J Clin Oncol. 2020;38(15_suppl):7502.
68. Quintas-Cardama A, Kantarjian H, Jones D, et al. Dasatinib (BMS-354825) is active in Philadelphia chromosome-positive chronic myelogenous leukemia after imatinib and nilotinib (AMN107) therapy failure. Blood. 2007;109(2):497–9.
69. Garg RJ, Kantarjian H, O'Brien S, et al. The use of nilotinib or dasatinib after failure to 2 prior tyrosine kinase inhibitors: long-term follow-up. Blood. 2009;114(20):4361–8.
70. Khoury HJ, Cortes JE, Kantarjian HM, et al. Bosutinib is active in chronic phase chronic myeloid leukemia after imatinib and dasatinib and/or nilotinib therapy failure. Blood. 2012;119(15):3403–12.
71. Russo Rossi A, Breccia M, Abruzzese E, et al. Outcome of 82 chronic myeloid leukemia patients treated with nilotinib or dasatinib after failure of two prior tyrosine kinase inhibitors. Haematologica. 2013;98(3):399–403.
72. Lipton JH, Bryden P, Sidhu MK, et al. Comparative efficacy of tyrosine kinase inhibitor treatments in the third-line setting, for chronic-phase chronic myelogenous leukemia after failure of second-generation tyrosine kinase inhibitors. Leuk Res. 2015;39(1):58–64.
73. Hughes TP, Mauro MJ, Cortes JE, et al. Asciminib in chronic myeloid leukemia after ABL kinase inhibitor failure. N Engl J Med. 2019;381(24):2315–26.
74. Sasaki K, Strom SS, O'Brien S, et al. Relative survival in patients with chronic-phase chronic myeloid leukaemia in the tyrosine-kinase inhibitor era: analysis of patient data from six prospective clinical trials. Lancet Haematol. 2015;2(5):e186–93.
75. Elrick LJ, Jorgensen HG, Mountford JC, Holyoake TL. Punish the parent not the progeny. Blood. 2005;105(5):1862–6.
76. Kavalerchik E, Goff D, Jamieson CH. Chronic myeloid leukemia stem cells. J Clin Oncol. 2008;26(17):2911–5.
77. Chen Y, Peng C, Sullivan C, Li D, Li S. Critical molecular pathways in cancer stem cells of chronic myeloid leukemia. Leukemia. 2010;24(9):1545–54.
78. Ross DM, Hughes TP, Melo JV. Do we have to kill the last CML cell? Leukemia. 2011;25(2):193–200.
79. Egan JM. Targeting stem cells in chronic myeloid Leukemia with a PPAR-gamma agonist. N Engl J Med. 2015;373(20):1973–5.
80. Graham SM, Jorgensen HG, Allan E, et al. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. Blood. 2002;99(1):319–25.
81. Copland M, Hamilton A, Elrick LJ, et al. Dasatinib (BMS-354825) targets an earlier progenitor population than imatinib in primary CML but does not eliminate the quiescent fraction. Blood. 2006;107(11):4532–9.
82. Jorgensen HG, Copland M, Allan EK, et al. Intermittent exposure of primitive quiescent chronic myeloid leukemia cells to granulocyte-colony stimulating factor in vitro promotes their elimination by imatinib mesylate. Clin Cancer Res. 2006;12(2):626–33.
83. Jiang X, Zhao Y, Smith C, et al. Chronic myeloid leukemia stem cells possess multiple unique features of resistance to BCR-ABL targeted therapies. Leukemia. 2007;21(5):926–35.
84. Jiang X, Forrest D, Nicolini F, et al. Properties of CD34+ CML stem/progenitor cells that correlate with different clinical responses to imatinib mesylate. Blood. 2010;116(12):2112–21.
85. Lemoli RM, Salvestrini V, Bianchi E, et al. Molecular and functional analysis of the stem cell compartment of chronic myelogenous leukemia reveals the presence of a CD34- cell population with intrinsic resistance to imatinib. Blood. 2009;114(25):5191–200.
86. Chomel JC, Bonnet ML, Sorel N, et al. Leukemic stem cell persistence in chronic myeloid leukemia patients with sustained undetectable molecular residual disease. Blood. 2011;118(13):3657–60.
87. Chu S, McDonald T, Lin A, et al. Persistence of leukemia stem cells in chronic myelogenous leukemia patients in prolonged remission with imatinib treatment. Blood. 2011;118(20):5565–72.
88. Corbin AS, Agarwal A, Loriaux M, Cortes J, Deininger MW, Druker BJ. Human chronic myeloid leukemia stem cells are insensitive to imatinib despite inhibition of BCR-ABL activity. J Clin Invest. 2011;121(1):396–409.
89. Hamilton A, Helgason GV, Schemionek M, et al. Chronic myeloid leukemia stem cells are not dependent on Bcr-Abl kinase activity for their survival. Blood. 2012;119(6):1501–10.
90. Rousselot P, Huguet F, Rea D, et al. Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. Blood. 2007;109(1):58–60.
91. Mahon FX, Rea D, Guilhot J, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular

remission for at least 2 years: the prospective, multicentre stop imatinib (STIM) trial. Lancet Oncol. 2010;11(11):1029–35.
92. Takahashi N, Kyo T, Maeda Y, et al. Discontinuation of imatinib in Japanese patients with chronic myeloid leukemia. Haematologica. 2012;97(6):903–6.
93. Tang M, Foo J, Gonen M, Guilhot J, Mahon FX, Michor F. Selection pressure exerted by imatinib therapy leads to disparate outcomes of imatinib discontinuation trials. Haematologica. 2012;97(10):1553–61.
94. Mori S, Vagge E, le Coutre P, et al. Age and dPCR can predict relapse in CML patients who discontinued imatinib: the ISAV study. Am J Hematol. 2015;90(10):910–4.
95. Etienne G, Guilhot J, Rea D, et al. Long-term follow-up of the French stop imatinib (STIM1) study in patients with chronic myeloid leukemia. J Clin Oncol. 2017;35(3):298–305.
96. Saussele S, Richter J, Guilhot J, et al. Discontinuation of tyrosine kinase inhibitor therapy in chronic myeloid leukaemia (EURO-SKI): a prespecified interim analysis of a prospective, multicentre, non-randomised, trial. Lancet Oncol. 2018;19(6):747–57.
97. Fava C, Rege-Cambrin G, Dogliotti I, et al. Observational study of chronic myeloid leukemia Italian patients who discontinued tyrosine kinase inhibitors in clinical practice. Haematologica. 2019;104(8):1589–96.
98. Ross DM, Masszi T, Gomez Casares MT, et al. Durable treatment-free remission in patients with chronic myeloid leukemia in chronic phase following frontline nilotinib: 96-week update of the ENESTfreedom study. J Cancer Res Clin Oncol. 2018;144(5):945–54.
99. Shah NP, Garcia-Gutierrez V, Jimenez-Velasco A, et al. Dasatinib discontinuation in patients with chronic-phase chronic myeloid leukemia and stable deep molecular response: the DASFREE study. Leuk Lymphoma. 2020;61(3):650–9.
100. Kimura S, Imagawa J, Murai K, et al. Treatment-free remission after first-line dasatinib discontinuation in patients with chronic myeloid leukaemia (first-line DADI trial): a single-arm, multicentre, phase 2 trial. Lancet Haematol. 2020;7(3):e218–25.
101. Machova Polakova K, Zizkova H, Zuna J, et al. Analysis of chronic myeloid leukaemia during deep molecular response by genomic PCR: a traffic light stratification model with impact on treatment-free remission. Leukemia. 2020;34(8):2113–24.
102. Houshmand M, Simonetti G, Circosta P, et al. Chronic myeloid leukemia stem cells. Leukemia. 2019;33(7):1543–56.
103. Bhatia R. Novel approaches to therapy in CML. Hematology Am Soc Hematol Educ Program. 2017;2017(1):115–20.
104. Butt NM, Rojas JM, Wang L, Christmas SE, Abu-Eisha HM, Clark RE. Circulating bcr-abl-specific CD8+ T cells in chronic myeloid leukemia patients and healthy subjects. Haematologica. 2005;90(10):1315–23.
105. Quintarelli C, Dotti G, De Angelis B, et al. Cytotoxic T lymphocytes directed to the preferentially expressed antigen of melanoma (PRAME) target chronic myeloid leukemia. Blood. 2008;112(5):1876–85.
106. Kreutzman A, Juvonen V, Kairisto V, et al. Mono/oligoclonal T and NK cells are common in chronic myeloid leukemia patients at diagnosis and expand during dasatinib therapy. Blood. 2010;116(5):772–82.
107. Clapp GD, Lepoutre T, El Cheikh R, et al. Implication of the autologous immune system in BCR-ABL transcript variations in chronic myelogenous leukemia patients treated with imatinib. Cancer Res. 2015;75(19):4053–62.
108. Hughes A, Yong ASM. Immune effector recovery in chronic myeloid Leukemia and treatment-free remission. Front Immunol. 2017;8:469.
109. Hjorth-Hansen H, Stentoft J, Richter J, et al. Safety and efficacy of the combination of pegylated interferon-alpha2b and dasatinib in newly diagnosed chronic-phase chronic myeloid leukemia patients. Leukemia. 2016;30(9):1853–60.
110. Nicolini FE, Etienne G, Dubruille V, et al. Nilotinib and peginterferon alfa-2a for newly diagnosed chronic-phase chronic myeloid leukaemia (NiloPeg): a multicentre, non-randomised, open-label phase 2 study. Lancet Haematol. 2015;2(1):e37–46.
111. Simonsson B, Gedde-Dahl T, Markevarn B, et al. Combination of pegylated IFN-alpha2b with imatinib increases molecular response rates in patients with low- or intermediate-risk chronic myeloid leukemia. Blood. 2011;118(12):3228–35.
112. Efficace F, Cannella L. The value of quality of life assessment in chronic myeloid leukemia patients receiving tyrosine kinase inhibitors. Hematology Am Soc Hematol Educ Program. 2016;2016(1):170–9.
113. Clark RE, Polydoros F, Apperley JF, et al. De-escalation of tyrosine kinase inhibitor therapy before complete treatment discontinuation in patients with chronic myeloid leukaemia (DESTINY): a non-randomised, phase 2 trial. Lancet Haematol. 2019;6(7):e375–83.
114. Russo D, Martinelli G, Malagola M, et al. Effects and outcome of a policy of intermittent imatinib treatment in elderly patients with chronic myeloid leukemia. Blood. 2013;121(26):5138–44.



11 The Role of Hematopoietic Stem Cell Transplantation in CML

Jane F. Apperley and A. Gratwohl

11.1 Introduction

The introduction of tyrosine kinase inhibitors (TKI) has changed the outlook for patients with chronic myeloid leukemia (CML), a previously uniformly fatal disease, and spearheaded the introduction of "precision medicine" for this and other malignant diseases [1–13]. The success of the TKI not only changed the course of the disease but also its treatment algorithms over a very short period of time, no better evidenced than with the dramatic decline in the use of hematopoietic stem cell transplantation (HSCT) for CML (Fig. 11.1). HSCT lost its former importance as the "only curative therapy" [14–17] and was rapidly consigned to use only as a last resort when everything else had failed and often when the patient had experienced disease progression. However HSCT remains a powerful intervention with the potential for "cure." As the use of TKI has been optimized over the past 20 years so has the outcome of HSCT considerably improved over the same period, with more sophisticated tools for donor and patient selection, a reduction in the intensity of preparative regimens, better supportive care, and the introduction of quality standards for transplant units ([18–20]). With this new knowledge it is now possible to integrate HSCT into the treatment of the small but nevertheless important cohort of patients who do not respond to TKI but may achieve long-term survival with HSCT if recognized early in their disease course.

The use of HSCT for CML has been a role model for all other diseases amenable to transplant, and it is worth reflecting on the lessons learnt to understand how the technology may be utilized in the future.

J. F. Apperley (✉)
Centre for Haematology, Imperial College London, Hammersmith Hospital, London, UK
e-mail: j.apperley@imperial.ac.uk

A. Gratwohl
Hematology, Medical Faculty, University of Basel, Basel, Switzerland

11.2 Evolution of HSCT for CML

11.2.1 Historical Perspective

The first report of a successful HSCT from a syngeneic donor to a patient with CML was more than 50 years ago and introduced a new concept into the treatment of the disease [21]. There followed the discovery of circulating leukemia progenitors in the peripheral blood and the start of the autografting era. Using progenitors collected from the bone marrow [22] or blood during the chronic phase [23], patients were treated at the time of blast transformation by high dose chemo- or chemoradiotherapy followed by infusion of cryopreserved chronic phase cells. The aim was

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
R. Hehlmann (ed.), *Chronic Myeloid Leukemia*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-71913-5_11

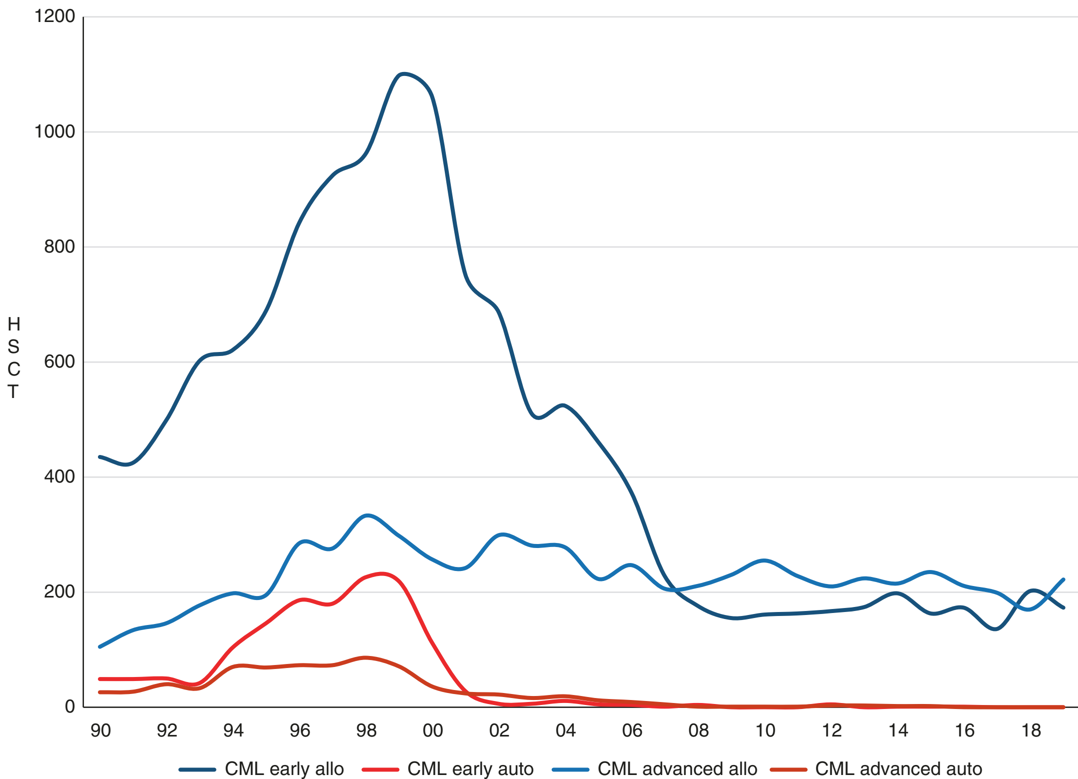


Fig. 11.1 Numbers of transplants performed for CML in Europe 1990–2019

to restore the chronic phase and prolong survival. Although the impact on survival was hard to judge, an important finding was that the majority of patients recovered Ph-negative (and putatively normal) hemopoiesis that persisted for variable lengths of time. The ability to restore normal hemopoiesis in patients with CML, albeit temporarily, through the use of high-dose chemotherapy was further confirmed by treatment with AML-like combination chemotherapy [24]. The same year saw the first series of patients who underwent transplantation with bone marrow from their identical twins who were clinically well 22–31 months later. In contrast to the previous strategies, Ph-negative hemopoiesis was consistently and durably achieved [25]. Transplantation from HLA-identical sibling donors followed rapidly thereafter [26–29].

The first autologous transplants for CML were reported to the EBMT database in 1979–80 from France and by the1990s this was a popular strategy to attempt to improve survival in selected patients. A number of prospective randomized trials were designed in Europe [30, 31] but none was completed as they coincided with the introduction of TKI. A retrospective meta-analysis of six multicenter trials in Europe and the United States showed no advantage of autologous HSCT compared to concurrent drug treatment [32]. This together with the success of TKI resulted in a rapid decrease in autologous transplant numbers since 2006, although it is fair to say that their potential role remains unclear [16] (Fig. 11.1).

The first allogeneic HSCT for CML was reported to the European Group for Blood and Marrow Transplantation (EBMT) database in 1975 from France, soon to be followed in 1978 by a patient from Switzerland and by 10 patients in 1979 from France, Italy, and the UK (personal communication; EBMT database, Leiden NL). CML soon became the most frequent indication for an allogeneic HSCT in Europe and worldwide (Fig. 11.1) [16, 33].

11.2.2 Lessons Learnt from Allogeneic HSCT for CML

The experience gained from HSCT for CML has been instructive in many ways, most of which are applicable to all hematological malignancies [34].

11.2.2.1 Disease Stage

An early observation was the importance of disease phase, rather than tumor bulk in determining outcome. Splenectomy, considered initially as essential, showed no advantage neither did splenic irradiation [35]. In contrast data from the Center for Blood and Marrow Transplant Research (CIBMTR) relating to 138 patients treated between 1978 and 1982 showed 3-year survivals of 63%, 56%, and 16% after transplant in the chronic, accelerated, and blast phases, respectively. Relapse rates for transplant in the chronic phase were remarkably low at 7% [17].

11.2.2.2 Expansion of Unrelated Donor HSCT

Only 30%–35% of patients who could benefit from allo-SCT have an HLA-identical family donor and in order to expand the applicability of transplant to more patients the next step was to utilize matched unrelated volunteer donors. In adults, the first successful unrelated allo-SCTs were reported for acute leukemia in 1980 and later for CML [36, 37]. Further development of the unrelated donor registries during the 1980s led to expansion of transplantation with CML becoming the commonest indication throughout the world [38]. With the introduction of high-resolution HLA typing, the outcomes of allografting using stem cells from matched unrelated donors are now comparable with those of HLA-matched siblings. The use of cord blood as the donor source has been successful in children but experience in adults is more limited. The largest series came from the Japan Cord Blood Bank Network, who described the outcome of transplant in 86 patients of median age 39 years. The 2-year survival for patients in chronic phase (n = 38), accelerated phase (n = 13), and blast crisis (n = 35) was 71%, 59%, and 32%, respectively ($P = 0.0004$). Results of multivariate analysis indicated that older patients (>50 years) had a higher incidence of transplant-related mortality and advanced-disease stage, and lower doses of nucleated cells were significantly associated with lower leukemia-free-survival (LFS) [39]. The Valencia group reported an LFS of 41% in 26 adults with CML of whom only 7 were in the first chronic phase at the time of a single-unit transplantation. All 8 patients transplanted in the advanced phase died [40].

11.2.2.3 GvHD and GvL

The major barrier to successful HSCT was then, as now, graft versus host disease (GvHD). The 1980s saw the introduction of T-cell depletion, which was effective in decreasing both the severity and frequency of GVHD, but was associated with higher frequencies of graft failure and relapse. The increased rate of relapse after T-cell depletion was more obvious in chronic phase CML than in other malignancies [41], and it provided direct evidence of the T-cell–mediated GVL effect of HSCT. The observation of an increased relapse rate in recipients of cells from identical twins compared with HLA-matched siblings further supported a graft versus leukemia (GvL) hypothesis [42]. The final proof of the necessity of an alloimmune effect came with the ability of additional donor lymphocyte infusions (DLI) at the time of relapse to restore remission [43, 44] in 60–90% of patients with CML who were transplanted and relapsed in the chronic phase. Optimization of the use of DLI through dose escalation minimized the risk of GvHD [45, 46].

11.2.2.4 The Advent of Reduced Intensity Conditioning

The realization that the curative power of allogeneic HSCT lay in large part in this alloimmunity paved the way for reduced intensity conditioning (RIC) which permits the expansion of transplant practice to older patients and/or those with co-morbidities. Although RIC approaches with preemptive (based on chimerism) or early (based on molecular monitoring of MRD) use of DLI might have been predicted to be most effective

in CML [47], the introduction of TKI into clinical practice abrogated the immediate need for randomized studies of RIC versus myeloablative conditioning, and the question of the best conditioning regimen for CML in the chronic phase remains unresolved. An early attempt to combine RIC, immunotherapy, and TKI was reported in a study of 22 patients who were transplanted using a RIC regimen and in vivo T-cell depletion to minimize non-relapse mortality. To mitigate against the expected increase in relapse rate, imatinib was given at engraftment and continued for 12 months. After this time, any patient with residual or recurrent disease was treated with DLI. At 36 months, 19 patients were alive and 15 were in molecular remission [48].

Retrospective comparisons of the outcome of myeloablative and reduced intensity approaches are always confounded by the fact that the two patient groups are not matched for factors such as age, disease phase, donor type, or comorbidity that directly impact transplant outcome. In CML an early attempt at a retrospective study showed a reduction in the early treatment mortality but failed to demonstrate significantly improved 3-year survivals in patients with EBMT scores of 0–2. Survival was improved albeit with relatively short follow-up in scores of >3 < 6 [49].

11.2.2.5 The Introduction of MRD Monitoring

The recognition that donor lymphocyte infusions were most effective at the time of minimal residual disease (MRD) burden identified the need for a technology to identify early relapse and led directly to the development of MRD detection through reverse-transcriptase polymerase chain reaction (RT-PCR) assays for BCR-ABL1 [50]. The subsequent value of this technology to measuring response to TKI cannot be under-estimated.

11.2.2.6 Risk Assessment in HSCT

CML also provided the first example for risk assessment with the EBMT risk score [51, 52]. The EBMT score is based on five variables: donor type, disease phase, recipient age, donor/recipient sex combination, and interval from diagnosis to transplantation, from which can be derived the probabilities of non-relapse mortality and overall survival. The EBMT score was later tested in patients with various hematological disorders and was also shown to stratify risks of mortality after allogeneic HCT for diseases other than CML. More than two decades on there are recognized limitations of the EBMT score. It was derived at a time where allogeneic HSCT was rarely applied to individuals over the age of 50 years, when HLA-matching had historically not used high-resolution technology and before the introduction of reduced intensity conditioning regimens. The level of risk according disease stage does not take into account cytogenetic or molecular markers of prognosis, although these may be less relevant in CML than in acute leukemia. For CML in particular, the effect of a delay to transplant may no longer be a risk in the era of TKI. It is highly unlikely that any patient with chronic phase CML will come to transplant less than 12 months from diagnosis as most will have received at least three TKI before referral to the transplant unit. The EBMT has presented an analysis demonstrating that time to HSCT is no longer an adverse risk factor for patients previously treated with imatinib [53].

A risk score for the effect of 17 relevant comorbidities on transplantation outcome [54] has provided additional information to assist in the prediction of survival post-transplant. The HCT-CI provides specific information about patient tolerability to the transplant process and assesses the risk of non-relapse mortality. Although the score has wide applicability in hematological malignancies other than CML, in conjunction with the EBMT score, it may help to reassure patients with none or few co-morbidities of the value of HSCT if their response to TKI is less than optimal (for a fuller review of the current risk assessment tools please see [55]).

11.2.2.7 The Impact of Macroeconomics

Last but not least, in no other disease became the impact of macroeconomic factors on use of HSCT as clear as in CML. Rates of HSCT for CML dropped already in the year 2000, 2 years before

the release of imatinib in high-income countries, illustrating how expectations drive medical decision making. Until very recently they remained at a stable level in middle- and low-income countries where costs of drug therapy became higher than costs for a transplant [33, 56–59].

11.3 HSCT for CML in 2021

Data from the EBMT activity survey from 2018, the last completed and validated year, report a total of 372 allogeneic HSCT, 202 in early phase of the disease, 170 in advanced phase, and no autologous HSCT [60]. Allogeneic HSCT was performed in 35 of 51 participating EBMT countries. Their distribution over disease stage, donor type, and stem cell source, together with the near final figures from 2019, is illustrated in Table 11.1. Total numbers have remained stable for several years. There continue to be some differences in transplant rates (numbers of HSCT per ten million inhabitants) between reporting countries although these differences have diminished over recent years (Fig. 11.2). Of note, bone marrow was used as primary stem cell source for allogeneic HSCT in the first chronic phase in only 25 of 150 HLA-identical sibling and volunteer unrelated transplants, despite its survival advantage.

These consistent numbers over the past decade represent 10–15% of the numbers performed for CML in the late 1990s, i.e., shortly before the introduction of TKI into clinical practice and when CML was the commonest indication for HSCT. In fact this number fits well with models that predict the proportion of patients who will fare poorly with TKI irrespective of whether they commence treatment with first- or second-generation drugs (Fig. 11.3a, b).

Further evidence for the on-going need for HSCT in CML comes from the impressive Swedish population-based registries of all cancers [62]. The CML registry comprises 98% of all cases, 97% of which have a cytogenetically confirmed diagnosis. One hundred and eighteen patients diagnosed from 2002 to 2016 had received allogeneic HSCT by August 2017. Almost every patient (114/118) had received a TKI prior to transplant. Per 5-year periods, 34–43 patients underwent HSCT, and this number was stable during successive 5-year periods. The estimated probability that a newly diagnosed patient under the age of 65 years would receive a transplant was 9.7%. Equal numbers were transplanted in chronic and more advanced phases but most patients transplanted in the advanced phase had been diagnosed in the chronic phase, highlighting the possibility that the need for transplant in some of these patients might have been recognized before progression. Indeed the most frequent indication for HSCT in chronic phase was TKI resistance. Of the 48 patients in a second or subsequent chronic phase at time of transplant, 31 had received chemotherapy and 15 a TKI alone.

Predictably, 5-year survival was higher in early-phase disease with survivals of 96.2%, (91.4–100%),

Table 11.1 Number of HCT for CML reported to EBMT for 2018 (full data) and 2019 (near final data). Information provided by Helen Baldomero on behalf of EBMT

	Numbers of HCT in Europe 2018 by indication, donor type, and stem cell source												
	Number of patients												Total
Disease	Family									Unrelated			
	HLA-identical			Twin	Haplo ≥2MM		Other family						
	BM	PBSC	Cord	All	BM	PBSC	BM	PBSC	Cord	BM	PBSC	Cord	
2018													
CML	12	107	0	0	13	34	0	1	0	21	176	8	372
CML CP1	8	60			6	15		1		17	90	0	202
CML > CP1	4	47			7	19		0		4	86	3	170
2019													
CML	7	103	1	1	6	46	0	0	0	20	209	2	395
CML CP1	3	54	1	0	2	13	0	0	0	7	91	2	173
CML > CP1	4	49	0	1	4	33	0	0	0	13	118	0	222

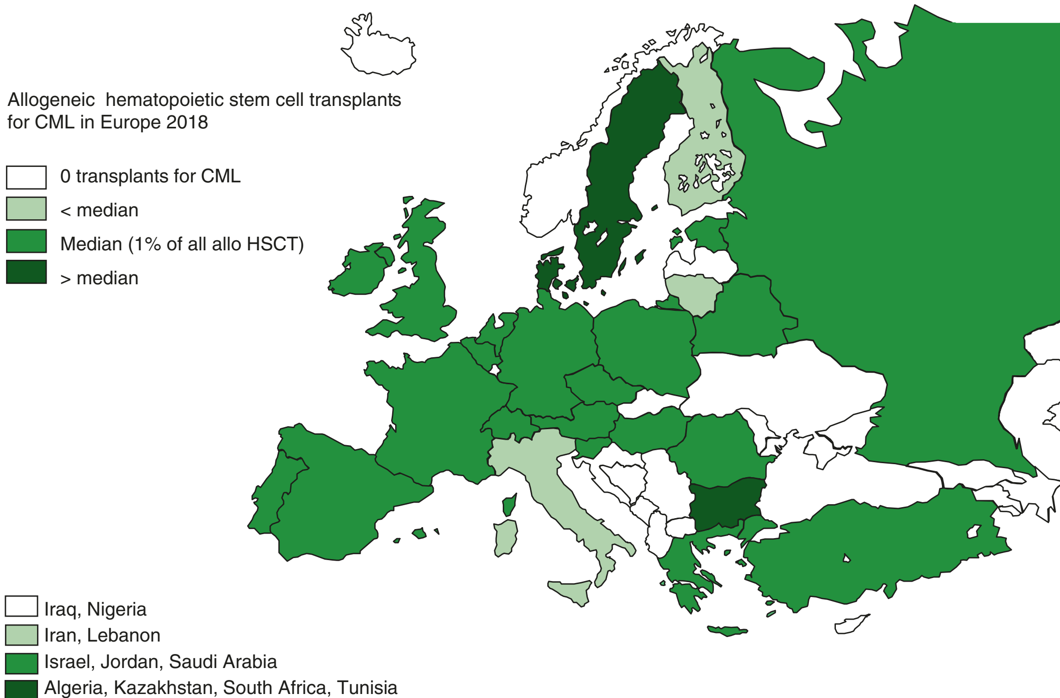


Fig. 11.2 Transplant rates for CML in Europe in 2018. The figure depicts number of HSCT for CML per ten million inhabitants for each country and depicts heterogeneity between countries. Data adapted from [61]) and kindly updated by Helen Baldomero, EBMT activity survey office

70.1% (57.4–85.5%), and 36.9% (17.7–76.8%) in the first chronic phase (CP1), second, or subsequent chronic phase (CP > 1) or advanced phase (AP – includes both acceleration and blast crisis). The excellent results achieved in the chronic phase are an important reminder of the value of HSCT in TKI non-responders. Twelve of the 56 patients transplanted in first chronic phase relapsed, most frequently detected by cytogenetic or molecular studies, and if destined to relapse, 66.7% did so within the first 2 years. Notably, 10 of 12 patients in this group achieved MR3 with TKI and/or DLI. All 7 patients transplanted in blast crisis relapsed within 6 months. Risk factors for relapse were an EBMT score > 2 and reduced intensity conditioning.

11.3.1 Factors Associated with Outcome

Risk assessment in HSCT is a complex task. The composite end points, overall survival, and relapse-free survival are influenced by two other independent keys: transplant-related mortality and relapse incidence. Some risk factors have congruent effects on transplant-related mortality and relapse incidence, hence affecting overall survival uniformly in the same direction. Disease stage is one such example. Other risk factors have discordant effects and the result then depends on the sum of all other risk factors. T-cell depletion reduces the risk of graft versus host disease but increases the risk of relapse. The net benefit on overall survival will differ between patients transplanted in early stages compared to those transplanted for advanced phase disease. Reduced intensity conditioning might be of benefit in an older patient with comorbidities and transplanted for early disease, but in contrast might be of no benefit in the same patient with no comorbidities and a transplant in advanced disease [51, 52]. As a general concept, risk factors act additively but not in a symmetrical way. A negative CMV serostatus might further improve

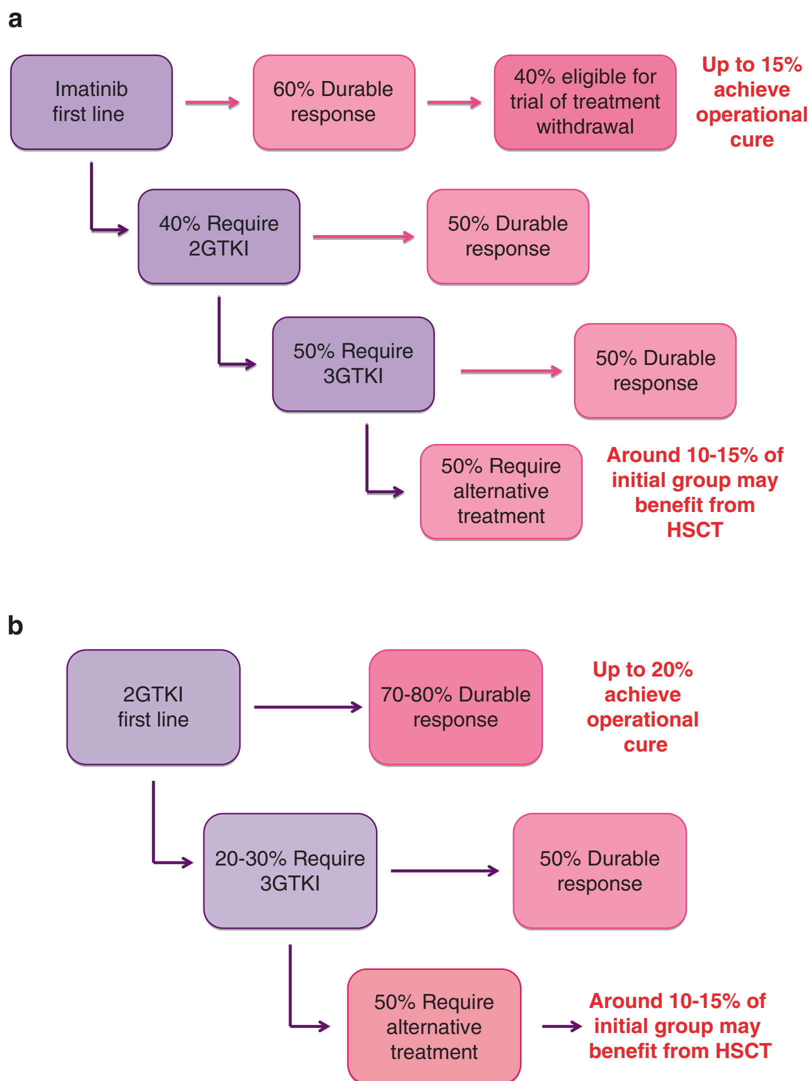


Fig. 11.3 (**a**) Predicted outcome of patients treated with first-line imatinib. (**b**) Predicted outcome of patients treated with first-line second-generation TKI

outlook for a low-risk patient but will have no additional beneficial effect in a high-risk patient; in contrast, a reduced Karnofsky score might be of minimal impact in a low-risk patient but deleterious in a high-risk patient. Hence, the general statement that probability of survival after an allogeneic HSCT for CML at 5 years is 60% is of limited value; it might range from more than 90% to less than 5%.

Assessing risk and predicting outcome for HSCT in CML in the TKI era has some additional complications. Because the numbers of transplants have fallen so dramatically, most publications in the past 15 years have been unable to identify a homogenous group of patients where the results might be of value to individual patient discussions. Studies have attempted to address important questions, for example, the impact of TKI therapy pre- or post-transplant, the role of reduced intensity versus myeloablative conditioning regimens, the stem cell source, etc. However, in order to achieve the numbers necessary for statistical analysis, they tend to use all the patients within their dataset merging risk factors other than that under study, that have a profound impact on outcome, such as disease phase, donor type, GvHD prophylaxis, conditioning regimens, stem cell source, etc. Larger numbers of patients

are available in the international transplant registries but they often lack important information, such as co-morbidities, the nature of therapy pre-transplant, the indication for transplant, the use of TKI post-transplant, and the reason for choosing reduced intensity over myeloablative preparative regimens. As a result there remain many unanswered questions regarding the optimal approach in individual patients.

11.3.2 Impact of Pre-Transplant Treatment

Most patients will have pre-treatment with a TKI for their CML before HSCT. To date there is no suggestion that a TKI given before the transplant has a deleterious effect on outcome after HSCT [63, 64]. More recently Turkish colleagues described 65 patients transplanted in the post-TKI era, defined as after 2002: 48 (73%) had received a TKI prior to the procedure and they were unable to identify any adverse impact [65].

An interesting study was reported from China where the use of a TKI prior to transplant was often a financial rather than a medical decision. They described 106 patients, of whom 36 had received imatinib before HSCT and 83 were in first chronic phase at transplant The estimated 10-year LFS and overall survivals (OS) were not statistically significant between the imatinib-treated and the imatinib-naive groups (79.6% vs. 62.4% P = 0.432, 68.9% vs. 55.5% P = 0.086, respectively). There was a suggestion of higher early non-relapse mortality in the imatinib-treated group but this did not affect long-term outcome. Interestingly the imatinib-exposed cohort contained higher proportions of patients with advanced-phase disease, and a longer duration from diagnosis to HSCT resulting in higher EBMT scores. This, in turn, influenced the choice of conditioning regimen [66].

Early papers addressing the potential impact of TKI therapy prior to transplant invariably contained patients who had been treated only with imatinib. Now patients come to HSCT often having received three or more TKI, and it becomes difficult to distinguish a possible negative effect of TKI from the poor biology of a patient who has failed successive TKIs. A study of 28 patients in different disease phases who had all received at least two TKIs was unable to show an adverse impact on outcome compared to historical controls [67]. In contrast a Japanese group reported 237 patients of whom 153, 49, and 35 had received one, two, or three TKIs prior to HSCT. Ninety-seven, 57, 32, and 51 patients were in the first chronic phase, second, or subsequent chronic accelerated phase and blast crisis, respectively, at the time of transplant; the overall and leukemia-free-survivals were 67% and 54% in patients exposed to fewer than three TKIs and 61% and 54% in patients who had received at least three TKIs. The relapse incidence in patients treated with three TKIs was twice (34%) that of patients exposed to fewer TKIs (17%). This is unlikely to be explained by TKI exposure per se and more likely reflects more resistant disease [68].

11.3.3 Impact of HSCT Methodology

Despite more than 40 years' experience, the best conditioning regimen and the best GvHD prophylaxis for HSCT in CML are still undefined. No other conditioning regimens produce a better long-term overall survival than cyclophosphamide and total body irradiation or busulfan and cyclophosphamide; no other GVHD prophylaxis has been shown to be superior to cyclosporine and methotrexate. In a large observational retrospective study by the CIBMTR, RIC gave a better overall survival in elderly patients compared to non-myeloablative conditioning; no comparison was made with standard conditioning [69].

Peripheral blood-derived stem cells (PBSC) have largely replaced bone marrow as the stem cell source for sibling, unrelated, and haploidentical transplants in adults. Initial studies showed a clear early advantage of peripheral blood with more rapid engraftment, and a slightly higher incidence of graft versus host disease but overall similar survival. Today, several studies have demonstrated an advantage of bone marrow as stem cell source in early disease, and of peripheral blood in advanced disease [70, 71]. In a

large CIBMTR study of unrelated donor HSCT, patients transplanted in the first chronic phase had 5-year rates of survival of 35% with PBSC compared to 56% with bone marrow. Relapse rates were low with both graft types suggesting that there was no advantage in higher rates of chronic GVHD after PBSC transplant. In contrast, for patients with CML transplanted in the second chronic, accelerated, or blast phase, there were no significant differences in rates of overall survival, non-relapse mortality, or relapse, which differs from HLA-matched sibling transplantation where mortality is lower using PBSCT in those with advanced CML [72]. Despite these differences, use of stem cell source still appears erratic, with major differences between European countries.

11.3.4 Impact of Maintenance TKI Post-Transplant

The value of using a TKI post-transplant is unclear and is further compounded by the current availability of at least five TKIs for use prior to transplant referral. In 2021 most patients in the first chronic phase will come to transplant having failed both second- and third-generation TKIs (2G-TKI, 3G-TKI), and the rationale for continuing treatment post-transplant with a drug to which the patient was resistant or intolerant is unclear. The situation might be different in patients transplanted for advanced-phase disease where exposure may be limited to none or just one TKI, with the patient being restored to a second chronic phase using AML-like chemotherapy, or where the patient was transplanted after the detection of a T315I mutation and who has been restored to varying levels of remission with ponatinib.

Furthermore the administration of TKI post-transplant may not be straightforward. A phase I/II study investigating the use of nilotinib after HSCT for high-risk Ph-positive leukemias reported 2-year overall and leukemia-free survivals of 69% and 56%, respectively. In this small study of 16 patients, 38% discontinued therapy, mainly because of gastrointestinal and/or hepatic toxicities [73]. In a separate phase I/II study, only a third of patients eligible for nilotinib maintenance completed the year of intended therapy [74].

A recent CIBMTR study compared 89 patients who received a TKI post-transplant with 301 who had no maintenance therapy. All patients received TKI therapy before HCT. In this landmark analysis from Day +100 the adjusted estimates for 5-year relapse (maintenance, 35% vs. no maintenance, 26%; $P = 0.11$), LFS (maintenance, 42% vs. no maintenance, 44%; $P = 0.65$), or overall survival (maintenance, 61% vs. no maintenance, 57%; $P = 0.61$) did not differ significantly between patients receiving TKI maintenance or not. These results were not affected by disease status at transplant [75]. However the results are difficult to interpret because the two groups were not comparable: for instance there was a higher percentage of patients in second or subsequent chronic phase in the maintenance group ($P < 0.001$) and there was no information regarding prior response to individual TKI or the indication for giving post-transplant maintenance.

11.3.5 Management of Relapse Post-Transplant

The most appropriate management of relapse post-transplant is also contentious. DLI have been used for 30 years to restore remission in patients who have relapsed in chronic phase after transplant in chronic phase and are most effective when given at a time of low disease burden, i.e., cytogenetic or molecular evidence of residual disease [44, 45]. DLI are not without complications having the capacity to cause pancytopenia and to induce potentially fatal GvHD, the latter being more frequent if the DLI are given within the first 12 months of transplant. In contrast TKI are relatively easy to give, and side effects should they occur are reversible on discontinuation. This has encouraged many investigators to give TKI for relapse which in the early years showed good results, apart from the dilemma of when they should be stopped. Giving a TKI for relapse in a patient who required their transplant for resistance to multiple TKIs is more problematic.

A study from the EBMT retrospectively analyzed 500 patients who received DLI for relapse (16% molecular, 30% cytogenetic, and 54% hematological) after HSCT for CML. Complete cytogenetic remission was achieved in 341 patients (71%) at a median of 7.5 months. A total of 222 (44%) patients developed secondary GVHD at a median of 3 months from first DLI with 61, 70, 40, and 20 patients being diagnosed with secondary acute GVHD grades 1, 2, 3, and 4, respectively. Secondary chronic GVHD occurred in 87 (17%) patients. The estimated probabilities of survival at 5 and 10 years from DLI were 64% and 59%, respectively. However the estimated probabilities of failure-free and GvHD free survival (FGFS) at 5 and 10 years were considerably less at 29% and 27%, respectively. The probability of survival in remission without secondary GVHD was highest (>50% at 5 years) when DLI were given beyond 1 year from HSCT for molecular and/or cytogenetic relapse that was not preceded by cGvHD [76]. Such information can help guide the choice of DLI or TKI in individual patients, particularly in those with prior and/or current GvHD and relapsing soon after transplant.

11.4 Outcome of HSCT in CML in the TKI Era

11.4.1 Chronic Phase Disease

Early after the introduction of TKI into clinical practice many patients continued to be transplanted. The reasons were varied and included lack of access to TKI, patient and physician choice, a lack of long-term follow-up from TKI treatment, a favorable EBMT risk score with or without a high Sokal/Euro score, resistance and/or intolerance to the only available TKI, imatinib, and concern regarding the long-term costs of life-long TKI. This situation enabled a number of comparisons of transplant versus TKI in retrospective cohorts. In 2021 these are of limited value as TKIs are the treatment of choice for newly diagnosed patients in the chronic phase, and most patients will be given multiple TKIs before transplant referral.

Just before the widespread availability of TKIs, the German CML study group tested the hypothesis that HSCT would be associated with early mortality but a subsequent survival benefit might compensate for the "early years of life lost" in the CML III trial. Availability of a matched family donor was used as "genetic randomization." In this study with 349 patients, survival was significantly better in patients on drug treatment after a median observation time of 8 years, in no small part because patients were able to access TKI therapy later in their disease course. The conclusion was clear: "the general recommendation of HSCT as first-line treatment option in chronic phase CML can no longer be maintained" [77]. These results formed the basis for the subsequent ELN guidelines on the use of HSCT in TKI-treated patients, currently in their fourth iteration [78]. Allogeneic HSCT is considered a third- or subsequent-line therapy for chronic phase disease and the preferred option for patients with advanced phase.

The German Swiss CML IV study permitted early HSCT in their first TKI-based study [79]. A total of 84 patients (median age, 37 years) received HSCT, either first line (19 patients) or after imatinib failure (37 and 28 patients in chronic and accelerated phases, respectively). Overall survival of this cohort was 88% for all, 94% when treated in the chronic phase, and 59% for those transplanted in the accelerated phase. Transplant-related mortality was 8%; chronic graft versus host disease occurred in 46%. Of note, overall survival of the patients transplanted in CP was no different from that of the concomitantly imatinib-treated patient cohort. This study serves as a reminder of the excellent outcome of HSCT in selected patients.

More recently a Chinese group reported the outcomes of imatinib treatment (n = 292) versus HSCT (n = 141) for CML, in a situation where the choice of HSCT over imatinib may have been driven by financial constraints rather than medical advice. In CP1, patients treated with imatinib (n = 278) had superior event-free and overall survivals at 5 years at 84% and 92% compared to transplanted patients at 75% and 79% ($P < 0.05$), respectively. In contrast they were unable to

demonstrate differences in outcome for patients treated in the accelerated phase or blast crisis [80].

11.4.2 Advanced-Phase Disease

Patients presenting in or progressing to the accelerated phase are heterogeneous in terms of disease biology and response to treatment, leaving some to question whether acceleration is a separate entity that can be clearly defined or is simply part of the spectrum of chronic phase with features (such as the blast cell count) that might identify the patient as high risk in the same way as a diagnostic risk score such as Sokal. There are limited data available for HSCT in acceleration. A study form Beijing compared HSCT versus imatinib in 132 patients, of whom 87 received imatinib and 45 allogeneic HSCT. Multivariate analysis found a CML duration ≥12 months, hemoglobin <100 g/L, and peripheral blood blasts ≥5% to be independent adverse prognostic factors for overall and progression-free survival. They developed low (no adverse factors), intermediate (anyone factor), and high (two or more factors) scores and showed that HSCT provided significant overall and/or progression-free survival advantages for high- and intermediate-risk patients: the outcome for low-risk accelerated phase was excellent and similar for imatinib and HSCT [81].

A study from the same group comprising 83 patients in blast crisis, 45 who received TKI and 38 who were treated with HSCT after TKI, showed that TKI-HSCT significantly improved the 4-year overall (46.7% vs. 9.7%, $P = 0.001$) and event-free survivals (EFS) (47.1% vs. 6.7%, P = 0.001) compared to TKI alone. Hemoglobin <100 g/L, failure to return to chronic phase after TKI therapy, and TKI treatment alone were independent adverse predictors of OS and EFS. The HSCT group comprised 27 patients who presented de novo and 11 patients who progressed on TKI. All 27 received a TKI and 21 achieved a second chronic phase. Those who had progressed on TKI were treated either with an alternative TKI or with chemotherapy and 9 returned to a chronic phase, such that 30 of the 38 transplanted patients were in a second chronic phase at the time of transplant. Eighteen patients survived with 12 patients dying of non-relapse mortality and 8 of relapse. In contrast, although a similar proportion of the 45 patients treated with TKI alone achieved chronic phase, there were only four survivors. Of the 23 patients who failed to achieve a second chronic phase there were no survivors in the group that did not proceed to HSCT [82].

The EBMT have recently reported a retrospective study of 171 patients allografted for blast crisis after TKI therapy. At transplant, 95 patients were in a second or subsequent chronic phase and 75 patients had active blast crisis. In multivariable analysis, active blast crisis at transplant was the strongest factor associated with decreased overall and leukemia-free survival. For patients in second or subsequent chronic phase at transplant, age > 45 years, Karnofsky <80%, time from blast crisis to HSCT >12 months, myeloablative conditioning, and unrelated donor transplant were risk factors for inferior survival [83].

11.4.3 Patients with a T315I Mutation

Outcomes for transplant were reported in 22 patients with T315I mutations who received HSCT (mostly haploidentical) as ponatinib was unavailable. At the time of HSCT 7, 8 and 7 patients were in first chronic, accelerated/second chronic and blastic phases, respectively. The estimated 2-year LFS was 80.0%, 72.9%, and 0% in the three groups confirming the poor outcome of HSCT in blast crisis and the need to transplant patients with adverse prognostic features such as the T315I mutation before disease progression [84].

The outcome of 184 patients with T315I mutations, in which 128 received ponatinib and 56 an allogeneic HSCT showed that 2- and 4-year survivals were significantly higher in patients with chronic-phase CML who received ponatinib at 84% compared with those who underwent HSCT at 60.5%. In patients in the accelerated phase, survival rates were not significantly dif-

ferent between the groups and in those in blast crisis ponatinib was associated with a shorter survival compared with HSCT. The authors concluded that ponatinib was a valuable treatment option for patients with a T315I mutation who remained in the chronic phase but in those who had progressed to an advanced phase, HSCT was the preferred therapy [85]. It is entirely possible, however, that with additional follow-up, the durability of response to ponatinib will be inferior to that of HSCT even in the chronic phase.

11.5 Timing of HSCT in 2021

The ASBMT and EBMT are very consistent in their most recent recommendations and consider HSCT as a standard indication for patients with failed TKI response and for patients in advanced disease [86, 87]. They emphasize that additional risk factors other than stage of the disease and response to TKI have to be integrated into the final decision to proceed or to abstain from HSCT.

11.5.1 Advanced-Phase Disease

The response to TKI in patients presenting in accelerated phase is highly variable, with many achieving deep and durable molecular responses and a minority displaying early TKI resistance and acquisition of additional chromosomal abnormalities. The general consensus is to treat patients as in the chronic phase but have a low threshold for transplant referral if responses are less than optimal. The more difficult question is the necessity to try to achieve a second chronic phase prior to transplant and here there is no clear answer. In the pre-TKI era, the outcome for HSCT in the accelerated phase was similar to that of the second chronic phase. However, if the accelerated phase cohort contained then, as now, a group of patients with disease biology more similar to chronic phase, then the transplant outcome might have been overly optimistic, in which case returning a patient to a second chronic phase would be advisable. There are currently no data to support this hypothesis.

In contrast there is general agreement that presentation in, or progression to, advanced phase disease is associated with very poor outcome, whether the patient is treated with TKI or HSCT or both. Fortunately blast crisis is now a rare event. In the pre-TKI era the rates of blast transformation per annum were approximately 1-5-4% and were consistent year on year. The estimated 10-year cumulative incidence of blast crisis in the pivotal IRIS study was 7.9% but the majority of the progressions occurred in the first 4 years. The use of 2G-TKI in first-line therapy appears to further reduce this risk with progression rates at 5 years of 0.7–1.3% for nilotinib and 3.0% for dasatinib compared to 4.8–5.7% for imatinib in the randomized Enestnd and Dasision studies [10, 12].

Once blast crisis is established the only treatment to offer any possibility of long-term survival is allogeneic HSCT, ideally after a return to a second chronic phase. This can be achieved by the use of TKI alone or in combination with AML-like chemotherapy, although the consensus is that combination treatment offers the higher probability of response. Recent data obtained from a small group of patients using the third-generation TKI, ponatinib, have demonstrated the feasibility of using this in combination with FLAG-Ida and showed encouraging outcomes for patients who were able to proceed to HSCT [88]. The actual choice may depend on factors including age, co-morbidities, and performance score but these periods of stability are short lived and patients should proceed to transplant as soon as possible. The results of haploidentical HSCT have improved very considerably over recent years following the introduction of post-transplant cyclophosphamide and have the advantage that almost all patients will have a suitable donor. If a fully matched family or unrelated donor cannot be identified in a timely manner, haploidentical transplant is now a very real alternative.

The MD Anderson Cancer Center reported their experience in 477 patients presenting in or progressing to blast crisis. Treatment modalities were TKI alone (n = 149; 35%), TKI plus chemotherapy (n = 195; 46%), and non-TKI based therapies (n = 82; 19%). Patients treated with

a combination of TKI with chemotherapy had significantly higher rates of major hematological, complete cytogenetic, and major molecular remissions compared to other modalities. One hundred and four patients (22%) proceeded to HSCT and the proportion of patients who received a transplant was higher among those treated with TKI-based combinations (21%) than those treated with TKI alone or non-TKI therapy (3% and 10%, respectively). Patients who received HSCT after their initial treatment for CML-BP had a significantly longer survival than patients who did not receive transplant [89].

11.5.2 Chronic-Phase Disease

Once progression has occurred treatment strategies are limited and the focus must be on the prevention of blast transformation through identification of high-risk patients at diagnosis, currently via ELTS and Sokal scores, and the presence of additional chromosomal abnormalities but perhaps in the future by next-generation sequencing for prognostic mutations in other somatic genes, rigorous molecular monitoring, and adherence to international guidelines for triggers for changes in management.

There are rare patients who, although responsive to TKI, are unable to tolerate the drugs in the long-term. Such patients may benefit from HSCT although often the reasons that they cannot tolerate TKI are often related to comorbidities that may also preclude transplant. Younger patients with poor compliance and a real risk of disease progression form another significant minority who may come to transplant early.

A Canadian study described 51 patients with CML underwent HSCT, including 15 in advanced phase at diagnosis, 30 with TKI resistance as defined by the European LeukemiaNet guidelines, 2 with TKI intolerance, and 4 because of physician preference. At diagnosis, 33 of the 51 patients were in first chronic phase but by the time of HSCT, 16 of the 33 had progressed on imatinib. The 8-year overall and event-free survivals were 68% and 46%, respectively. Predictors for overall survival included first chronic phase at the time of HSCT, an EBMT score of 1–4, and complete molecular remission after HSCT [90].

As experience in the use of TKI increases, there is increasing evidence that resistance to a 2G-TKI, unless associated with poor compliance and/or the presence of a kinase domain mutation sensitive to an alternative agent, is a poor prognostic factor. Switching the patient to an alternative 2G-TKI is associated with a low probability of attaining the major molecular remission consistent with a prolonged survival. At this point and irrespective of whether the 2G-TKI has been used in first or second-line treatment, the patient should be switched to a third-generation drug, co-morbidities permitting, and referred to the transplant unit for donor identification and consideration of HSCT (Fig. 11.4). At the time of starting a 3G-TKI, it is difficult to pre-

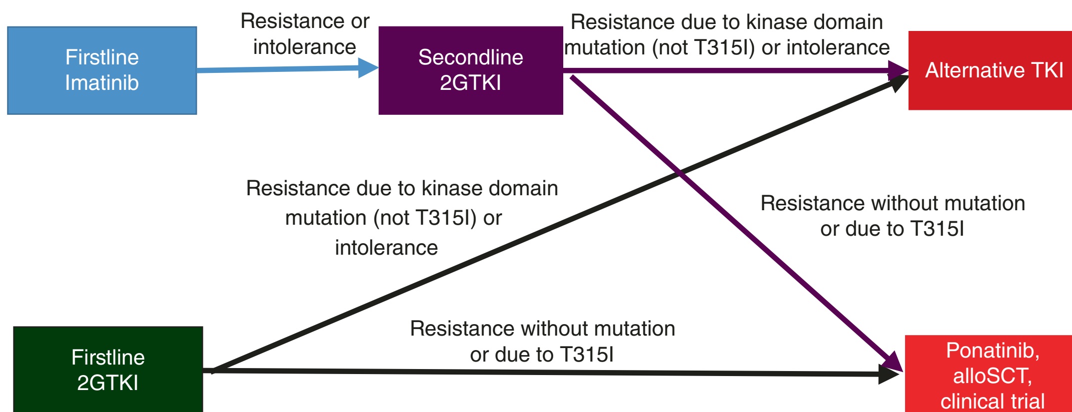


Fig. 11.4 Algorithm for management of patients with CML in 2021

dict response but the outcome of patients who discontinue ponatinib for resistance or intolerance is poor with one study demonstrating a median survival for all patients of 16.6 months after stopping ponatinib (31, 9, and 13 months for patients stopping in chronic, accelerated, and blast phases, respectively). Predictably there was a trend for better survival in patients who discontinued ponatinib for toxicity rather than resistance [91].

11.6 Concluding Remarks

The introduction of TKI as targeted therapy has eased and improved the treatment of CML in an unprecedented way. It has increased the understanding of the disease and changed attitudes but complicated decision trees. The astonishing results with TKI have interrupted many comparative trials and focused multicenter research interests on comparative trials of different drugs. In parallel, interest in the HSCT community has moved to questions of novel transplant technologies rather than comparisons with non-HSCT approaches. It is highly unlikely that there will ever be a comparative study of HSCT versus no HSCT in CML at any disease phase. As a consequence, all recommendations are based on individual interpretation of past results.

The ease of drug administration has shifted the patient community from major university centers toward decentralized medical practice, which is appropriate for the majority of patients. However there is a risk that the busy generalist can miss the signs of poor response and delay or defer changes in treatment and/or referral for HSCT. This is reflected in the better survival of patients on drug treatment for advanced disease treated in a university-affiliated center compared to those in a community practice [61].

During the TKI era, the outcome of HSCT has also substantially improved; the numbers of HLA-typed volunteer-unrelated donors has increased to more than 22 million worldwide, and improvements in haploidentical transplant mean that suitable donors can be identified promptly. The outcome of HSCT is substantially better in centers with longer disease experience and higher patient volumes. Experience in complications and disease management is essential in order to ascertain optimal survival. In the case of early TKI failure, HSCT should be considered early for those with minimal transplant risks and the drug treatment changed for those with less favorable transplant options. The same applies to patients experiencing transformation at any time and for those with failure to respond to second- or third-line therapy. In contrast patients with high transplant risks and aggressive disease should not be referred for HSCT without any reasonable likelihood for success. Continued drug therapy, experimental approaches, or palliation might be the wiser option. In order to arrive at such a policy, patients and patient advocacy groups need to be informed, cooperation should be established between the local medical community and the transplant centers, and professional organizations must continue to adapt their recommendations as appropriate. In this way, more patients will profit from a safe transplant; fewer patients will undergo a futile transplant procedure.

References

1. Abrams J, Conley B, Mooney M, Zwiebel J, Chen A, Welch JJ, Takebe N, Malik S, McShane L, Korn E, Williams M, Staudt L, Doroshow J. National Cancer Institute's precision medicine initiatives for the new National Clinical Trials Network. Am Soc Clin Oncol Educ Book. 2014:71–6. https://doi.org/10.14694/EdBook_AM.2014.34.71.
2. Cortes JE, Kantarjian HM, Brümmendorf TH, Kim DW, Turkina AG, Shen ZX, Pasquini R, Khoury HJ, Arkin S, Volkert A, Besson N, Abbas R, Wang J, Leip E, Gambacorti-Passerini C. Safety and efficacy of bosutinib (SKI-606) in chronic phase Philadelphia chromosome-positive chronic myeloid leukemia patients with resistance or intolerance to imatinib. Blood. 2011;118:4567–76. https://doi.org/10.1182/blood-2011-05-355594.
3. Cortes JE, Kim DW, Pinilla-Ibarz J, le Coutre P, Paquette R, Chuah C, Nicolini FE, Apperley JF, Khoury HJ, Talpaz M, Di Persio J, DJ DA, Abruzzese E, Rea D, Baccarani M, Müller MC, Gambacorti-Passerini C, Wong S, Lustgarten S, Rivera VM, Clackson T, Turner CD, Haluska FG, Guilhot F, Deininger MW, Hochhaus A, Hughes T, Goldman JM, Shah NP, Kantarjian H, PACE Investigators. A phase 2 trial of ponatinib in Philadelphia chromosome-

positive leukemias. N Engl J Med. 2013;369:1783–96. https://doi.org/10.1056/NEJMoa1306494.
4. Cortes JE, Gambacorti-Passerini C, Deininger MW, Mauro MJ, Chuah C, Kim DW, Dyagil I, Glushko N, Milojkovic D, le Coutre P, Garcia-Gutierrez V, Reilly L, Jeynes-Ellis A, Leip E, Bardy-Bouxin N, Hochhaus A, Brümmendorf TH. Bosutinib versus Imatinib for newly diagnosed chronic myeloid leukemia: results from the randomized BFORE trial. J Clin Oncol. 2018;36:231–7. https://doi.org/10.1200/JCO.2017.74.7162.
5. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, Zimmermann J, Lydon NB. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med. 1996;2:561–6. https://doi.org/10.1038/nm0596-561.
6. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers CL. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med. 2001;344:1031–7. https://doi.org/10.1056/NEJM200104053441401.
7. Hughes TP, Hochhaus A, Branford S, Müller MC, Kaeda JS, Foroni L, Druker BJ, Guilhot F, Larson RA, O'Brien SG, Rudoltz MS, Mone M, Wehrle E, Modur V, Goldman JM, Radich JP, IRIS investigators. Long-term prognostic significance of early molecular response to imatinib in newly diagnosed chronic myeloid leukemia: an analysis from the international randomized study of interferon and STI571 (IRIS). Blood. 2010;116:3758–65. https://doi.org/10.1182/blood-2010-03-273979.
8. Hughes TP, Mauro MJ, Cortes JE, Minami H, Rea D, DeAngelo DJ, Breccia M, Goh YT, Talpaz M, Hochhaus A, le Coutre P, Ottmann O, Heinrich MC, Steegmann JL, Deininger MWN, Janssen JJWM, Mahon FX, Minami Y, Yeung D, Ross DM, Tallman MS, Park JH, Druker BJ, Hynds D, Duan Y, Meille C, Hourcade-Potelleret F, Vanasse KG, Lang F, Kim DW. Asciminib in chronic myeloid leukemia after ABL kinase inhibitor failure. N Engl J Med. 2019;381:2315–26. https://doi.org/10.1056/NEJMoa1902328.
9. Kantarjian H, Giles F, Wunderle L, Bhalla K, O'Brien S, Wassmann B, Tanaka C, Manley P, Rae P, Mietlowski W, Bochinski K, Hochhaus A, Griffin JD, Hoelzer D, Albitar M, Dugan M, Cortes J, Alland L, Ottmann OG. Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. N Engl J Med. 2006;354:2542–51. https://doi.org/10.1056/NEJMoa055104.
10. Kantarjian H, Shah NP, Hochhaus A, et al. Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2010;362:2260–70. https://doi.org/10.1056/NEJMoa1002315.
11. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, Cornelissen JJ, Fischer T, Hochhaus A, Hughes T, Lechner K, Nielsen JL, Rousselot P, Reiffers J, Saglio G, Shepherd J, Simonsson B, Gratwohl A, Goldman JM, Kantarjian H, Taylor K, Verhoef G, Bolton AE, Capdeville R, Druker BJ, Investigators IRIS. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2003;348:994–1004.
12. Saglio G, Kim DW, Issaragrisil S, le Coutre P, Etienne G, Lobo C, Pasquini R, Clark RE, Hochhaus A, Hughes TP, Gallagher N, Hoenekopp A, Dong M, Haque A, Larson RA, Kantarjian HM, ENESTnd Investigators. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med. 2010;362:2251–9. https://doi.org/10.1056/NEJMoa0912614.
13. Talpaz M, Shah NP, Kantarjian H, Donato N, Nicoll J, Paquette R, Cortes J, O'Brien S, Nicaise C, Bleickardt E, Blackwood-Chirchir MA, Iyer V, Chen TT, Huang F, Decillis AP, Sawyers CL. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. N Engl J Med. 2006;354:2531–41. https://doi.org/10.1056/NEJMoa055229.
14. Appelbaum FR. Hematopoietic-cell transplantation at 50. N Engl J Med. 2007;357:1472–5.
15. Goldman JM, Apperley JF, Jones L, Marcus R, Goolden AW, Batchelor R, Hale G, Waldmann H, Reid CD, Hows J, et al. Bone marrow transplantation for patients with chronic myeloid leukemia. N Engl J Med. 1986;314:202–7. https://doi.org/10.1056/NEJM198601233140403.
16. Passweg JR, Baldomero H, Bader P, Bonini C, Cesaro S, Dreger P, Duarte RF, Dufour C, Falkenburg JH, Farge-Bancel D, Gennery A, Kröger N, Lanza F, Nagler A, Sureda A, Mohty M, European Society for Blood and Marrow Transplantation (EBMT). Hematopoietic SCT in Europe 2013: recent trends in the use of alternative donors showing more haploidentical donors but fewer cord blood transplants. Bone Marrow Transplant. 2015;50:476–82. https://doi.org/10.1038/bmt.2014.312.
17. Speck B, Bortin MM, Champlin R, Goldman JM, Herzig RH, McGlave PB, Messner HA, Weiner RS, Rimm AA. Allogeneic bone-marrow transplantation for chronic myelogenous leukaemia. Lancet. 1984;1(8378):665–8.
18. Gooley TA, Chien JW, Pergam SA, Hingorani S, Sorror ML, Boeckh M, Martin PJ, Sandmaier BM, Marr KA, Appelbaum FR, Storb R, McDonald GB. Reduced mortality after allogeneic hematopoietic-cell transplantation. N Engl J Med. 2010;363:2091–101. https://doi.org/10.1056/NEJMoa1004383.
19. Sorror ML Comorbidities and hematopoietic cell transplantation outcomes. Hematology Am Soc Hematol Educ Program. 2010:237–47.
20. Gratwohl A, Brand R, McGrath E, van Biezen A, Sureda A, Ljungman P, Baldomero H, Chabannon C, Apperley J, Joint Accreditation Committee (JACIE) of the International Society for Cellular Therapy and the European Group for Blood and Marrow Transplantation, and the European Leukemia Net. Use of the quality management system "JACIE" and

outcome after hematopoietic stem cell transplantation. Haematologica. 2014;99:908–15.
21. Holt JA, Woodliff HJ, Davis RE, Neal JR. Radiation and marrow infusion in leukaemia. Observations on a patient with chronic granulocytic leukaemia treated with whole-body radiation and infusion of isogenic marrow. Australas Radiol. 1967;11:63–6.
22. Buckner CD, Clift RA, Fefer A, Neiman PE, Storb R, Thomas ED. Treatment of blastic transformation of chronic granulocytic leukemia by high dose cyclophosphamide, total body irradiation and infusion of cryopreserved autologous marrow. Exp Hematol. 1974;2:138–46.
23. Goldman JM, Catovsky D, Galton DA. Reversal of blast-cell crisis in C.G.L. by transfusion of stored autologous buffy-coat cells. Lancet. 1978;1:437–8. https://doi.org/10.1016/s0140-6736(78)91221-7.
24. Cunningham I, Gee T, Dowling M, Chaganti R, Bailey R, Hopfan S, Bowden L, Turnbull A, Knapper W, Clarkson B. Results of treatment of Ph'+ chronic myelogenous leukemia with an intensive treatment regimen (L-5 protocol). Blood. 1979;53:375–95.
25. Fefer A, Cheever MA, Thomas ED, Boyd C, Ramberg R, Glucksberg H, Buckner CD, Storb R. Disappearance of Ph1-positive cells in four patients with chronic granulocytic leukemia after chemotherapy, irradiation and marrow transplantation from an identical twin. N Engl J Med. 1979;300:333–7. https://doi.org/10.1056/NEJM197902153000702.
26. Champlin R, Ho W, Arenson E, Gale RP. Allogeneic bone marrow transplantation for chronic myelogenous leukemia in chronic or accelerated phase. Blood. 1982;60:1038–41.
27. Clift RA, Buckner CD, Thomas ED, Doney K, Fefer A, Neiman PE, Singer J, Sanders J, Stewart P, Sullivan KM, Deeg J, Storb R. Treatment of chronic granulocytic leukaemia in chronic phase by allogeneic marrow transplantation. Lancet. 1982;2:621–3. https://doi.org/10.1016/s0140-6736(82)92735-0.
28. Goldman JM, Baughan ASJ, McCarthy DM, Worsley AM, Hows JM, Gordon-Smith EC, Catovsky D, Batchelor JR, Goolden AW, Galton DA. Marrow transplantation for patients in the chronic phase of chronic granulocytic leukaemia. Lancet. 1982;2:623–5. https://doi.org/10.1016/s0140-6736(82)92736-2.
29. Speck B, Gratwohl A, Nissen C, Osterwalder B, Müller M, Bannert P, Müller H, Jeannet M. Allogeneic marrow transplantation for chronic granulocytic leukemia. Blut. 1982;45:237–42. https://doi.org/10.1007/BF00320190.
30. Butturini A, Keating A, Goldman J, Gale RP. Autotransplants in chronic myelogenous leukaemia: strategies and results. Lancet. 1990;335(8700):1255–8.
31. Reiffers J, Goldman J, Meloni G, Cahn JY, Gratwohl A. Autologous stem cell transplantation in chronic myelogenous leukemia: a retrospective analysis of the European Group for Bone Marrow Transplantation Chronic Leukemia Working Party of the EBMT. Bone Marrow Transplant. 1994;14:407–10.
32. Autograft Trials Collaboration. CML autologous stem cell transplantation in chronic myeloid leukaemia: a meta-analysis of six randomized trials. Cancer Treat Rev. 2007;33:39–47.
33. Gratwohl A, Schwendener A, Baldomero H, et al. Changes in the use of hematopoietic stem cell transplantation: a model for diffusion of medical technology. Haematologica. 2010a;95:637–43.
34. Pavlu J, Szydlo RM, Goldman JM, Apperley JF. Three decades of transplantation for chronic myeloid leukemia: what have we learned? Blood. 2011;117(3):755–63. https://doi.org/10.1182/blood-2010-08-301341.
35. Gratwohl A, Goldman J, Gluckman E, Zwaan F. Effect of splenectomy before bone-marrow transplantation on survival in chronic granulocytic leukaemia. Lancet. 1985;2(8467):1290–1.
36. Hansen JA, Clift RA, Thomas ED, Buckner CD, Storb R, Giblett ER. Transplantation of marrow from an unrelated donor to a patient with acute leukemia. N Engl J Med. 1980;303:565–7. https://doi.org/10.1056/NEJM198009043031007.
37. Mackinnon S, Hows JM, Goldman JM, Arthur CK, Hughes T, Apperley JF, Jones L, Batchelor JR, Brookes P, Catovsky D, et al. Bone marrow transplantation for chronic myeloid leukemia: the use of histocompatible unrelated volunteer donors. Exp Hematol. 1990;18:421–5.
38. Gratwohl A, Baldomero H, Horisberger B, Schmid C, Passweg J, Urbano-Ispizua A. Accreditation Committee of the European Group for blood and marrow transplantation (EBMT). Current trends in hematopoietic stem cell transplantation in Europe. Blood. 2002;100:2374–86. https://doi.org/10.1182/blood-2002-03-0675.
39. Nagamura-Inoue T, Kai S, Azuma H, Takanashi M, Isoyama K, Kato K, Takahashi S, Taniguchi S, Miyamura K, Aoki K, Hidaka M, Nagamura F, Tojo A, Fang X, Kato S, Japan Cord Blood Bank Network. Unrelated cord blood transplantation in CML: Japan cord blood Bank network analysis. Bone Marrow Transplant. 2008;42:241–51. https://doi.org/10.1038/bmt.2008.164.
40. Sanz J, Montesinos P, Saavedra S, Lorenzo I, Senent L, Planelles D, Larrea L, Martín G, Palau J, Jarque I, Martínez J, de la Rubia J, Moscardó F, Martinez D, Gómez I, López M, Sanz MA, Sanz GF. Single-unit umbilical cord blood transplantation from unrelated donors in adult patients with chronic myeloid leukemia. Biol Blood Marrow Transplant. 2010;16:1589–95. https://doi.org/10.1016/j.bbmt.2010.05.014.
41. Apperley JF, Jones L, Hale G, Waldmann H, Hows J, Rombos Y, Tsatalas C, Marcus RE, Goolden AW, Gordon-Smith EC, Goldman JM. Bone marrow transplantation for patients with chronic myeloid leukaemia: T-cell depletion with Campath-1 reduces the incidence of graft-versus-host disease but may increase the risk of leukaemic relapse. Bone Marrow Transplant. 1986;1:53–66.

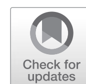
42. Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ, Rimm AA, Ringdén O, Rozman C, Speck B, Truitt RL, Zwaan FE, Bortin MM. Graft-versus-leukemia reactions after bone marrow transplantation. Blood. 1990;75:555–62.
43. Chalandon Y, Passweg JR, Guglielmi C, Iacobelli S, Apperley J, Schaap NP, Finke J, Robin M, Fedele R, Bron D, Yakoub-Agha I, van Biezen A, de Witte T, Kröger N, Olavarria E, Chronic Malignancies Working Party of the European Group for Blood and Marrow Transplantation (EBMT). Early administration of donor lymphocyte infusions upon molecular relapse after allogeneic hematopoietic stem cell transplantation for chronic myeloid leukemia: a study by the chronic malignancies working party of the EBMT. Haematologica. 2014;99:1492–8. https://doi.org/10.3324/haematol.2013.100198.
44. Kolb HJ, Mittermüller J, Clemm C, Holler E, Ledderose G, Brehm G, Heim M, Wilmanns W. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. Blood. 1990;76:2462–5.
45. Dazzi F, Szydlo RM, Craddock C, Cross NCP, Kaeda J, Chase A, Olavarria E, van Rhee F, Kanfer E, Apperley JF, Goldman JM. Comparison of single-dose and escalating-dose regimens of donor lymphocyte infusion for relapse after allografting for chronic myeloid leukemia. Blood. 2000;95:67–71. https://doi.org/10.1182/blood.V95.1.67.
46. Mackinnon S, Papadopoulos EB, Carabasi MH, Reich L, Collins NH, Boulad F, Castro-Malaspina H, Childs BH, Gillio AP, Kernan NA, Small TN, Young JW, O'Reilly RJ. Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: separation of graft-versus-leukemia responses from graft-versus-host disease. Blood. 1995;86:1261–8.
47. Niederwieser D, Maris M, Shizuru JA, Petersdorf E, Hegenbart U, Sandmaier BM, Maloney DG, Storer B, Lange T, Chauncey T, Deininger M, Pönisch W, Anasetti C, Woolfrey A, Little MT, Blume KG, McSweeney PA, Storb RF. Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolatemofetil (MMF) can induce durable complete chimerism and sustained remissions in patients with hematological diseases. Blood. 2003;101:1620–9.
48. Olavarria E, Siddique S, Griffiths MJ, Avery S, Byrne JL, Piper K, Lennard A, Pallan L, Arrazi K, Perz J, O'Shea D, Goldman J, Apperley JF, Craddock C. Post-transplant imatinib as a strategy to postpone the requirement for immunotherapy in patients undergoing reduced intensity allografts for chronic myeloid leukemia. Blood. 2007;110:4614–7. https://doi.org/10.1182/blood-2007-04-082990.
49. Crawley C, Szydlo R, Lalancette M, Bacigalupo A, Lange A, Brune M, Juliusson G, Nagler A, Gratwohl A, Passweg J, Komarnicki M, Vitek A, Mayer J, Zander A, Sierra J, Rambaldi A, Ringden O, Niederwieser D, Apperley JF. Outcomes of reduced intensity transplantation for chronic myeloid leukaemia: an analysis of prognostic factors from the chronic leukemia working party of the EBMT. Blood. 2005;106:2969–76. https://doi.org/10.1182/blood-2004-09-3544.
50. Cross NC, Feng L, Chase A, Bungey J, Hughes TP, Goldman JM. Competitive polymerase chain reaction to estimate the number of BCR-ABL transcripts in chronic myeloid leukemia patients after bone marrow transplantation. Blood. 1993;82:1929–36.
51. Gratwohl A, Hermans J, Goldman JM, Arcese W, Carreras E, Devergie A, Frassoni F, Gahrton G, Kolb HJ, Niederwieser D, Ruutu T, Vernant JP, de Witte T, Apperley J. Risk assessment for patients with chronic myeloid leukaemia before allogeneic blood or marrow transplantation. Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Lancet. 1998;352:1087–92.
52. Gratwohl A, Stern M, Brand R, Apperley J, Baldomero H, de Witte T, Dini G, Rocha V, Passweg J, Sureda A, Tichelli A, Niederwieser D. Risk score for outcome after allogeneic hematopoietic stem cell transplantation: a retrospective analysis. Cancer. 2009;115:4715–26. https://doi.org/10.1002/cncr.24531.
53. Milojkovic D, Szydlo R, Hoek J, Beelen D, Hamladji R, Kyrcz-Krzemien S, Bacigalupo A, Niederwieser D, Tischer J, Schwerdtfeger R, Volin L, Gurman G, Greinix H, Chalandon Y, Kroeger N, Olavarria E. Prognostic significance of EBML score for CML patients in the era of tyrosine kinase inhibitor therapy: a retrospective study from the Chronic Malignancies Working Party of the European Group for Blood and Marrow Transplantation, 2014, 40th Annual Meeting of the European Group for Blood and Marrow Transplantation, Publisher: Nature Publishing Group, S34-S35, ISSN: 0268–3369.
54. Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG, Storer B. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood. 2005;106:2912–9. https://doi.org/10.1182/blood-2005-05-2004.
55. Elsawy M, Sorror ML. Up-to-date tools for risk assessment before allogeneic hematopoietic cell transplantation. Bone Marrow Transplant. 2016;51:1283–300. https://doi.org/10.1038/bmt.2016.141.
56. Gratwohl A, Baldomero H, Aljurf M, et al. Hematopoietic stem cell transplantation: a global perspective. JAMA. 2010b;303:1617–24.
57. Gratwohl A, Baldomero H, Gratwohl M, Aljurf M, Bouzas LF, Horowitz M, Kodera Y, Lipton J, Iida M, Pasquini MC, Passweg J, Szer J, Madrigal A, Frauendorfer K, Niederwieser D. Worldwide Network of Blood and Marrow Transplantation (WBMT)/Quantitative and qualitative differences in use and trends of hematopoietic stem cell transplantation: a global observational study. Haematologica. 2013;98:1282–90. https://doi.org/10.3324/haematol.2012.076349.

58. Gratwohl A, Pasquini MC, Aljurf M, For the Worldwide Network of Blood and Marrow Transplantation WBMT, et al. One million haemopoietic stem- cell transplants: a retrospective observational study. Lancet Haematol. 2015a;2:e91–100.
59. Pasquini MC. Hematopoietic cell transplantation for chronic myeloid leukemia in developing countries: perspectives from Latin America in the post-tyrosine kinase inhibitor era. Hematology. 2012;17(Suppl 1):S79–82.
60. Passweg JR, Baldomero H, Chabannon C, Basak GW, Corbacioglu S, Duarte R, Dolstra H, Lankester AC, Mohty M, Montoto S, Peffault de Latour R, Snowden JA, Styczynski J, Yakoub-Agha I, Kröger N, European Society for Blood and Marrow Transplantation (EBMT). The EBMT activity survey on hematopoietic-cell transplantation and cellular therapy 2018: CAR-Ts come into focus. Bone Marrow Transplant. 2020;55:1604–13. https://doi.org/10.1038/s41409-020-0826-4.
61. Gratwohl A, Baldomero H, Passweg J. The role of hematopoietic stem cell transplantation in chronic myeloid leukemia. Ann Hematol. 2015b;94(Suppl 2):S177–86. https://doi.org/10.1007/s00277-015-2313-3.
62. Lubking A, Dreimane A, Sandin F, Isaksson C, Märkevärn B, Brune M, Ljungman P, Lenhoff S, Stenke L, Höglund M, Richter J, Olsson-Strömberg U. Allogeneic stem cell transplantation for chronic myeloid leukemia in the TKI era: population-based data from the Swedish CML registry. Bone Marrow Transplant. 2019;54:1764–74.
63. Khoury HJ, Kukreja M, Goldman JM, Wang T, Halter J, Arora M, Gupta V, Rizzieri DA, George B, Keating A, Gale RP, Marks DI, McCarthy PL, Woolfrey A, Szer J, Giralt SA, Maziarz RT, Cortes J, Horowitz MM, Lee SJ. Prognostic factors for outcomes in allogeneic transplantation for CML in the imatinib era: a CIBMTR analysis. Bone Marrow Transplant. 2012;47:810–6.
64. Lee SJ, Kukreja M, WT LSJ, Kukreja M, Wang T, Giralt SA, Szer J, Arora M, Woolfrey AE, Cervantes F, Champlin RE, Gale RP, Halter J, Keating A, Marks DI, McCarthy PL, Olavarria E, Stadtmauer EA, Abecasis M, Gupta V, Khoury HJ, George B, Hale GA, Liesveld JL, Rizzieri DA, Antin JH, Bolwell BJ, Carabasi MH, Copelan E, Ilhan O, Litzow MR, Schouten HC, Zander AR, Horowitz MM, Maziarz RT. Impact of prior imatinib mesylate on the outcome of hematopoietic cell transplantation for chronic myeloid leukemia. Blood. 2008:3500–7.
65. Özen M, Üstün C, Öztürk B, Topçuoğlu P, Arat M, Gündüz M, Atilla E, Bolat G, Arslan Ö, Demirer T, Akan H, İlhan O, Beksaç M, Gürman G, Özcan M. Allogeneic transplantation in chronic myeloid Leukemia and the effect of tyrosine kinase inhibitors on survival: a quasi-experimental study. Turk J Haematol. 2017;34:16–26. https://doi.org/10.4274/tjh.2015.0346.
66. Shen K, Liu O, Sun J, Jiang Q, Ye Y, Huang H, Meng F, Zhou Y, Yang M. Prior exposure to imatinib does not impact outcome of allogeneic hematopoietic transplantation for chronic myeloid leukemia patients: a single-center experience in China. Int J Clin Exp Med. 2015;8:2495–505.
67. Piekarska A, Gil L, Prejzner W, Wiśniewski P, Leszczyńska A, Gniot M, Komarnicki M, Hellmann A. Pretransplantation use of the second-generation tyrosine kinase inhibitors has no negative impact on the HCT outcome. Ann Hematol. 2015;94:1891–7.
68. Kondo T, Nagamura-Inoue T, Tojo A, Nagamura F, Uchida N, Nakamae H, Fukuda T, Mori T, Yano S, Kurokawa M, Ueno H, Kanamori H, Hashimoto H, Onizuka M, Takanashi M, Ichinohe T, Atsuta Y, Ohashi K. Clinical impact of pre-transplant TKI. Am J Hematol. 2017;92:902–8. https://doi.org/10.1002/ajh.24793.
69. Warlick E, Ahn KW, Pedersen TL, Artz A, de Lima M, Pulsipher M, Akpek G, Aljurf M, Cahn JY, Cairo M, Chen YB, Cooper B, Deol A, Giralt S, Gupta V, Khoury HJ, Kohrt H, Lazarus HM, Lewis I, Olsson R, Pidala J, Savani BN, Seftel M, Socié G, Tallman M, Ustun C, Vij R, Vindeløv L, Weisdorf D. Reduced intensity conditioning is superior to nonmyeloablative conditioning for older chronic myelogenous leukemia patients undergoing hematopoietic cell transplant during the tyrosine kinase inhibitor era. Blood. 2012;119:4083–90.
70. Holtick U, Albrecht M, Chemnitz JM, Theurich S, Skoetz N, Scheid C, von Bergwelt-Baildon M. Bone marrow versus peripheral blood allogeneic haematopoietic stem cell transplantation for haematological malignancies in adults. Cochrane Database Syst Rev. 2014;4:CD010189. https://doi.org/10.1002/14651858.CD010189.pub2.
71. Ohashi K, Nagamura-Inoue T, Nagamura F, Tojo A, Miyamura K, Mori T, Kurokawa M, Taniguchi S, Ishikawa J, Morishima Y, Atsuta Y, Sakamaki H. Effect of graft sources on allogeneic hematopoietic stem cell transplantation outcome in adults with chronic myeloid leukemia in the era of tyrosine kinase inhibitors: a Japanese Society of Hematopoietic Cell Transplantation retrospective analysis. Int J Hematol. 2014;100:296–306. https://doi.org/10.1007/s12185-014-1632-9.
72. Eapen M, Logan BR, Appelbaum FR, Antin JH, Anasetti C, Couriel DR, Chen J, Maziarz RT, McCarthy PL, Nakamura R, Ratanatharathorn V, Vij R, Champlin RE. Long-term survival after transplantation of unrelated donor peripheral blood or bone marrow hematopoietic cells for hematologic malignancy. Biol Blood Marrow Transplant. 2015;21:55–9. https://doi.org/10.1016/j.bbmt.2014.09.006.
73. Shimoni A, Volchek Y, Koren-Michowitz M, Varda-Bloom N, Somech R, Shem-Tov N, Yerushalmi R, Nagler A. Phase 1/2 study of nilotinib prophylaxis after allogeneic stem cell transplantation in patients with advanced chronic myeloid leukemia or Philadelphia chromosome-positive acute lymphoblastic leukemia. Cancer. 2015;121:863–71. https://doi.org/10.1002/cncr.29141.

74. Carpenter PA, Johnston L, Fernandez HF, Radich JP, Mauro MJ, Flowers MED, Martin PJ, Gooley TA. Posttransplant feasibility study of nilotinib prophylaxis for high-risk Philadelphia chromosome positive leukemia. Blood. 2017;130:1170–2. https://doi.org/10.1182/blood-2017-03-771121.
75. DeFilipp Z, Ancheta R, Liu Y, Hu ZH, Gale RP, Snyder D, Schouten HC, Kalaycio M, Hildebrandt GC, Ustun C, Daly A, Ganguly S, Inamoto Y, Litzow M, Szer J, Savoie ML, Hossain N, Kharfan-Dabaja MA, Hamadani M, Reshef R, Bajel A, Schultz KR, Gadalla S, Gerds A, Liesveld J, Juckett MB, Kamble R, Hashmi S, Abdel-Azim H, Solh M, Bacher U, Lazarus H, Olsson R, Cahn JY, Grunwald MR, Savani BN, Yared J, Rowe JM, Cerny J, Chaudhri NA, Aljurf M, Beitinjaneh A, Seo S, Nishihori T, Hsu JW, Ramanathan M, Alyea E, Popat U, Sobecks R, Saber W. Maintenance tyrosine kinase inhibitors following allogeneic hematopoietic stem cell transplantation for chronic myelogenous leukemia: a Center for International Blood and Marrow Transplant Research Study. Biol Blood Marrow Transplant. 2020;26:472–9. https://doi.org/10.1016/j.bbmt.2019.10.017.
76. Radujkovic A, Guglielmi C, Bergantini S, Iacobelli S, van Biezen A, Milojkovic D, Gratwohl A, Schattenberg AV, Verdonck LF, Niederwieser DW, de Witte T, Kröger N, Olavarria E, Chronic Malignancies Working Party of the European Society for Blood and Marrow Transplantation. Donor lymphocyte infusions for chronic myeloid leukemia relapsing after allogeneic stem cell transplantation: may we predict graft-versus-leukemia without graft-versus-host disease? Biol Blood Marrow Transplant. 2015;21:1230–6. https://doi.org/10.1016/j.bbmt.2015.03.012.
77. Hehlmann R, Berger U, Pfirrmann M, Heimpel H, Hochhaus A, Hasford J, Kolb HJ, Lahaye T, Maywald O, Reiter A, Hossfeld DK, Huber C, Loffler H, Pralle H, Queisser W, Tobler A, Nerl C, Solenthaler M, Goebeler ME, Griesshammer M, Fischer T, Kremers S, Eimermacher H, Pfreundschuh M, Hirschmann WD, Lechner K, Wassmann B, Falge C, Kirchner HH, Gratwohl A. Drug treatment is superior to allografting as first-line therapy in chronic myeloid leukemia. Blood. 2007;109:4686–92. https://doi.org/10.1182/blood-2006-11-055186.
78. Hochhaus A, Baccarani M, Silver RT, Schiffer C, Apperley JF, Cervantes F, Clark RE, Cortes JE, Deininger MW, Guilhot F, Hjorth-Hansen H, Hughes TP, JJWM J, Kantarjian HM, Kim DW, Larson RA, Lipton JH, Mahon FX, Mayer J, Nicolini F, Niederwieser D, Pane F, Radich JP, Rea D, Richter J, Rosti G, Rousselot P, Saglio G, Saußele S, Soverini S, Steegmann JL, Turkina A, Zaritskey A, Hehlmann R. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020;34:966–84. https://doi.org/10.1038/s41375-020-0776-2.
79. Saussele S, Lauseker M, Gratwohl A, Beelen DW, Bunjes D, Schwerdtfeger R, Kolb HJ, Ho AD, Falge C, Holler E, Schlimok G, Zander AR, Arnold R, Kanz L, Dengler R, Haferlach C, Schlegelberger B, Pfirrmann M, Muller MC, Schnittger S, Leitner A, Pletsch N, Hochhaus A, Hasford J, Hehlmann R. Allogeneic hematopoietic stem cell transplantation (Allo SCT) for chronic myeloid leukemia in the imatinib era: evaluation of its impact within a subgroup of the randomized German CML study IV. Blood. 2010;115:1880–5. https://doi.org/10.1182/blood-2009-08-237115.
80. Zhang GF, Zhou M, Bao XB, Qiu HY, Li Z, Xue SL. Imatinib mesylate versus allogeneic hematopoietic stem cell transplantation for patients with chronic myelogenous Leukemia. Asian Pac J Cancer Prev. 2016;17:4477–81.
81. Jiang Q, Xu LP, Liu DH, Liu KY, Chen SS, Jiang B, Jiang H, Chen H, Chen YH, Han W, Zhang XH, Wang Y, Qin YZ, Liu YR, Lai YY, Huang XJ. Imatinib mesylate versus allogeneic hematopoietic stem cell transplantation for patients with chronic myelogenous leukemia in the accelerated phase. Blood. 2011;117:3032–40.
82. Jiang H, Xu LP, Liu DH, Liu KY, Chen SS, Jiang B, Jiang Q, Chen H, Chen YH, Han W, Zhang XH, Wang Y, Wang JZ, Wang FR, Qin YZ, Lai YY, Huang XJ. Allogeneic hematopoietic SCT in combination with tyrosine kinase inhibitor treatment compared with TKI treatment alone in CML blast crisis. Bone Marrow Transplant. 2014;49:1146–54. https://doi.org/10.1038/bmt.2014.
83. Radujkovic A, Dietrich S, Blok HJ, Nagler A, Ayuk F, Finke J, Tischer J, Mayer J, Koc Y, Sorà F, Passweg J, Byrne JL, Jindra P, Veelken JH, Socié G, Maertens J, Schaap N, Stadler M, Itälä-Remes M, Tholouli E, Arat M, Rocha V, Ljungman P, Yakoub-Agha I, Kröger N, Chalandon Y. Allogeneic stem cell transplantation for blast crisis chronic myeloid leukemia in the era of tyrosine kinase inhibitors: a retrospective study by the EBMT Chronic Malignancies Working Party. Biol Blood Marrow Transplant. 2019;25:2008–16. https://doi.org/10.1016/j.bbmt.2019.06.028.
84. Xu LP, Xu ZL, Zhang XH, Chen H, Chen YH, Han W, Chen Y, Wang FR, Wang JZ, Wang Y, Yan CH, Mo XD, Liu KY, Huang XJ. Allogeneic stem cell transplantation for patients with T315I BCR-ABL mutated chronic myeloid leukemia. Biol Blood Marrow Transplant. 2016;22:1080–6. https://doi.org/10.1016/j.bbmt.2016.03.012.
85. Nicolini FE, Basak GW, Kim DW, Olavarria E, Pinilla-Ibarz J, Apperley JF, Hughes T, Niederwieser D, Mauro MJ, Chuah C, Hochhaus A, Martinelli G, DerSarkissian M, Duh MS, McGarry LJ, Kantarjian HM, Cortes JE. Overall survival with ponatinib versus allogeneic stem cell transplantation in Philadelphia chromosome-positive leukemias with the T315I mutation. Cancer. 2017;123:2875–80. https://doi.org/10.1002/cncr.30558.
86. Majhail NS, Farnia SH, Carpenter PA, Champlin RE, Crawford S, Marks DI, Omel JL, Orchard PJ, Palmer J, Saber W, Savani BN, Veys PA, Bredeson CN, Giralt SA, LeMaistre CF. Indications for autolo-

gous and allogeneic hematopoietic cell transplantation: guidelines from the American Society for Blood and Marrow Transplantation. Biol Blood Marrow Transplant. 2015;21:1863–9. https://doi.org/10.1016/j.bbmt.2015.07.032.

87. Sureda A, Bader P, Cesaro S, Dreger P, Duarte RF, Dufour C, Falkenburg JH, Farge-Bancel D, Gennery A, Kröger N, Lanza F, Marsh JC, Nagler A, Peters C, Velardi A, Mohty M, Madrigal A. Indications for allo- and auto-SCT for haematological diseases, solid tumours and immune disorders: current practice in Europe. Bone Marrow Transplant. 2015;50:1037–56. https://doi.org/10.1038/bmt.2015.6.
88. Copland M, Slade D, Byrne J, Brock K, De Lavallade H, Craddock C, Clark R, Smith M, Bishop R, Milojkovic D, Yap C. FLAG-IDA and Ponatinib in patients with blast phase chronic myeloid leukaemia: results from the phase I/II UK trials acceleration programme Matchpoint trial. Blood. 2019;134(Supplt1):497. https://doi.org/10.1182/blood-2019-125591.
89. Jain P, Kantarjian HM, Ghorab A, Sasaki K, Jabbour EJ, Nogueras Gonzalez G, Kanagal-Shamanna R, Issa GC, Garcia-Manero G, Kc D, Dellasala S, Pierce S, Konopleva M, Wierda WG, Verstovsek S, Daver NG, Kadia TM, Borthakur G, O'Brien S, Estrov Z, Ravandi F, Cortes JE. Prognostic factors and survival outcomes in patients with chronic myeloid leukemia in blast phase in the tyrosine kinase inhibitor era: cohort study of 477 patients. Cancer. 2017;123:4391–402. https://doi.org/10.1002/cncr.30864.
90. Nair AP, Barnett MJ, Broady RC, Hogge DE, Song KW, Toze CL, Nantel SH, Power MM, Sutherland HJ, Nevill TJ, Abou Mourad Y, Narayanan S, Gerrie AS, Forrest DL. Allogeneic hematopoietic stem cell transplantation is an effective salvage therapy for patients with chronic myeloid leukemia presenting with advanced disease or failing treatment with tyrosine kinase inhibitors. Biol Blood Marrow Transplant. 2015;21:1437–44. https://doi.org/10.1016/j.bbmt.2015.04.005.
91. Boddu P, Shah AR, Borthakur G, Verstovsek S, Garcia-Manero G, Daver N, Kadia T, Ravandi F, Jain N, Alhuraiji A, Burger J, Kornblau S, Pierce S, Dellasala S, Jabbour E, Kantarjian H, Cortes J. Life after ponatinib failure: outcomes of chronic and accelerated phase CML patients who discontinued ponatinib in the salvage setting. Leuk Lymphoma. 2018;59:1312–22. https://doi.org/10.1080/10428194.2017.1379076.



12 CML End Phase and Blast Crisis: Implications and Management

Rüdiger Hehlmann, Susanne Saußele, Astghik Voskanyan, and Richard T. Silver

12.1 Introduction

Blast crisis (BC) is among the remaining challenges in the management of CML. Although an acute or terminal phase of CML has been mentioned in the beginning of the twentieth century [1], it was only in the 1960s that a definition of the terminal phase of CML was attempted. Morrow Jr. et al. [2] defined the terminal phase as the interim extending from the first clinical change heralding the onset of the final phase of disease to the time of death. Defining signs and symptoms were mainly fever, abdominal discomfort in the left upper quadrant, weakness, and dyspnea (without cardiac failure). Karanas and Silver [3] included laboratory values and determined that 30% myeloblasts and promyelocytes or more in the peripheral blood predicted death within 6 months more accurately than 20- < 30% myeloblasts and promyelocytes, hemoglobin <9 g%, <100.000/ml platelets, an increase of WBC after treatment of 2 weeks, or otherwise unexplained fever. In 1971 Canellos et al. [4] reported a subset of patients with blastic transformation that responded to vincristine and prednisone which was followed by the detection of terminal deoxynucleotidyl transferase (TdT) [5, 6] defining lymphoid as opposed to myeloid subtypes of BC.

BC is a malignancy that, as a rule, develops under the eyes of the treating physician. Indicators are clonal evolution with additional chromosomal abnormalities (ACA) reaching levels of up to 90% [7] and mutation levels including resistance mutations to tyrosine kinase inhibitor (TKI) treatment in up to 80% [8]. CML end phase comprises early progression with emerging high-risk ACA and late progression with failing hematopoiesis and blast cell proliferation (Fig. 12.1). BC is the end stage of this evolution. The incidence of BC has been greatly reduced by the introduction of TKI which demonstrates that BC can be prevented by effective therapy. Once BC has occurred, no effective therapy exists to date, except for the occasional return to a second chronic phase (CP2) after chemotherapy followed by transplantation [9]. Without transplantation, survival is generally less than 1 year with death due to infection or bleeding. Prevention of BC by careful monitoring treatment response and intensification of treatment, if response milestones are not reached, remain the mainstay of the treatment strategy.

R. Hehlmann (✉)
ELN Foundation, Weinheim, Germany

Medizinische Fakultät Mannheim, Universität Heidelberg, III.Medizinische Klink, Mannheim, Germany

S. Saußele · A. Voskanyan
Medizinische Fakultät Mannheim, Universität Heidelberg, III.Medizinische Klink, Mannheim, Germany

R. T. Silver
Division of Hematology/Medical Oncology, Weill Cornell Medical College, New York, NY, USA

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
R. Hehlmann (ed.), *Chronic Myeloid Leukemia*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-71913-5_12

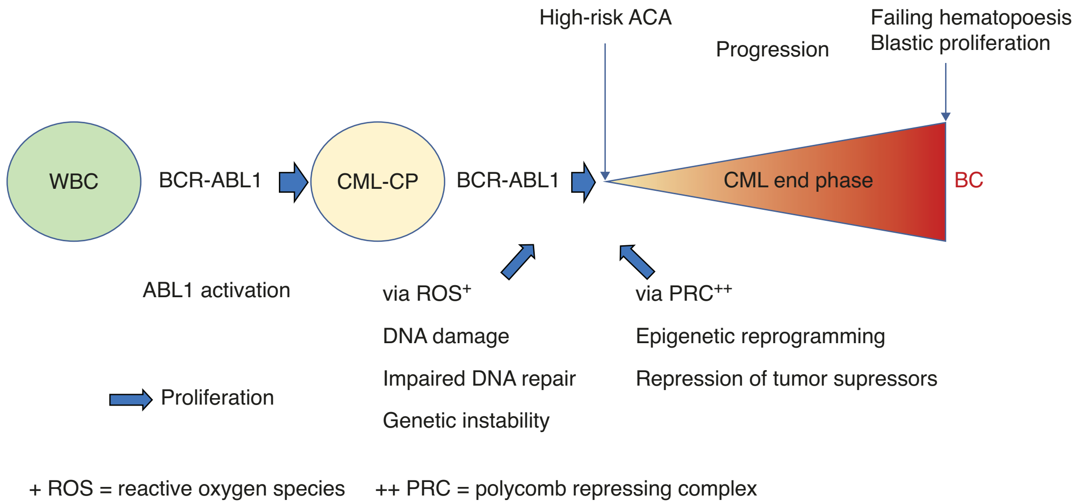


Fig. 12.1 Role of BCR-ABL1 in CML and progression to BC

For the advancement of prevention and treatment, several open questions need to be addressed:

1. Can we prevent progression to BC better by early treatment intensification according to response milestones and genetic markers (ACA, mutations)? Answer: carefully designed clinical trials with early treatment intensification could provide the answer (second generation TKI in ENESTnd, Dasision?).
2. Can we define a point in the course of the disease after which drug treatment cannot reverse clonal evolution (point of no return)? Possible answer: by systematic aligning genetic with hematologic and clinical findings.
3. What indicators precede an increase of blasts? Possible answer: careful dissection of the course of disease after the appearance of prognostically relevant genetic markers and as a proof of principle, following with targeted intervention.
4. Is genetic instability by BCR-ABL the single causative factor for clonal evolution or disease progression, or are there other predisposing factors? Answer: comparative analysis of clinical course and appearance of genetic markers with whole genome sequencing may be helpful.

This review gives a broad overview of diagnosis of BC, therapy, clonal evolution and early prediction of progression, and prevention of BC, as well as our opinion regarding the open questions.

12.2 Diagnosis

To diagnose BC, complete blood and differential counts, marrow cytology, and cytogenetics are required. Cytogenetic evolution is the most consistent predictor of blast transformation. Flow cytometry or cytochemistry is needed to determine the type of BC (myeloid or lymphoid). Molecular genetics with mutation analysis are needed to choose the appropriate TKI. Consensus recommendations for performing mutation analyses have been published by the European LeukemiaNet [8]. Tests at diagnosis and during follow-up are shown in Table 12.1.

Currently, diagnosis of BC rests on the percentage of blasts (20% or 30%) in blood or marrow [10–12], but not all patients dying of CML reach the BC-defining blast levels [13]. Earlier recognition of CML end phase might enable earlier intervention to improve prospects for BC.

Clinically, BC may present with night sweats, weight loss, fever, bone pain, or symptoms of anemia. An increased risk of infections and of bleeding is also observed. The common laboratory features include high white blood and blast cell counts, features of hematopoietic failure,

Table 12.1 Tests for BC diagnosis and monitoring of treatment

Test	Test rational
At diagnosis	
CBC with differential and marrow cytology	Proportions of blasts, promyelocytes, and basophils
Flow cytometry and/or cytochemistry	Myeloid or lymphoid phenotype
Cytogenetics	Baseline for follow-up and prognosis High-risk ACA
Molecular genetics	KD-mutation profile for choice of TKI Somatic mutations
Donor search (if applicable)	Preparation for Allo-SCT
For monitoring	
CBC with differential	Return to CP (CP2)
Marrow cytology with cytogenetics	Ascertainment of second CP or remission
Molecular genetics	Monitoring of BCR-ABL transcript levels under TKI treatment and after Allo-SCT
In lymphoid BC: CSF cytology	Intrathecal neuroprophylaxis

BC blast crisis, *CP* chronic phase, *CSF* cerebrospinal fluid, *CBC* complete blood count, *TKI* tyrosine kinase inhibitor, *SCT* stem cell transplantation, *ACA* additional cytogenetic aberrations, *PCR* polymerase chain reaction

additional cytogenetic aberrations (ACA) in addition to the Philadelphia (Ph) chromosome [14–22], and somatic mutations [23, 24].

Up to 90% of BC patients show chromosomal aberrations (termed major or minor route by Mitelman dependent on their frequency in BC) in addition to the Ph chromosome [7, 25] and up to 80% BCR-ABL1 KD mutations [8]. Various somatic mutations have been detected in BC or associated with poor risk disease when detected at diagnosis [23, 24]. Blast increase in blood or marrow represents the end stage of progression.

12.3 Genetically Based Risk Assessment

Genetically based risk assessment by ACA and somatic mutations has been proposed for a better recognition of patients at risk for progression to end phase CML and BC [26–29]. Analyzing single chromosome changes, Wang et al. [29] stratified the six most common ACA into two prognostic groups: a good risk group comprising +8, +Ph, and -Y and a poor risk group comprising i17(q10), −7/7q-, and 3q26.2 rearrangements. Based on BC-risk associated with each ACA, Gong et al. analyzed the time intervals from diagnosis to emergence of ACA, from emergence of ACA to onset of BC and survival with BC, and stratified ACA into three risk groups (high risk: 3q26.2; −7/7q-; i17(q10); complex karyotypes with high-risk ACA; intermediate 1: +8; +Ph; other single ACA; intermediate 2: other complex ACA). Hehlmann et al. suggested two groups: high-risk ACA with unfavorable impact on survival and low-risk ACA with no or little impact on survival. High-risk ACA are defined as the major route ACA +8, +Ph, i(17q), +19, +21 and + 17 (the ACA most frequently observed in BC) [7]; the minor route ACA −7/7q-, 3q26.2, and 11q23 rearrangements (less frequently observed, but negative impact on prognosis) [27, 29]; and complex aberrant karyotypes (Table 12.2). If present at low-blast counts, high-risk ACA herald death by CML [28].

Somatic mutations observed in BC and in poor-risk patients include mutations of genes associated with poor outcome also in other malignancies [30]. Also, they might enable early identification of patients at risk of progression. Frequently mutated genes include RUNX1, ASXL1, and IKZF1 [23, 24] (Table 12.2).

Mutations of the BCR-ABL tyrosine kinase domain have been observed in as many 80% of patients [8]. ABL mutations in late CP with initial imatinib resistance have been associated with a greater likelihood of progression to BC [31]. Other mutations associated with BC include p53 mutations in approximately 24% of myeloid BC, p16 mutations in approximately 50% of lymphoid BC [32, 33], and somatic mutations, such as RUNX-1, IKZF1 (Ikaros), ASXL1, WT1, TET2, IDH1, NRAS, KRAS, and CBL in 3–33% of myeloid and/or lymphoid BC [23, 24, 34, 35]. In addition, a profoundly altered gene expression profile has been reported in CD34+ BC cells compared with CP cells [36, 37]. Genes overexpressed, downregulated, or deregulated in BC

Table 12.2 Genetically based risk assessment

Chromosomal abnormalities	Somatic mutations		
High-risk ACA [29] [27] [28]	Mutated genes, selection	Frequency of mutation in BC (%) [24] $n = 39$	[23] $n = 46$
+8	RUNX 1	33,3	28
+Ph	ASXL 1	20,5	23
i(17q)	IKZF 1	17,9	33
+19	WT 1	15,4	NA
+21	TET 2	7,7	NA
+17	IDH 1/2	7,7	8
−7/7q-	CBFB/MYH11	NA	6
3q26.2	TP 53	2,6	3
11q23	ABL1-KD	33,3	58
Complex aberrant			

include SOCS2, CD52, HLA antigens, PRAME, JunB, Fos, FosB, Il8, and genes of the Wnt/β--catenin pathway [38]. Also, the evolution of gene expression profiles may allow diagnosis of disease progression [39, 40].

12.4 Pathogenetic Basis of Therapy

Treatment of BC is guided by our understanding of BC pathogenesis. Good in-depth reviews on the biology of BC have been published [41–43]. According to current evidence, BC is the direct consequence of continued BCR-ABL activity [41, 42], possibly via oxidative stress and reactive oxygen species [44, 45], causing DNA damage and impaired DNA repair [46] and, in a vicious circle, genomic instability by more mutations, gene doublings, translocations, and chromosomal breakages [47]. The latter effect of BCR-ABL would explain what is observed during clonal evolution and progression to BC. BCR-ABL has been shown to produce reactive oxygen species in hematopoietic cells [48].

An alternative model [49, 50] makes use of the observation that the polycomb repressive complex (PRC) gene BMI1 is a marker for predicting prognosis of CML [51]. Based on an integrated multiomics analysis, this model proposes pathway convergence in genetically heterogeneous BC by PRC-driven epigenetic reprogramming of BC progenitors. A PRC2-related gene set including EZH2 directs BC DNA hypermethylation silencing myeloid differentiation, whereas PRC1 including BMI1 represses tumor suppressors and maintains the BC transcriptome. Since BMI1 inhibitors (e.g., PTC596) de-repress genes involved in apoptosis, proliferation, and differentiation and since hypomethylating agents (decitabine) revert EZH2-directed hypermethylation, the model predicts that a combination of PTC596 and decitabine might prove effective for treating BC.

Figure 12.1 summarizes our current understanding of CP and BC pathogenesis.

12.5 Intensive Chemotherapy

Once BC has been diagnosed, management depends on prior therapy and type of leukemia (myeloid or lymphoid). In the late 1960s/early 1970s, attempts were made to treat BC with treatment protocols designed for acute leukemia (AL). It was observed that 30% of the patients responded to a combination of vincristine and prednisone as used for acute lymphoblastic leukemia (ALL) [4, 52]. The cells of the responding BC frequently showed features of lymphoid morphology and were TdT+ [5]. These observations have led to the distinction of lymphoid and myeloid variants of BC. The response rates to vincristine and prednisone and other drugs used for ALL, such as 6-thioguanine, 6-mercaptopurine, cytosine arabinoside, and methotrexate, ranged between 15 and 50%. Response was only of short duration. Responders survived a median of 3–10 months compared with 1–5 months in nonresponders.

Between 1980 and 1990, AML-type induction therapies were applied, including various combinations of anthracyclines, cytosine arabinoside, 5-azacytidine, etoposide, carboplatin, fludarabine, and decitabine [53]. A return to CP (CP2) was observed in approximately 10% of patients, opening a window for transplantation. No cures

in the absence of stem cell transplantation were observed. Overall, treatment of BC was less successful than that of de novo acute leukemias despite considerable intensity (and toxicity), but the advantage offered by a second CP prior to allo-SCT was recognized. Best results are probably achieved for patients who return to CP and are then successfully transplanted.

12.6 TKI Therapy

The clinical improvement with more effective treatment (10-year BC incidence 5.8–6.9% in CML study IV [54] and in the IRIS trial [55] compares to 70% BC incidence 25 years ago) is shown in Fig. 12.2. This decrease in BC incidence occurs in parallel with a reduction of BCR-ABL1 indicating that BC can be prevented by effective therapy. Treatment outcome supports the conclusion that BCR-ABL1 is the driving force behind disease progression (Fig. 12.1). Currently, most BC cases occur early after start of therapy (Fig. 12.3) indicating the disease had progressed to an advanced phase even though it appeared phenotypically early. A minority of patients progress to BC later during the course of the disease suggesting continued disease activity in some patients. Population-based progression rates are similar to those in clinical trials [56].

The transient nature of response to TKI in BC shows that most cells are still sensitive to BCR-ABL1 inhibition but that BCR-ABL1 independence has been achieved in some cells which have a growth advantage. It follows that the most effective management of BC would be its prevention by early reduction of tumor burden and elimination of BCR-ABL1.

12.7 Imatinib

1. Treatment of de novo BC should be started with imatinib, 600–800 mg/day. If the response is unsatisfactory, dasatinib 140 mg once daily or nilotinib 400 mg twice daily according to mutation profile (Table 12.3) should be tried A sensitive detection of BCR-ABL1 mutations is now possible by NGS [57]. If the profile indicates the T315I mutation, ponatinib should be

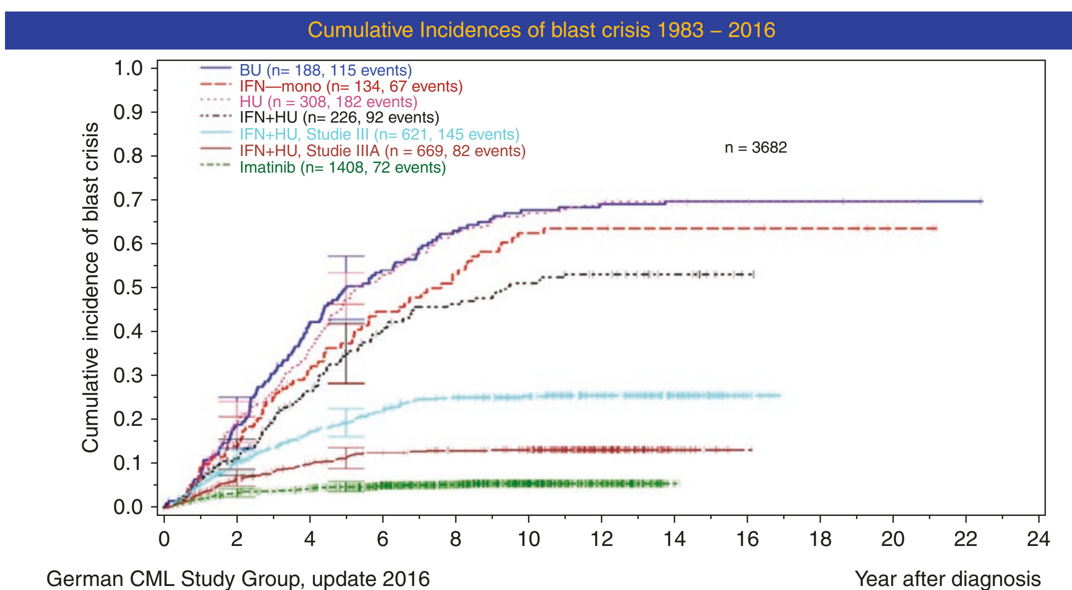


Fig. 12.2 Prevention of BC by more effective therapy. Update to [58]. *Bu* busulfan, *HU* hydroxyurea, *IFN* interferon alpha

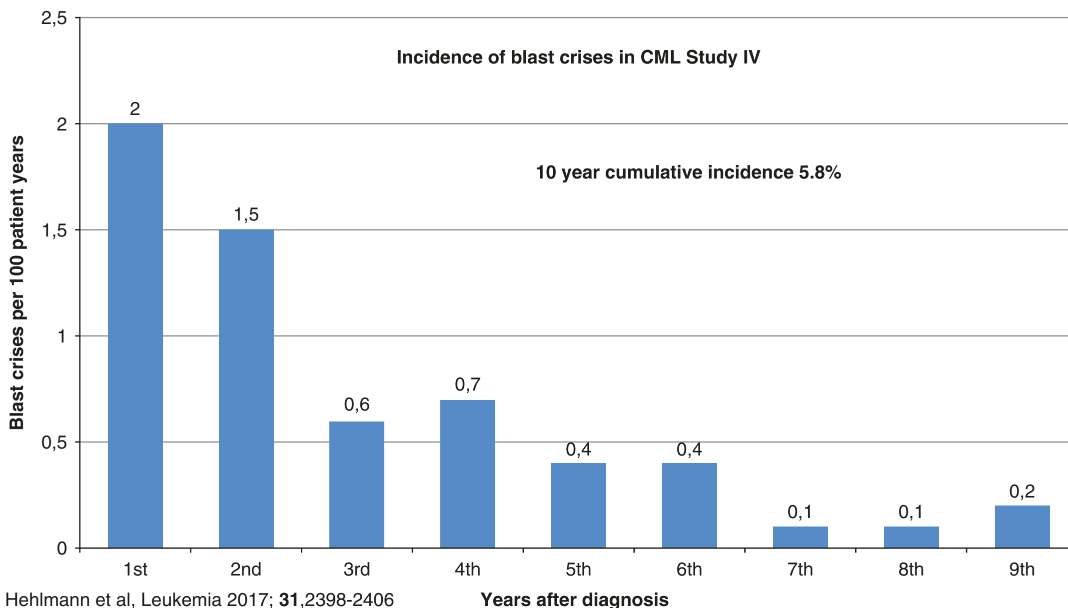


Fig. 12.3 Incidence of BC over time. Ten-year cumulative incidence of BC is 5.8% (Hehlmann et al. 2017)

Table 12.3 TKI indications based on KD mutation profile

F317L/V/I/C, T315A	Nilotinib or ponatinib
V299L	Nilotinib or ponatinib
Y253H, E255V/K, F359V/I/C	Dasatinib, bosutinib[a] or ponatinib
T315I	Ponatinib

[a]In vitro data suggest that E255K and, to a lesser extent, E255V might be poorly sensitive to bosutinib

given at a dose of 45 mg daily. Allo-SCT should be planned as early as possible [9]. Imatinib, dasatinib, bosutinib, and ponatinib have been approved for all phases of CML, including BC, by the Food and Drug Administration and the European Medicine Agency.

Five studies of 484 BC patients, 50 with lymphoid BC, showed hematologic remission rates of 50–70% (70% in patients with lymphoid BC), cytogenetic response rates of 12–17% (all responses), a 1-year survival of 22–36%, and a median survival of 6.5–10 months [59–63].

2. If BC evolves during imatinib therapy, treatment with a second- or third-generation TKI (dasatinib 140 mg, nilotinib 400 mg twice daily, bosutinib 500 mg, or ponatinib 45 mg each daily, respectively, according to mutation profile) combined with intensive chemotherapy as necessary should be given such as combinations of dasatinib or ponatinib + FLAG-IDA [64, 65], or with high-dose cytarabine and daunorubicin (“7 plus 3,” [66]), for myeloid BC, or combinations of imatinib or dasatinib + hyperfractionated CVAD for lymphoid BC [67] and allo-SCT planned as quickly as possible. Cytopenias may necessitate TKI dose reduction or treatment interruption, transfusion of erythrocytes and platelets, or, in case of neutropenia, treatment with G-CSF.

12.8 Dasatinib

Three studies of 400 BC patients who had been previously treated with imatinib, including 119 with lymphoid BC, showed hematologic remission rates of 33–61% (lymphoid BC, 36–80%), major (MCR) cytogenetic remission rates of 35–56%, a 1-year survival of 42–50%, a 2-year survival of 20–30%, and a median survival of 8–11 months [68–70].

The largest of the studies, a randomized open-label phase 3 study of 214 BC patients stratified for myeloid or lymphoid (61) type, attempted to

optimize the dose schedule of dasatinib, comparing dasatinib at 140 mg once daily with 70 mg twice daily. The study yielded similar efficacy for both doses and had improved tolerability for the once-daily regimen [69]. Pleural effusion, which was observed in as many as one-third of the patients, necessitated dose reduction, diuretics, and, in some cases, corticosteroids.

Dasatinib crosses the blood-brain barrier and shows long-lasting responses in Ph + CNS disease [71]. It is speculated that these effects, which differ from imatinib, are the result of the dual specific SRC/BCR-ABL TK-inhibitory property of dasatinib. Dasatinib maintenance is recommended in responders not suitable for allo-SCT.

12.9 Nilotinib

Two studies of 169 patients including 40 with lymphoid BC [72, 73] reported a hematologic response rate of 60% in all patients (59% in lymphoid BC), major cytogenetic response rates of 38% in myeloid BC and 52% in lymphoid BC, a 1-year survival of 42%, a 2-year survival of 27%, and a median survival of 10 months (7.9 months for lymphoid BC). Hyperglycemia, which is observed in as many as 40% of nilotinib-treated patients, required monitoring and may necessitate dose adjustment. Nilotinib has been approved for treating CP and accelerated phase (AP) CML, but not BC.

The outcomes with dasatinib and nilotinib are similar to those with imatinib.

12.10 Imatinib in Combination

Several small studies have focused on the combination of imatinib at 600–800 mg with chemotherapy or other agents. In a phase 1/2 trial of 16 BC patients, imatinib 600 mg daily was combined with mitoxantrone/etoposide [74]. Hematologic response rate was 81% with a 1-year survival of approximately 50%, including six patients who had an allo-SCT. Another study combined imatinib 600 mg with decitabine in ten patients and reported a median survival of 15 weeks [75]. The combination of imatinib 600 mg with low-dose cytosine arabinoside and idarubicin in 19 patients with myeloid BC showed hematologic remissions in 47%. Median survival was 5 months [76]. In a phase 1 study with the combination of the farnesyltransferase inhibitor lonafarnib with imatinib, two of three BC patients showed hematologic improvement [77]. A study of 12 patients combining imatinib and homoharringtonine after priming with G-CSF reported hematologic or cytogenetic response in all patients [78]. Rea et al. [79] reported results of 31 patients with Ph-positive ALL or lymphoid BC treated with imatinib 800 mg/day, vincristine, and dexamethasone. Twenty-eight of 30 evaluable patients achieved complete cytogenetic remissions at a major molecular response level or better. Of 19 patients under 55 years, nine were transplanted and eight were alive 7–23 months afterward. Deau et al. [80] evaluated 36 patients with myeloid BC treated with imatinib 600 mg/day, cytosine arabinoside over 7 days, and daunorubicin up to 45 mg/m^2/day over 3 days. A complete hematologic response of 55.5% was achieved, median survival of all patients was 16 months, for responders 35.4 months, and for transplanted patients the median survival has not been reached.

None of these studies has provided convincing evidence that any of the combinations are superior to imatinib alone.

12.11 Dasatinib or Nilotinib in Combination

Milojkovic et al. [65] reported four patients who progressed to BC while on imatinib and were successfully treated with dasatinib 100 mg daily combined with fludarabine 30 mg/m^2 IV, days 1–5; cytosine arabinoside 2 g/m^2 IV, days 1–5; idarubicin 12 mg/m^2 IV, days 1–3; and G-CSF 300 mg/day sc, days 0–6 (FLAG-IDA). All patients were alive, three after and one prior to SCT. Strati et al. treated 42 BC patients with hyperfractionated cyclophosphamide, vincristine, adriamycin, dexamethasone (HCVAD) plus imatinib, or dasatinib. CCR was achieved in

58%, complete molecular remission in 25% of patients. Eighteen patients received allo-SCT in hematological remission. Median survival was 17 months and was longer in SCT recipients [67]. Ghez et al. reported on five BC patients treated with a combination of 5-azacytidine and dasatinib or nilotinib. Two patients were transplanted; one died of relapse. All other patients are alive and in hematologic remission after 11–33 months [81].

12.12 Bosutinib and Ponatinib

Bosutinib, a third second-generation TKI, shows in preliminary analyses of 48 BC patients similar activity (CCR, 29%; MMR, 28%; PFS, 7.8 months) as dasatinib and nilotinib [58, 82].

The pan-BCR-ABL third generation TKI ponatinib has, in addition to recognizing the T315I mutation, efficacy in BC and Ph + ALL. A phase 2 study of 449 ponatinib-treated patients included 62 patients in BC. After a 6 months median follow-up of the BC patients, a major cytogenetic remission rate of 18% was observed [83]. OS at 12 months was 20%.

In a recent UK study of 17 BC patients, ponatinib was given at 30 mg/day in combination with FLAG-IDA followed by allo-SCT and ponatinib maintenance [64]. One-year OS was 45.8% as estimated by the Kaplan-Meier method.

A drawback of ponatinib is its toxicity profile which requires a thorough risk-benefit assessment [84, 85]. Vascular events at a dose of 45 mg/d may be reduced by smaller doses (15, 30 mg/d).

12.13 Prognostic Factors

A cohort study of 477 BC patients [86] treated with any TKI approved for CML (imatinib, dasatinib, nilotinib, bosutinib, ponatinib) and in part combined with chemotherapy (46%) and allo-SCT (22%) showed a median OS of 12 months. By multivariate analysis, prognostic factors were analyzed for risk of death. Myeloid BC, prior TKI, age $\geq$ 58 years, high LDH, low platelets, no history of SCT, secondary BC, and chromosome 15 abnormalities were found to predict for an increased risk of death. The findings await confirmation.

12.14 Overall Treatment Strategy

If TKIs fail, conventional approaches remain an option, such as AL induction protocols with cytosine arabinoside and anthracyclines in myeloid BC or with vincristine and prednisone (combined with dasatinib) in lymphoid BC.

Patients with suboptimal responses by ELN criteria [87] and with less than DMR after 2–3 years (less than MR^4) should have a genetic evaluation. In patients with high-risk ACA, more intensive treatment, e.g., by allo-SCT, may be indicated. Current treatment approaches to end phase CML are summarized in Fig. 12.4.

Treatment depends on disease stage: Elimination of BCR-ABL1 by effective TKI treatment is expected to prevent progression. When high-risk ACA emerge, intensification of treatment should be considered. Also, there is evidence that earlier allo-SCT is more successful in patients with high-risk ACA. An appropriate time for changing treatment may occur when high-risk ACA emerge rather than waiting for the appearance of or an increase in blasts. Cytogenetic monitoring is indicated when response to therapy is unsatisfactory. AP should be treated as high-risk CML. Allo-SCT is recommended if response to drug treatment is not optimal. Treatment of BC consists of intensive combination chemotherapy based on AML regimens for myeloid, and ALL regimens for lymphoid, BC with or without a TKI in preparation for a prompt allo-SCT if possible. Lymphoid BC has more treatment options and a better outcome than myeloid BC.

In patients who cannot tolerate intensive chemotherapy regimens, a more palliative approach using less intensive therapy according to immunophenotype should be considered such as vincristine and prednisone in lymphoid BC.

There is emerging evidence that high-risk ACA is an indication for a timelier change of treatment which may result in a better outcome [28]. Comparing allo-SCT outcome in early with late end phase, a clinically relevant,

Stage	Management
Prevention of progression	Elimination of BCR-ABL1 by effective TKI treatment
Emergence of high-risk ACA	High-risk patients, observe closely, consider intensification of treatment (ponatinib, early allo-SCT)
AP	To be treated as high-risk CML; proceed to allo-SCT if response not optimal
Primary BC	Start with imatinib, change to a 2G-TKI according to KD-mutation profile. Assessment for allo-SCT, donor search
Resistance to 2G-TKI (first or second-line)	Ponatinib or experimental agent
Failure to ponatinib	High-risk of progression, early allo-SCT recommended
Progress to BC	Attempt at return to CP2 Outcome with currently available TKI poor Addition of chemotherapy based on AML regimens for myeloid BC (such as dasatinib or ponatinib + FLAG-IDA or "7 plus 3") or ALL regimens for lymphoid BC (such as imatinib or dasatinib + hyperfractionated CVAD) recommended Choice of TKI should be based on prior therapy and BCR-ABL1 KD-mutational status After CP2 is achieved proceed to allo-SCT without delay.

Fig. 12.4 Management strategy for end phase CML. The arrow indicates worse progression. *CP2* second chronic phase

though not statistically significant difference of 30% in 2-year survival suggests that outcome of transplanted patients with high-risk ACA depends on disease stage similar to patients without ACA [88].

In summary, survival after BC is better after treatment with TKI than after conventional therapies, but with a median survival of less than 1 year, outcome is still unsatisfactory. A 10-year survival of 19% after TKI versus 3% after conventional treatment is promising. This is illustrated in Fig. 12.5 which depicts the German CML Study Group experience. The majority of BC survivors have received a transplant.

12.15 Allo-SCT

Allo-SCT is successful in only a minority of BC patients after achieving a chemotherapy-induced remission. Nevertheless, it probably has the best outcome in BC, if the patient can tolerate the procedure and if a donor is available (Fig. 12.5). The search for a donor should be started as early as possible. In an overview of the European Group for Blood and Marrow Transplantation from 1980 to 2003, 2-year survival rates were 16–22% [89]. Most patients were transplanted in the pre-imatinib era. In a report from the German CML Study Group which was updated in 2014, the 6-year survival of 28 imatinib-pretreated patients transplanted in advanced phases (25 in BC) was 49% [9, 90]. Similar data were reported in a retrospective analysis of 83 BC patients by a Chinese group [91]. 38 BC patients were treated with allo-SCT after TKI and 45 received TKI only. After a follow-up of 30–126 months, 4-year OS was significantly better for the allo-SCT group compared to the group with only a TKI (47% vs. 10%). Another German group analyzed 40 advanced-phase patients and reported a 43% OS after 3–5 years [92].

Data suggest that allo-SCT represents the best chance of long-term survival after BC, if a second CP has been achieved.

Current experience recommends allo-SCT in primary BC after an attempt has been made with a suitable TKI selected according to mutation profile in combination with chemotherapy as

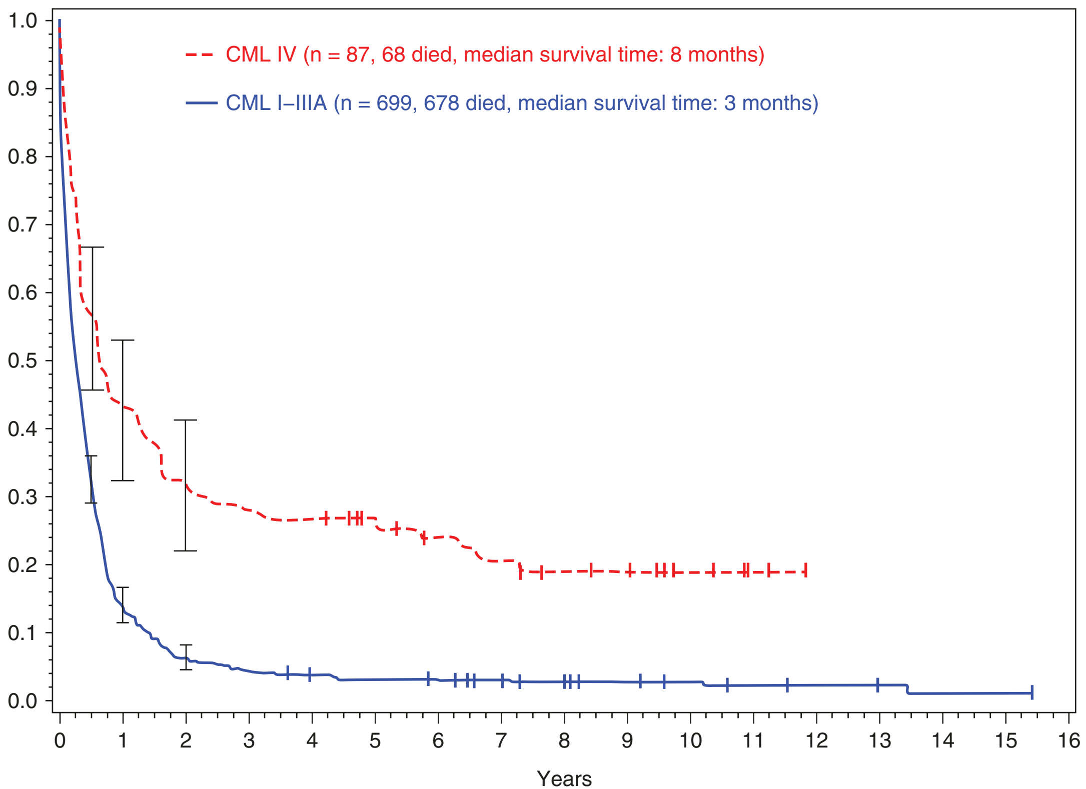


Fig. 12.5 Survival of BC patients under conventional therapy and TKI. German CML Study Group experience, updated (M. Lauseker, 2020 unpublished). Ten year survival after TKI is 19%, after conventional therapy 3%. Fifteen of 20 living patients (75%) have been transplanted

needed to achieve a second CP. In lymphoid BC, dasatinib should be combined with vincristine, prednisone, and HCVAD.

Transplantation should be performed with an HLA-identical related or matched unrelated or, if unavailable, haploidentical donor and an EBMT score of 0–4 [93]. Standard conditioning with busulfan and cyclophosphamide or total body irradiation should be used. Reduced intensity conditioning is not recommended in this situation unless it is a clinical study. Sudden-onset BC during imatinib treatment is a rare event, but full disease eradication by allo-SCT may be successful [94] and is warranted. Posttransplantation maintenance with TKI appears reasonable. Maintenance with dasatinib is recommended in lymphoid BC for neuroprophylaxis, since as mentioned, it crosses the blood-brain barrier. Monitoring of BCR-ABL transcript levels should be done at regular intervals: 3 months initially, 6 months later on, if transcripts are not detectable or stable.

As a consequence of these observations and recommendations, more CML patients are now transplanted in second chronic or advanced phases than in first CP [95].

12.16 Investigational Agents

A number of new approaches are under investigation. A selection is presented in Table 12.4. The approaches include activation of the tumor suppressor protein phosphatase 2A (PP2A), which has decreased activity in BC [111] through upregulation of its inhibitor suppressor of variegation, enhancer of zeste and trithorax (SET), and cancerous inhibitor of PP2A (CIP2A) [98, 99], or in combination with TKI [100]; inhibition of self-renewal of leukemia stem cells (LSCs) by

Table 12.4 Investigational approaches (selection)

Principle/mode of action	Agent (s)	Target (s)
PP2A activation	Fingolimod (FTY720) [96]	PP2A
	SET antagonist OP449 [97]	SET
	CIP2A inhibitor [98, 99]	CIP2A
PP2A inhibition	Sensitization of LSC to TKI [100]	Drug-insensitive LSC
Survival of LSC	BCL6 + TK inhibitors [101]	BCL6 + BCR-ABL
	HIF1α inhibitor [102]	HIF1α
	Smoothened inhibitors in combination with TKI (dasatinib, nilotinib) [103]	Smoothened (hedgehog pathway) + BCR-ABL
	Jak2 inhibitor SAR 302503+ dasatinib [104]	Jak2 + BCR-ABL, LSC
	Jak2/STAT 5 inhibition by nilotinib + ruxolitinib [105]	CML CD34+ cells
Activation of apoptosis	BCL2 inhibitor ABT-737 [106]	Anti-apoptotic proteins
	Triptolide [106] Venetoclax [118]	Anti-apoptotic proteins
	MEK inhibitor PD184352 + farnesyltransferase inhibitor BMS-214662 [107]	MEK1, MEK2, RAS
Repurposing	Axitinib (approved for renal cell cancer) [108]	BCR-ABL, T315I, BC
High-throughput sensitivity and resistance testing (DSRT)	295 anticancer agents screened: VEGFR, NAMPT inhibitors identified [109]	CML-BC
Induction of differentiation	Nilotinib + arsenic trioxide [110]	CML-BC
Epigenetic reprogramming and repression of tumor suppressors	BMI1 inhibitor PTC596 + Hypomethylating agent decitabine [50]	BMI1 EZH2

TKI tyrosine kinase inhibitor, *PP2A* protein phosphatase 2A, *LSC* leukemia stem cells, *MEK* mitogen-activated protein kinase, *VEGFR* vascular endothelial growth factor receptor, *NAMPT* nicotinamide phosphoribosyltransferase

pharmacologic inhibition of BCL6 in combination with BCR-ABL inhibition [101], of hypoxia-inducible factor 1α [102], or of smoothened which plays a role in the hedgehog pathway and is essential for the maintenance of LSC [112]; and induction of apoptosis [106, 107]. Targeting the phosphatidylinositol-3 kinase/AKT/mammalian target of rapamycin (mTOR) activation, Xie et al. reported successful treatment of a patient with myeloid BC by the combination of rapamycin and imatinib [113]. Another approach is repurposing of already approved drugs as has been proposed for axitinib, an antiangiogenic agent for treating renal cell carcinoma which also inhibits T315I mutant BCR-ABL [108]. A novel concept is the search for drug candidates effective in BC by high-throughput testing. Candidate drugs include vascular endothelial growth factor receptor (VEGFR) and nicotinamide phosphoribosyltransferase (NAMPT) inhibitors [109]. Immune checkpoint inhibitors which have been shown to improve prognosis in a variety of cancers [114, 115] are thought to offer promise also for myeloid antigens [116] and high-risk CML. After failure of at least two previous TKIs, the allosteric BCR-ABL1 inhibitor asciminib has shown efficacy in some patients with the T315I mutation or in AP [117]. Venetoclax, in combination with BCR-ABL1 TKI, has been studied in 16 Ph + heavily pretreated patients with AML (7) and myeloid BC (9). The median OS of 10.9 months for BC patients indicates some efficacy of the combination in view of the heavy pretreatment [118]. Because of the numerous blastic genotypes and their instability, no single therapeutic approach can soon be expected to be successful in all patients.

12.17 Prevention

The reduction of BC incidence with more effective therapy indicates that BC can be prevented (Fig. 12.2). Also, it is well known that very low or undetectable BCR-ABL transcripts after allo-SCT correlate with low relapse rates [119].

Further, imatinib-treated patients who have achieved DMR enjoy durable responses with virtually no current progression to AP or BC [120]. Patients who have achieved stable complete molecular remission may experience continued remission in the absence of maintenance treatment in approximately 40% of cases [121]. The challenge therefore is to identify those patients who are at early risk to develop to BC and to be able to offer more effective treatment to this special patient group.

12.18 Early Predictors of Progression

At diagnosis, risk scores provide information on the likelihood of progression [122–125]. The EUTOS score [123], developed from imatinib-treated patients, has a predictive value of 34% of not reaching a CCR by 18 months. It also recognizes a small group of high-risk patients (~12%) with a significantly higher progression rate. Distinct markers such as high-risk ACA [28], p190$^{BCR\text{-}ABL}$ [126], and signs of acceleration may also be suitable for early prediction of progression. CIP2A levels at diagnosis have been reported predictive of BC [98, 99].

The relevance of clonal evolution has not changed in the imatinib era [14, 15]. The types of chromosome abnormalities associated with progression are not altered by TKI treatment [16]. Patients with high-risk ACA are defined as high-risk patients by the ELN 2020 recommendations [87] and indicate treatment failure if they appear under therapy [127].

Failure to achieve defined response landmarks may detect high-risk patients as early as 3–12 months after diagnosis [128–131]. These include cytogenetic and molecular responses determined by monitoring all patients. Measurement of the velocity or halving time of the early decline of BCR-ABL transcripts may increase sensitivity and specificity of response measurement [132, 133]. Patients who do not respond satisfactorily and are classified as high risk may need alternative approaches, such as early second-generation TKI, treatment intensification, or an early allo-SCT [127]. If the patients have a donor and have no medical contraindications, the risk of progression to BC has to be weighed against the risks of early transplantation and of chronic GVHD. With the current progress in donor selection and posttransplantation management, the risk of transplantation seems acceptable if compared with the risk of BC. If the patient is too old or has other medical contraindications that preclude allo-SCT or has no donor, investigational agents may be tried.

12.19 Conclusion

The strategy outlined in Fig. 12.4 offers an overview of the management of a patient with BC. The treatment goal is to induce a second chronic phase (CP2) characterized by a cytogenetic or molecular remission. The main form of treatment should be a TKI followed promptly by allo-SCT if possible. If TKIs are not sufficient, for myeloid BC, cytosine arabinoside and anthracyclines in combination with dasatinib or ponatinib should be considered; for lymphoid BC, hyperfractionated CVAD plus imatinib or dasatinib (or prednisone and vincristine) may be used. Management of de novo BC follows the same principles, except that imatinib should be tried first. Treatment decisions are adapted to the need and situation of each patient. Hematologic, cytogenetic, and molecular monitoring are mandatory (Table 12.1). Cytopenias may necessitate dose adaptive substitution therapy and treatment with G-CSF. In lymphoid BC, intrathecal neuroprophylaxis is indicated. Investigational approaches are recommended only after all other options have failed.

In view of the limited therapeutic options once BC has developed, the best management is prevention by rigorous and early reduction or elimination of BCR-ABL1. Regular molecular monitoring is required. Patients with high-risk features at diagnosis, unsatisfactory response to therapy (e.g., no major cytogenetic response or less than 90% BCR-ABL reduction by 3 months), or signs of progression under therapy,

such as clonal evolution and high-risk ACA, should receive more intensive therapies. With the availability of second- and third-generation BCR-ABL inhibitors and allo-SCT as needed, every attempt should be made to eliminate BCR-ABL1 as early as possible. More efficacious therapies and early treatment intensification in patients with high-risk features or unsatisfactory responses will likely further reduce progression to BC.

12.20 Summary

TKIs have moderately prolonged survival after BC. The best prognosis is observed in patients who achieve a second CP (CP2). Allo-SCT probably further improves prognosis of patients in CP2. The choice of TKI should be directed by the mutation profile of the patient. If ponatinib is given, risk and benefit should be carefully weighed in view of its vascular risks. Likely, BC may be prevented. A careful analysis of risk factors for progression is therefore needed. Treatment intensification in patients at risk of progression may improve prognosis, but controlled studies are not available. Much is known of genetic instability and clonal evolution as causes of BC, but confirmation of our understanding by successful intervention as proof of principle is lacking.

12.21 Practice Points

- Initial diagnostics of BC should include immunophenotyping and mutation profile to direct choice of therapies.
- Cytogenetics is of prognostic value (high-risk ACA) with a more intensive approach encouraged for high-risk karyotypes.
- Treatment options include intensive chemotherapy, TKI, and allo-SCT. Treatment may improve survival but, overall, outcome remains unsatisfactory.
- Prevention of BC seems possible. The risk of progression requires careful assessment and treatment intensification in patients at risk, although prospective trials supporting this concept are still lacking.
- A better pathophysiologic understanding of clonal evolution and progression to BC is expected to result in improved outcome.

Acknowledgments This work was supported by the ELN Foundation, Weinheim. RS acknowledges support by the Cancer Research and Treatment Fund, Inc., New York, New York.

The contribution of Johannes Hehlmann M.A. is acknowledged.

References

1. Minot GR, Buckman TE, Isaacs R. CML: age, incidence, duration and benefit derived from irradiation. JAMA. 1924;82:1489–94.
2. Morrow GW Jr, Pease GL, Stroebel CF, Bennett WA. Terminal phase of chronic myelogenous leukemia. Cancer. 1965;18(3):369–74.
3. Karanas A, Silver RT. Characteristics of the terminal phase of chronic granulocytic leukemia. Blood. 1968;32(3):445–59.
4. Canellos GP, Devita VT, Whang-Peng J, Carbone PP. Hematologic and cytogenetic remission of blastic transformation in chronic granulocytic leukemia. Blood. 1971;38(6):671–9.
5. Marks SM, Baltimore D, McCaffrey R. Terminal transferase as a predictor of initial responsiveness to vincristine and prednisone in blastic chronic myelogenous leukemia. N Engl J Med. 1978;298:812–4.
6. McCaffrey R, Harrison TA, Parkman R, Baltimore D. Terminal deoxynucleotidyl transferase activity in human leukemic cells and in normal human thymocytes. N Engl J Med. 1975;292(15):775–80.
7. Johansson B, Fioretos T, Mitelman F. Cytogenetic and molecular genetic evolution of chronic myeloid leukemia. Acta Haematol. 2002;107:76–94.
8. Soverini S, Hochhaus A, Nicolini FE, et al. BCR-ABL kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: recommendations from an expert panel on behalf of European LeukemiaNet. Blood. 2011;118:1208–15.
9. Saussele S, Lauseker M, Gratwohl A, et al. Allogeneic hematopoietic stem cell transplantation (Allo SCT) for chronic myeloid leukemia in the imatinib era: evaluation of its impact within a subgroup of the randomized German CML study IV. Blood. 2010;115:1880–5.
10. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.

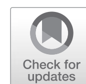
11. Baccarani M, Saglio G, Goldman J, et al. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. Blood. 2006;108(6):1809–20.
12. Radich JP, Deininger M, Abboud CN, et al. Chronic myeloid leukemia, version 1.2019, NCCN clinical practice guidelines in oncology. J Natl Compr Cancer Netw. 2018;16(9):1108–35.
13. Spiers AS. Metamorphosis of chronic granulocytic leukaemia: diagnosis, classification, and management. Br J Haematol. 1979;41:1–7.
14. Cortes JE, Talpaz M, Giles F, et al. Prognostic significance of cytogenetic clonal evolution in patients with chronic myelogenous leukemia on imatinib mesylate therapy. Blood. 2003;101:3794–800.
15. Schoch C, Haferlach T, Kern W, et al. Occurrence of additional chromosome aberrations in chronic myeloid leukemia patients treated with imatinib mesylate. Leukemia. 2003;17:461–3.
16. Haferlach C, Bacher U, Schnittger S, et al. Similar patterns of chromosome abnormalities in CML occur in addition to the Philadelphia chromosome with or without tyrosine kinase inhibitor treatment. Leukemia. 2010;24:638–40.
17. Alimena G, De Cuia MR, Diverio D, et al. The karyotype of blastic crisis. Cancer Genet Cytogenet. 1987;26:39–50.
18. Fabarius A, Leitner A, Hochhaus A, et al. Impact of additional cytogenetic aberrations at diagnosis on prognosis of CML: long-term observation of 1151 patients from the randomized CML Study IV. Blood. 2011;118:6760–8.
19. Fabarius A, Kalmanti L, Dietz CT, et al. Impact of unbalanced minor route versus major route karyotypes at diagnosis on prognosis of CML. Ann Hematol. 2015;94:2015–24.
20. Hehlmann R, Saußele S, Voskanyan A, Silver RT. Management of CML-blast crisis. Best Pract Res Clin Haematol. 2016;29(3):295–307.
21. Krulik M, Smadja N, Degramont A, et al. Sequential karyotype study on Ph-positive chronic myelocytic leukemia. Significance of additional chromosomal abnormalities during disease evolution. Cancer. 1987;60:974–9.
22. Verma D, Kantarjian H, Shan J, et al. Survival outcomes for clonal evolution in chronic myeloid leukemia patients on second generation tyrosine kinase inhibitor therapy. Cancer. 2010;116:2673–81.
23. Branford S, Wang P, Yeung DT, et al. Integrative genomic analysis reveals cancer-associated mutations at diagnosis of CML in patients with high-risk disease. Blood. 2018;132(9):948–61.
24. Grossmann V, Kohlmann A, Zenger M, et al. A deep-sequencing study of chronic myeloid leukemia patients in blast crisis (BC-CML) detects mutations in 76.9% of cases. Leukemia. 2011;25:557–60.
25. Mitelman F, Levan G, Nilsson PG, et al. Non-random karyotypic evolution in chronic myeloid leukemia. Int J Cancer. 1976;18:24–30.
26. Branford S, Kim DDH, Apperley J, et al. Laying the foundation for genomically-based risk assessment in chronic myeloid leukemia. Leukemia. 2019;33(8):1835–50.
27. Gong Z, Medeiros LJ, Cortes JE, et al. Cytogenetics-based risk prediction of blastic transformation of chronic myeloid leukemia in the era of TKI therapy. Blood Adv. 2017;1(26):2541–52.
28. Hehlmann R, Voskanyan A, Lauseker M, et al. High-risk additional chromosomal abnormalities at low blast counts herald death by CML. Leukemia 2020;34(8):2074–2086.
29. Wang W, Cortes JE, Tang G, et al. Risk stratification of chromosomal abnormalities in chronic myelogenous leukemia in the era of tyrosine kinase inhibitor therapy. Blood. 2016;127(22):2742–50.
30. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424–47.
31. Soverini S, Martinelli G, Rosti G, et al. ABL mutations in late chronic phase chronic myeloid leukemia patients with up-front cytogenetic resistance to imatinib are associated with a greater likelihood of progression to blast crisis and shorter survival: a study by the GIMEMA Working Party on Chronic Myeloid Leukemia. J Clin Oncol. 2005;23:4100–9.
32. Prokocimer M, Rotter V. Structure and function of p53 in normal cells and their aberrations in cancer cells: projection on the hematologic cell lineages. Blood. 1994;84:2391–411.
33. Sill H, Goldman JM, Cross NCP. Homozygous deletions of the p16 tumor-suppressor gene are associated with lymphoid transformation of chronic myeloid leukemia. Blood. 1995;85:2013–6.
34. Mullighan CG, Miller CB, Radtke I, et al. BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. Nature. 2008;453:110–5.
35. Roche-Lestienne C, Deluche L, Corm S, et al. RUNX1 DNA-binding mutations and RUNX1-PRDM16 cryptic fusions in BCR-ABL(+) leukemias are frequently associated with secondary trisomy 21 and may contribute to clonal evolution and imatinib resistance. Blood. 2008;111:3735–41.
36. Radich JP, Dai H, Mao M, et al. Gene expression changes associated with progression and response in chronic myeloid leukemia. Proc Natl Acad Sci U S A. 2006;103:2794–9.
37. Zheng C, Li L, Haak M, et al. Gene expression profiling of CD34+ cells identifies a molecular signature of chronic myeloid leukemia blast crisis. Leukemia. 2006;20:1028–34.
38. Jamieson CH, Ailles LE, Dylla SJ, et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. N Engl J Med. 2004;351:657–67.
39. Kok CH, Yeung DT, Lu L, et al. Gene expression signature that predicts early molecular response failure

in chronic-phase CML patients on frontline imatinib. Blood Adv. 2019;3(10):1610–21.
40. Oehler VG, Yeung KY, Choi YE, et al. The derivation of diagnostic markers of chronic myeloid leukemia progression from microarray data. Blood. 2009;114:3292–8.
41. Chereda B, Melo JV. Natural course and biology of CML. Ann Hematol. 2015;94(Suppl 2):107–21.
42. Perrotti D, Jamieson C, Goldman J, et al. Chronic myeloid leukemia: mechanisms of blastic transformation. J Clin Invest. 2010;120:2254–64.
43. Radich JP. The biology of CML blast crisis. ASH Education Program Book, 2007(1):384–91.
44. Koptyra M, Falinski R, Nowicki MO, et al. BCR/ABL kinase induces self-mutagenesis via reactive oxygen species to encode imatinib resistance. Blood. 2006;108:319–27.
45. Skorski T. Oncogenic tyrosine kinases and the DNA-damage response. Nat Rev Cancer. 2002;2:351–60.
46. Nowicki MO, Falinski R, Koptyra M, et al. BCR/ABL oncogenic kinase promotes unfaithful repair of the reactive oxygen species-dependent DNA double-strand breaks. Blood. 2004;104:3746–53.
47. Soverini S, Gnani A, Colarossi S, et al. Philadelphia-positive patients who already harbor imatinib-resistant Bcr-Abl kinase domain mutations have a higher likelihood of developing additional mutations associated with resistance to second- or third-line tyrosine kinase inhibitors. Blood. 2009;114:2168–71.
48. Sattler M, Verma S, Shrikhande G, et al. The BCR/ABL tyrosine kinase induces production of reactive oxygen species in hematopoietic cells. J Biol Chem. 2000;275:24273–8.
49. Giotopoulos G, Huntly BJ. CML: new tools to answer old questions. Blood. 2020;135(26):2327–8.
50. Ko TK, Javed A, Lee KL, et al. An integrative model of pathway convergence in genetically heterogeneous blast crisis chronic myeloid leukemia. Blood. 2020;135:2337–53.
51. Mohty M, Yong AS, Szydlo RM, Apperley JF, Melo JV. The polycomb group BMI1 gene is a molecular marker for predicting prognosis of chronic myeloid leukemia. Blood. 2007;110(1):380–3.
52. Marmont AM, Damasio EE. The treatment of terminal metamorphosis of chronic granulocytic leukaemia with corticosteroids and vincristine. Acta Haematol. 1973;50:1–8.
53. Iacoboni SJ, Plunkett W, Kantarjian HM, et al. High-dose cytosine arabinoside: treatment and cellular pharmacology of chronic myelogenous leukemia blast crisis. J Clin Oncol. 1986;4:1079–88.
54. Hehlmann R, Lauseker M, Saußele S, et al. Assessment of imatinib as first-line treatment of chronic myeloid leukemia: 10-year survival results of the randomized CML study IV and impact of non-CML determinants. Leukemia. 2017;31(11): 2398–2406.
55. Hochhaus A, Larson RA, Guilhot F, et al. Long-term outcomes of imatinib treatment for chronic myeloid leukemia. New England Journal of Medicine. 2017;376(10):917–927.
56. Söderlund S, Dahlén T, Sandin F, et al. Advanced phase chronic myeloid leukaemia (CML) in the tyrosine kinase inhibitor era–a report from the Swedish CML register. Eur J Haematol. 2017;98(1):57–66.
57. Soverini S, Bavaro L, Benedittis D, et al. Prospective assessment of NGS-detectable mutations in CML patients with nonoptimal response: the NEXT-in-CML study. Blood. 2020;135(8):534–41.
58. Hehlmann R. How I treat CML blast crisis. Blood. 2012;120:737–47.
59. Druker BJ, Sawyers CL, Kantarjian H, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med. 2001;344:1038–42.
60. Kantarjian HM, Cortes J, O'Brien S, et al. Imatinib mesylate (STI571) therapy for Philadelphia chromosome–positive chronic myelogenous leukemia in blast phase. Blood. 2002;99:3547–53.
61. Palandri F, Castagnetti F, Testoni N, et al. Chronic myeloid leukemia in blast crisis treated with imatinib 600 mg: outcome of the patients alive after a 6-year follow-up. Haematologica. 2008;93:1792–6.
62. Sawyers CL, Hochhaus A, Feldman E, et al. Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. Blood. 2002;99:3530–9.
63. Sureda A, Carrasco M, de Miguel M, et al. Imatinib mesylate as treatment for blastic transformation of Philadelphia chromosome positive chronic myelogenous leukemia. Haematologica. 2003;88:1213–20.
64. Copland M, Slade D, Byrne J. FLAG-IDA and ponatinib in patients with blast phase chronic myeloid leukaemia: results from the phase I/II UK Trials Acceleration Programme Matchpoint trial. Blood. 2019;134:497.
65. Milojkovic D, Ibrahim A, Reid A, et al. Efficacy of combining dasatinib and FLAG-IDA for patients with chronic myeloid leukemia in blastic transformation. Haematologica Hematol J. 2012;97:473–4.
66. Mayer RJ, Davis RB, Schiffer, et al. Intensive postremission chemotherapy in adults with acute myeloid leukemia. N Engl J Med. 1994;331(14):896–903.
67. Strati P, Kantarjian H, Thomas D, et al. HCVAD plus imatinib or dasatinib in lymphoid blastic phase chronic myeloid leukemia. Cancer. 2014;120:373–80.
68. Cortes J, Kim DW, Raffoux E, et al. Efficacy and safety of dasatinib in imatinib-resistant or -intolerant patients with chronic myeloid leukemia in blast phase. Leukemia. 2008;22:2176–83.
69. Saglio G, Hochhaus A, Goh YT, et al. Dasatinib in imatinib-resistant or imatinib-intolerant chronic myeloid leukemia in blast phase after 2 years of fol-

low-up in a phase 3 study: efficacy and tolerability of 140 milligrams once daily and 70 milligrams twice daily. Cancer. 2010;116:3852–61.
70. Talpaz M, Shah NP, Kantarjian H, et al. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. N Engl J Med. 2006;354:2531–41.
71. Porkka K, Koskenvesa P, Lundan T, et al. Dasatinib crosses the blood-brain barrier and is an efficient therapy for central nervous system Philadelphia chromosome-positive leukemia. Blood. 2008;112:1005–12.
72. Giles FJ, Kantarjian HM, le Coutre PD, et al. Nilotinib is effective in imatinib-resistant or -intolerant patients with chronic myeloid leukemia in blastic phase. Leukemia. 2012;26:959–62.
73. Kantarjian H, Giles F, Wunderle L, et al. Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. N Engl J Med. 2006;354:2542–51.
74. Fruehauf S, Topaly J, Buss EC, et al. Imatinib combined with mitoxantrone/etoposide and cytarabine is an effective induction therapy for patients with chronic myeloid leukemia in myeloid blast crisis. Cancer. 2007;109:1543–9.
75. Oki Y, Kantarjian HM, Gharibyan V, et al. Phase II study of low-dose decitabine in combination with imatinib mesylate in patients with accelerated or myeloid blastic phase of chronic myelogenous leukemia. Cancer. 2007;109:899–906.
76. Quintas-Cardama A, Kantarjian H, Garcia-Manero G, et al. A pilot study of imatinib, low-dose cytarabine and idarubicin for patients with chronic myeloid leukemia in myeloid blast phase. Leuk Lymphoma. 2007;48:283–9.
77. Cortes J, Jabbour E, Daley GQ, et al. Phase 1 study of lonafarnib (SCH 66336) and imatinib mesylate in patients with chronic myeloid leukemia who have failed prior single-agent therapy with imatinib. Cancer. 2007;110:1295–302.
78. Fang B, Li N, Song Y, et al. Standard-dose imatinib plus low-dose homoharringtonine and granulocyte colony-stimulating factor is an effective induction therapy for patients with chronic myeloid leukemia in myeloid blast crisis who have failed prior single-agent therapy with imatinib. Ann Hematol. 2010;89:1099–105.
79. Rea D, Legros L, Raffoux E, et al. High-dose imatinib mesylate combined with vincristine and dexamethasone (DIV regimen) as induction therapy in patients with resistant Philadelphia-positive acute lymphoblastic leukemia and lymphoid blast crisis of chronic myeloid leukemia. Leukemia. 2006;20:400–3.
80. Deau B, Nicolini FE, Guilhot J, et al. The addition of daunorubicin to imatinib mesylate in combination with cytarabine improves the response rate and the survival of patients with myeloid blast crisis chronic myelogenous leukemia (AFR01 study). Leuk Res. 2011;35:777–82.
81. Ghez D, Micol JB, Pasquier F, et al. Clinical efficacy of second generation tyrosine kinase inhibitor and 5-azacytidine combination in chronic myelogenous leukaemia in myeloid blast crisis. Eur J Cancer (Oxford, England 1990). 2013;49:3666–70.
82. Doan V, Wang A, Prescott H. Bosutinib for the treatment of chronic myeloid leukemia. Am J Health Syst Pharm. 2015;72:439–47.
83. Cortes JE, Kim DW, Pinilla-Ibarz J, et al. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. N Engl J Med. 2013;369:1783–96.
84. Valent P, Hadzijusufovic E, Schernthaner GH, et al. Vascular safety issues in CML patients treated with BCR/ABL1 kinase inhibitors. Blood. 2015;125:901–6.
85. Rea D, Mirault T, Raffoux E, et al. Usefulness of the 2012 European CVD risk assessment model to identify patients at high risk of cardiovascular events during nilotinib therapy in chronic myeloid leukemia. Leukemia. 2015;29(5):1206–9.
86. Jain P, Kantarjian HM, Ghorab A, et al. Prognostic factors and survival outcomes in patients with chronic myeloid leukemia in blast phase in the tyrosine kinase inhibitor era: cohort study of 477 patients. Cancer. 2017;123(22):4391–402.
87. Hochhaus A, Baccarani M, Silver RT, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020:1–19.
88. Gratwohl A, Pfirrmann M, Zander A, et al. Long-term outcome of patients with newly diagnosed chronic myeloid leukemia: a randomized comparison of stem cell transplantation with drug treatment. Leukemia. 2016;30(3):562–9.
89. Gratwohl A, Brand R, Apperley J, et al. Allogeneic hematopoietic stem cell transplantation for chronic myeloid leukemia in Europe 2006: transplant activity, long-term data and current results. An analysis by the chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). Haematologica. 2006;91(4):513–21.
90. Saussele S, Lauseker M, Muller MC, et al. Allogeneic hematopoietic stem cell transplantation (HSCT) in the Imatinib-era: update on the survival outcome following allogeneic HSCT after imatinib failure; results of the German CML Study IV. Blood. 2014;124:abstract no. 2567.
91. Jiang H, Xu LP, Liu DH, et al. Allogeneic hematopoietic SCT in combination with tyrosine kinase inhibitor treatment compared with TKI treatment alone in CML blast crisis. Bone Marrow Transplant. 2014;49(9):1146–54.
92. Oyekunle A, Zander AR, Binder M, et al. Outcome of allogeneic SCT in patients with chronic myeloid leukemia in the era of tyrosine kinase inhibitor therapy. Ann Hematol. 2013;92:487–96.
93. Gratwohl A, Heim D. Current role of stem cell transplantation in chronic myeloid leukaemia. Best Pract Res Clin Haematol. 2009;22:431–43.
94. Jabbour E, Kantarjian H, O'Brien S, et al. Sudden blastic transformation in patients with chronic myeloid leukemia treated with imatinib mesylate. Blood. 2006;107:480–2.

95. Gratwohl A, Baldomero H, Passweg J. The role of hematopoietic stem cell transplantation in chronic myeloid leukemia. Ann Hematol. 2015;94(Suppl 2):177–86.
96. Neviani P, Santhanam R, Oaks JJ, et al. FTY720, a new alternative for treating blast crisis chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphocytic leukemia. J Clin Invest. 2007;117:2408–21.
97. Agarwal A, MacKenzie R, Oddo J, et al. A novel SET antagonist (OP449) is cytotoxic to CML cells, including the highly-resistant BCR-ABLT315I mutant, and demonstrates enhanced efficacy in combination with ABL tyrosine kinase inhibitors. Blood. 2011;118:1603. Abstract 3757
98. Lucas CM, Harris RJ, Giannoudis A, et al. Cancerous inhibitor of PP2A (CIP2A) at diagnosis of chronic myeloid leukemia is a critical determinant of disease progression. Blood. 2011;117:6660–8.
99. Lucas CM, Milani M, Butterworth M, et al. High CIP2A levels correlate with an antiapoptotic phenotype that can be overcome by targeting BCL-X L in chronic myeloid leukemia. Leukemia. 2016;30(6):1273–81.
100. Lai D, Chen M, Su J, et al. PP2A inhibition sensitizes cancer stem cells to ABL tyrosine kinase inhibitors in BCR-ABL+ human leukemia. Sci Transl Med. 2018;10(427):eaan8735.
101. Hurtz C, Hatzi K, Cerchietti L, et al. BCL6-mediated repression of p53 is critical for leukemia stem cell survival in chronic myeloid leukemia. J Exp Med. 2011;208:2163–74.
102. Zhang H, Li H, Xi HS, et al. HIF1α is required for survival maintenance of chronic myeloid leukemia stem cells. Blood. 2012;119:2595–607.
103. Shah NP, Cortes JE, Martinelli G, et al. Dasatinib plus smoothened (SMO) inhibitor BMS-833923 in chronic myeloid Leukemia (CML) with resistance or suboptimal response to a prior tyrosine kinase inhibitor (TKI): phase I study CA180323. Blood (ASH Annu Meet Abstr). 2014;124:4539.
104. Court Recart AC, Sadarangani A, Goff D, et al. Combination targeted therapy to impair self-renewal capacity of human blast crisis leukemia stem cells. Blood. 2011;118:737. Abstract 1693
105. Gallipoli P, Cook A, Rhodes S, et al. JAK2/STAT5 inhibition by nilotinib with ruxolitinib contributes to the elimination of CML CD34+ cells in vitro and in vivo. Blood. 2014;124:1492–501.
106. Mak DH, Wang RY, Schober WD, et al. Activation of apoptosis signaling eliminates CD34+ progenitor cells in blast crisis CML independent of response to tyrosine kinase inhibitors. Leukemia. 2012;26:788–94.
107. Pellicano F, Simara P, Sinclair A, et al. The MEK inhibitor PD184352 enhances BMS-214662-induced apoptosis in CD34+ CML stem/progenitor cells. Leukemia. 2011;25:1159–67.
108. Pemovska T, Johnson E, Kontro M, et al. Axitinib effectively inhibits BCR-ABL1(T315I) with a distinct binding conformation. Nature. 2015;519:102–5.
109. Pietarinen PO, Pemovska T, Kontro M, et al. Novel drug candidates for blast phase chronic myeloid leukemia from high-throughput drug sensitivity and resistance testing. Blood Cancer J. 2015;5:e309.
110. Wang W, Lv FF, Du Y, et al. The effect of nilotinib plus arsenic trioxide on the proliferation and differentiation of primary leukemic cells from patients with chronic myeloid leukemia in blast crisis. Cancer Cell Int. 2015;15:10.
111. Neviani P, Santhanam R, Trotta R, et al. The tumor suppressor PP2A is functionally inactivated in blast crisis CML through the inhibitory activity of the BCR/ABL-regulated SET protein. Cancer Cell. 2005;8:355–68.
112. Zhao C, Chen A, Jamieson CH, et al. Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. Nature. 2009;458:776–U117.
113. Xie J, Zhang X, Fang BZ, et al. Combination of rapamycin and imatinib in treating refractory chronic myeloid leukemia myeloid blast crisis: a case report (025B3). Chin Med Sci J. 2013;28:127–8.
114. Postow MA, Chesney J, Pavlick AC, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. N Engl J Med. 2015;372:2006–17.
115. Wang H, Kaur G, Sankin AI, Chen F, Guan F, Zang X. Immune checkpoint blockade and CAR-T cell therapy in hematologic malignancies. J Hematol Oncol. 2019;12(1):59.
116. Pratap S, Zhao ZJ. Finding new lanes: chimeric antigen receptor (CAR) T-cells for myeloid leukemia. Cancer Rep. 2020;3(2):e1222.
117. Hughes TP, Mauro MJ, Cortes JE, et al. Asciminib in chronic myeloid Leukemia after ABL kinase inhibitor failure. N Engl J Med. 2019;381(24):2315–26.
118. Maiti A, Franquiz MJ, Ravandi F, et al. Venetoclax and BCR-ABL tyrosine kinase inhibitor combinations: outcome in patients with Philadelphia chromosome-positive advanced myeloid leukemias. Acta Haematol. 2020:1–7.
119. Kaeda J, O'Shea D, Szydlo RM, et al. Serial measurement of BCR-ABL transcripts in the peripheral blood after allogeneic stem cell transplantation for chronic myeloid leukemia: an attempt to define patients who may not require further therapy. Blood. 2006;107:4171–6.
120. Hehlmann R, Müller MC, Lauseker M, et al. Deep molecular response is reached by the majority of patients treated with imatinib, predicts survival, and is achieved more quickly by optimized high-dose imatinib: results from the randomized CML-study IV. J Clin Oncol. 2014;32:415–23.
121. Mahon FX, Rea D, Guilhot J, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular

remission for at least 2 years: the prospective, multicentre stop Imatinib (STIM) trial. Lancet Oncol. 2010;11:1029–35.
122. Hasford J, Pfirrmann M, Hehlmann R, et al. A new prognostic score for survival of patients with chronic myeloid leukemia treated with interferon alfa. Writing Committee for the Collaborative CML Prognostic Factors Project Group. J Natl Cancer Inst. 1998;90:850–8.
123. Hasford J, Baccarani M, Hoffmann V, et al. Predicting complete cytogenetic response and subsequent progression-free survival in 2060 patients with CML on imatinib treatment: the EUTOS score. Blood. 2011;118:686–92.
124. Pfirrmann M, Baccarani M, Saußele S, et al. Prognosis of long-term survival considering disease-specific death in patients with chronic myeloid leukemia. Leukemia. 2016;30(1):48–56.
125. Sokal JE, Cox EB, Baccarani M, et al. Prognostic discrimination in “good-risk” chronic granulocytic leukemia. Blood. 1984;63:789–99.
126. Verma D, Kantarjian HM, Jones D, et al. Chronic myeloid leukemia (CML) with P190BCR-ABL: analysis of characteristics, outcomes, and prognostic significance. Blood. 2009;114:2232–5.
127. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood. 2013;122:872–84.
128. Hanfstein B, Müller MC, Hehlmann R, et al. Early molecular and cytogenetic response is predictive for long-term progression-free and overall survival in chronic myeloid leukemia (CML). Leukemia. 2012;26:2096–102.
129. Hehlmann R, Lauseker M, Jung-Munkwitz S, et al. Tolerability-adapted imatinib 800 mg/d versus 400 mg/d versus 400 mg/d plus interferon-a in newly diagnosed chronic myeloid leukemia. J Clin Oncol. 2011;29(12):1634–42.
130. Jabbour E, Kantarjian H, O’Brien S, et al. The achievement of an early complete cytogenetic response is a major determinant for outcome in patients with early chronic phase chronic myeloid leukemia treated with tyrosine kinase inhibitors. Blood. 2011;118:4541–6.
131. Marin D, Ibrahim AR, Lucas C, et al. Assessment of BCR-ABL1 transcript levels at 3 months is the only requirement for predicting outcome for patients with chronic myeloid leukemia treated with tyrosine kinase inhibitors. J Clin Oncol. 2012;30:232–8.
132. Branford S, Yeung DT, Parker WT, et al. Prognosis for patients with CML and >10% BCR-ABL1 after 3 months of imatinib depends on the rate of BCR-ABL1 decline. Blood. 2014;124:511–8.
133. Hanfstein B, Shlyakhto V, Lauseker M, et al. Velocity of early BCR-ABL transcript elimination as an optimized predictor of outcome in chronic myeloid leukemia (CML) patients in chronic phase on treatment with imatinib. Leukemia. 2014;28:1988–92.



13 The Interferon-Alpha Revival in CML

Moshe Talpaz, Jessica Mercer, and Rüdiger Hehlmann

Interferon-alpha (IFNα) demonstrated activity against a variety of solid tumors and myeloid malignancies in the 1970s and 1980s. The first approved antitumor application for IFNα was hairy cell leukemia in 1986. Since then, IFNα has been used to treat a number of malignancies, although enthusiasm has waned due to its significant side effect profile. In chronic myeloid leukemia (CML), IFNα was eventually replaced by targeted therapy with the BCR-ABL tyrosine kinase inhibitors (TKIs). However, TKI therapy is seldom curative, and IFNα has a unique mechanism of action, as discussed below, which may complement the action of TKIs. Furthermore, the newer pegylated forms of IFNα are easier to administer and better tolerated than previous forms. These features make IFNα a promising candidate for combination therapy in CML.

M. Talpaz (✉) · J. Mercer
Department of Internal Medicine, Division of Hematology Oncology, University of Michigan Rogel Cancer Center, Ann Arbor, MI, USA
e-mail: mtalpaz@med.umich.edu

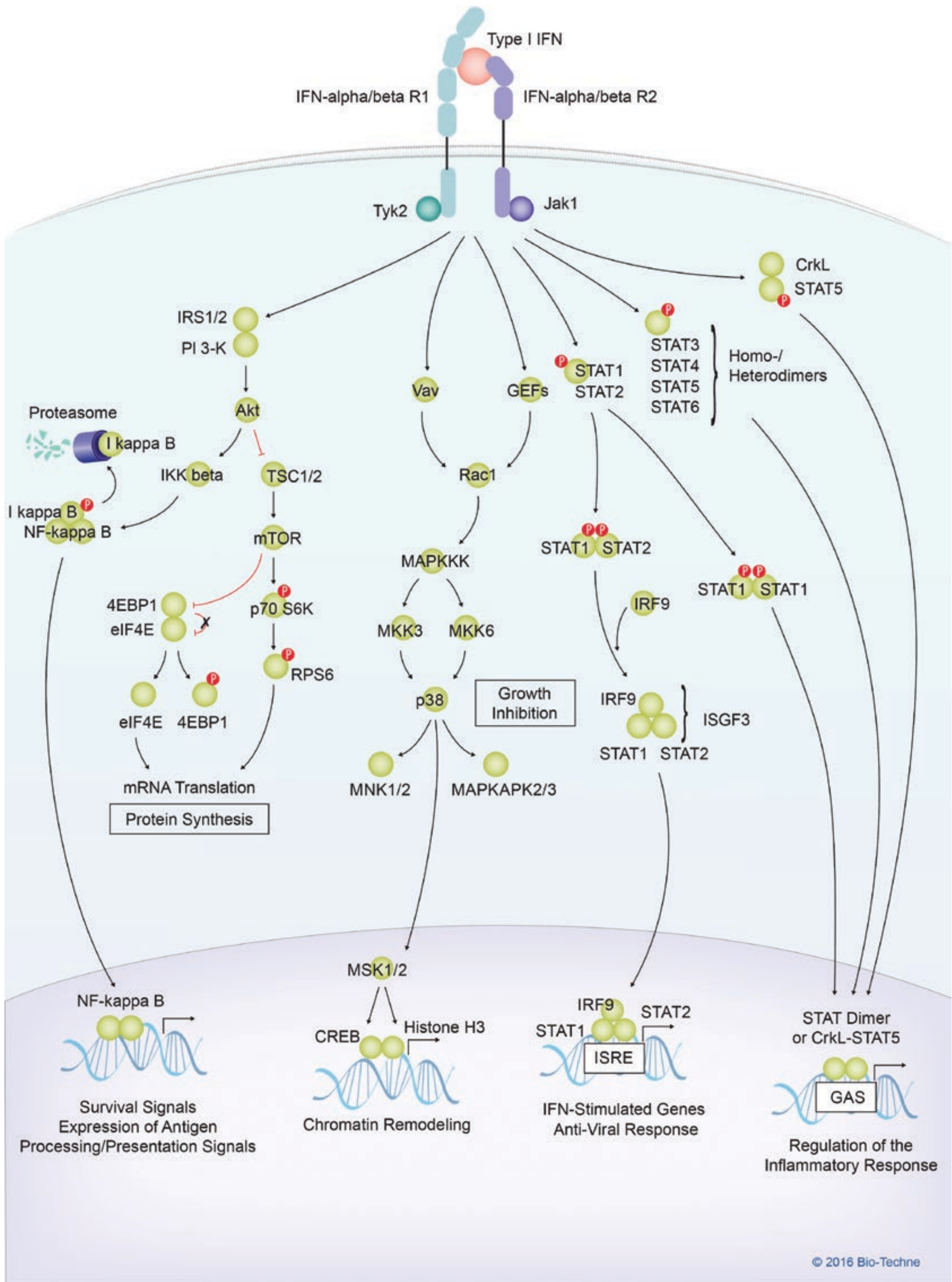
R. Hehlmann
ELN Foundation, Weinheim, Germany

Medizinische Fakultät Mannheim, Universität Heidelberg, III.Medizinische Klink, Mannheim, Germany

13.1 Mechanisms of IFNα Antitumor Effects

Interferons (IFNs) are α-helical glycoproteins secreted by almost every cell type [1]. They are classified as type I (α,β), type II (γ), or type III (λ1, λ2, λ3). Type I IFNs bind to the IFNAR complex, which consists of transmembrane subunits IFNα/β R1 and IFNα/β R2 (Fig. 13.1). These subunits signal through two Janus kinases (JAK), Tyk2 and Jak1 [2]. Binding of IFNα to IFNAR stimulates dimerization of the receptor subunits and autophosphorylation of JAKs. The activated JAKs phosphorylate STATs, which form homo- or heterodimers that translocate to the nucleus and activate transcription of IFN-stimulated genes (ISGs). STAT1-STAT2 heterodimers associate with the IFN regulatory factor 9 (IRF9, p48) and activate transcription of antiproliferative, antiviral and proapoptotic genes, characteristic of IFNα signaling [3]. Table 13.1 lists ISGs implicated in the anticancer effects of type I IFNs. IFNα can also activate non-STAT pathways including CrkL, MAP kinases, VAV, and PI3-kinases (Fig. 13.1) [3]. The outcome of IFNAR signaling depends on IFN binding affinity to receptors, the receptor composition, and the accessory molecules expressed by different cell types [4]. As reviewed in [5], multiple mechanisms can downregulate IFNAR1 in normal and tumor tissue, suppressing sensitivity to IFNα effects. Restoring sensitivity to IFNα may be an important obstacle to overcome before IFNα can be reinstated in standard therapy regimens.

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
R. Hehlmann (ed.), *Chronic Myeloid Leukemia*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-71913-5_13



Type I IFN
IFN-alpha/beta R1
IFN-alpha/beta R2
Tyk2
Jak1
CrkL
STAT5
IRS1/2
PI 3-K
STAT3
STAT4
STAT5
STAT6
Homo-/
Heterodimers
STAT1
STAT2
Vav
GEFs
Akt
Proteasome
I kappa B
IKK beta
TSC1/2
Rac1
I kappa B
NF-kappa B
mTOR
MAPKKK
STAT1
STAT2
STAT1
STAT1
4EBP1
eIF4E
p70 S6K
MKK3
MKK6
IRF9
RPS6
Growth
Inhibition
p38
IRF9
ISGF3
eIF4E
4EBP1
STAT1
STAT2
MNK1/2
MAPKAPK2/3
mRNA Translation
Protein Synthesis
MSK1/2
NF-kappa B
IRF9
STAT2
STAT1
ISRE
CREB
Histone H3
STAT Dimer
or CrkL-STAT5
GAS
Survival Signals
Expression of Antigen
Processing/Presentation Signals
Chromatin Remodeling
IFN-Stimulated Genes
Anti-Viral Response
Regulation of the
Inflammatory Response
© 2016 Bio-Techne

Microarray analyses have shown that IFNs can induce expression of over 300 different genes [6, 7]. These genes encode apoptotic, antiviral, immunomodulatory, host defense, cell cycle, and transcription factor proteins [7]. The diversity in this gene set underlies the pleiotropic and complex effects of IFNα, which are probably not attributable to any one gene product [8]. The antitumor effects of IFNα may involve direct apoptosis of tumor cells or indirect effects on immune effector cells or the vasculature [8]. The net effect of IFNα treatment depends on cell type, tumor environment, acquired genetic defects, sensitivity to different IFN isoforms, and other variables [9]. The biological processes that contribute most prominently to the antitumor effects of IFNα are addressed below and summarized in Fig. 13.2 [4].

13.2 IFNα Induces Apoptosis

IFNα can induce or suppress apoptosis, depending on cell type [10]. IFNα initiates the apoptotic signal through the JAK/STAT pathway [1]. Although the apoptotic mediators can vary, the mechanism always involves FADD/caspase-8 signaling, which leads to activation of the caspase cascade, release of cytochrome c, and disruption of the mitochondrial potential [8]. In malignant cells, IFNα induces apoptosis independently of cell cycle arrest, p53, or expression of Bcl2 members. Apoptosis occurs >48 h after IFNα treatment, indicating that intermediary genes are probably transcribed first [8]. More than 15 ISGs have been identified with proapoptotic function, including TRAIL/Apo2L and Fas/CD95 [7].

13.3 IFNα Inhibits Cell Growth

IFNα can interfere with all phases of the cell cycle, most commonly arresting cells in G1 [11]. The mechanism involves regulation of the serine/threonine kinases, cyclins, and cyclin-dependent kinases (cdks) that form complexes and control the passage of the cell through the cell cycle. Activated cyclin-cdk complexes normally phosphorylate retinoblastoma protein (pRb), an important cell cycle regulator. Hyperphosphorylated pRb releases bound transcription factor E2F, which then activates genes required for DNA replication. IFNα treatment downregulates cyclin D3, cyclin E, cyclin A, and cdc25A, leading to decreased phosphorylation of pRb [8]. This in turn prevents E2F from inducing entry into S phase. IFNα also causes G1 arrest by downregulating c-myc and inducing cdk inhibitors p21 and p27 via RIG-G [10]. C-myc is a transcription factor that activates cyclin-cdk complexes important for S phase entry and stimulates transcription of genes that control S phase progression. Its decreased expression by IFNα, in addition to the other IFN-mediated effects on cell cycle, can lead to cytostasis, increased cell size, and apoptosis [8].

13.4 IFNα Suppresses Angiogenesis

IFNα treatment is effective in angioproliferative diseases such as Kaposi's sarcoma and hemangiomas [12]. Its antiangiogenic activity derives from downregulation of proangiogenic factors including vascular endothelial growth factor (VEGF) [13], basic fibroblast growth factor, IL-8 [14], and matrix

Fig. 13.1 Major signaling pathways activated by type 1 IFNs and the genes and functions they regulate. Receptor engagement activates Tyk2 protein tyrosine kinase and Jak1 protein tyrosine kinase associated with IFNα/ß R1 and R2 receptors. The JAK-STAT signal pathway activates transcription of a variety of interferon-stimulated genes (ISGs), depending on the composition of the STAT homo- or heterodimer. In the CRKL pathway, activated CRKL forms a complex with STAT5, which translocates to the nucleus and binds specific GAS elements, stimulating transcription of specific ISGs. Activation of PI3K and AKT activates the NF-κB cascade via IKKβ or PKCθ, leading to enhanced expression of several proteins and prosurvival signals. The AKT pathway also leads to activation of mTOR, which regulates translation of mRNAs important in cell survival pathways [158]. Phosphorylation of Vav leads to the activation of several MAPKs, which regulate transcription and translation of ISGs involved in the antitumor effects of IFNα, including chromatin remodeling [159]. Figure and legend adapted from Ref. [160]

Table 13.1 ISGs involved in the anticancer effects of IFNs

Gene	Protein function	Mechanism of action	References
ADAR1	Adenosine deaminase for dsRNA	RNA editing, altered translation	[161]
CCL2, CCL3, CCL5, CXCL9, CXCL10, CXCL11	Chemoattractants	Recruits lymphocytes and monocytes	[162]
GBP1	GTPase	Angiogenesis inhibitor	[163]
IFI16	DNA binding, transcriptional regulation, and protein-protein interactions	Angiogenesis inhibitor	[164]
IFI27	Lamin binding	Apoptosis	[165, 166]
IFIT1	Binds EIF3	Blocks protein synthesis	[167, 168]
IFIT2	Inhibits expression of specific viral mRNAs	Inhibited motility of transformed cells	[169]
IL15	Cytokine	Primes natural killer cells	[170]
IRF7, MDA5, RIG-I, STAT1	Signaling to IFNα/β genes or to ISGs	Induction of type I IFNs	[7, 171, 172]
ISG15	ISGylation	Cytokine-like, protein modification	[173–175]
MHC class I genes	MHC class I components	Antigen-specific T-cell immunity	[176, 177]
MX1	GTPase	Inhibited motility of transformed cells	[178]
OAS, RNASEL	RNA cleavage	Induces IFNα/β expression and apoptosis	[179–181]
PKR	EIF2α phosphorylation	Blocks protein synthesis, transcriptional signaling	[182, 183]
PLSCR1	Phospholipid migration, DNA binding	Signals macrophages to engulf dying tumor cells	[184, 185]
PML	Transcription factor, tumor suppressor	Antitumor	[186]
PSMB8, *PSMB9, PSMB10*	Proteasome subunits	Processing antigenic peptides for loading on MHC class I molecules	[187, 188]
SECTM1	Type 1 transmembrane glycoprotein	Co-stimulatory ligand for T cells	[189, 190]
SLFN5	Hematopoietic cell differentiation	Inhibited invasiveness of transformed cells	[191]
TAP1, TAP2	ATP-binding cassette transporter	Loading antigenic peptides on MHC class I molecules	[192, 187]
TRAIL/APO2L	Ligand of death receptor	Apoptosis	[193, 194]
XAF1	Blocks inhibitor of apoptosis (XIAP)	Apoptosis	[195]

ADAR1 adenosine deaminase, RNA-specific, *CCL2* chemokine (C-C motif) ligand 2 (also known as *MCP-1*), *CCL3* chemokine (C-C motif) ligand 3 (also known as *MIP-1α*), *CCL5*, chemokine (C-C motif) ligand 5 (also known as *RANTES*), *CXCL9* chemokine (C-X-C motif) ligand 9 (also known as *MIG*), *CXCL10* chemokine (C-X-C motif) ligand 10 (also known as *IP-10*), *CXCL11* chemokine (C-X-C motif) ligand 11 (also known as *I-TAC*), dsRNA double-stranded RNA, *EIF2α/3* eukaryotic initiation factor 2α/3, *GBP1* guanylate binding protein 1, *IFI16* interferon gamma-inducible protein 16, *IFI27* interferon alpha-inducible protein 27 (also known as *ISG12*), *IFIT1* interferon-induced protein with tetratricopeptide repeats 1 (also known as *ISG56* and *p56*), *IFIT2* interferon-induced protein with tetratricopeptide repeats 2 (also known as *ISG54* and *p54*), *IL15* interleukin 15, *IRF7* interferon regulatory factor 7, *ISGs* interferon-stimulated genes, *MDA5* melanoma differentiation-associated protein 5 (also known as *IFIH1*), *MHC* major histocom-

(continued)

Table 13.1 (continued)

patibility complex, *MX1* myxovirus (influenza virus) resistance 1, *OAS* 2′-5′-oligoadenylate synthetase, *PKR* protein kinase R, *PLSCR1* phospholipid scramblase 1, *PML* promyelocytic leukemia, *PSMB8* proteasome subunit beta 8 (also known as *LMP7*), *PSMB9* proteasome subunit beta 9 (also known as *LMP2*), *PSMB10* proteasome subunit beta 10 (also known as *LMP10*), *RIG-I* retinoic acid-inducible gene I (also known as *DDX58*), *RNASEL* ribonuclease L, *SECTM1* secreted and transmembrane 1, *SLFN5* Schlafen 5, *STAT1* signal transducer and activator of transcription 1, *TAP1* transporter 1, ATP-binding cassette, subfamily B (MDR/TAP), *TAP2* transporter 2, ATP-binding cassette, subfamily B (MDR/TAP), *TRAIL/APO2L* tumor necrosis factor-related apoptosis-inducing ligand (also known as *TNFSF10*), *XAF1* X-linked inhibitor of apoptosis-associated factor 1, XIAP X-linked inhibitor of apoptosis protein (also known as *BIRC4*)
Table adapted from ref. [196]

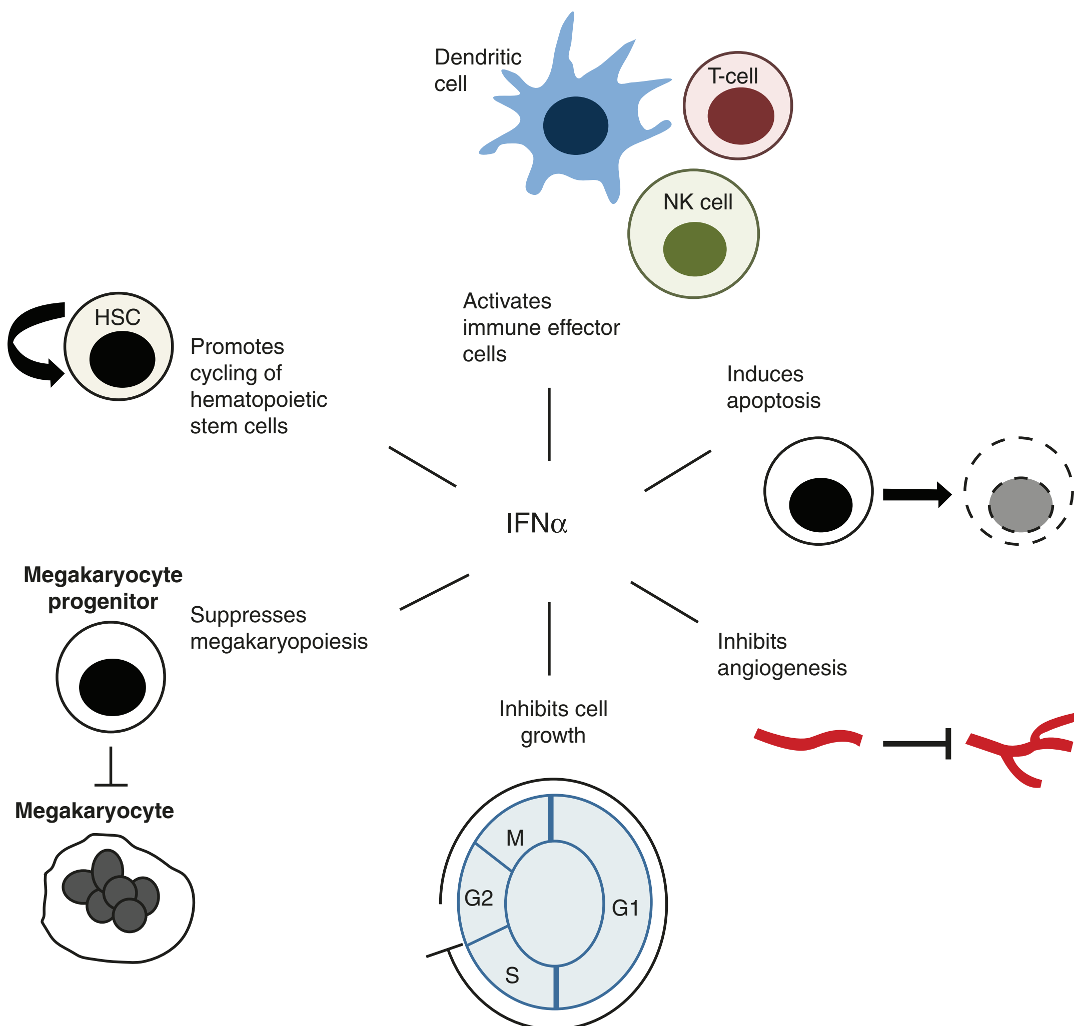


Fig. 13.2 Mechanisms of IFNα antitumor effects. IFNα affects several biological processes that contribute to its antitumor effects in hematological malignancies

metalloproteinase 9 expression [15]. CML patients treated with a combination of IFNα and imatinib exhibited lower levels of VEGF levels compared with imatinib alone or imatinib plus cytarabine treatment [16]. IFNα treatment may also suppress angiogenesis through direct effects on endothelial cells (ECs). Indeed, IFNα treatment in vitro directly impaired the proliferation and migration of ECs and upregulated transcription of angiostatic chemokines CXCL10 and CXCL11 in these cells [17].

13.5 IFNα Activates Immune Effector Cells

IFNα elicits an antitumor immune response that links innate and adaptive immunity [18–20]. Treatment with type I IFNs induces proliferation, expansion, and long-term survival of cytotoxic T cells (CTLs) in response to specific antigens in mice [21]. These effects are likely directed by a specific transcriptional program activated in naïve human CD8+ T cells [22]. Type I IFNs also enhance NK cell cytotoxic activity in vitro [23] and control the antitumor responses mediated by NK cells in experimental tumor models [24]. On the other hand, a study in CML patients found that adding IFNα to nilotinib decreased the maturation status of NK cells, without altering effector function, and increased specific early B cell subsets [25]. In addition, immunological profiling of CML patients revealed that IFNα combined with imatinib led to an enhanced immunosuppressive state, primarily through increase of myeloid-derived suppressor cells, compared with patients on imatinib alone [26]. The discrepancy with preclinical findings may be due to the long-term exposure to IFNα in patients and the interactive effects between IFNα and TKI therapy. Dendritic cells (DCs) are another important mediator of IFN's immunomodulatory effects. In vivo studies have shown that IFNα causes CML mononuclear cells to differentiate into DCs; these DCs are highly active, with the ability to take up apoptotic bodies and promote $CD8^+$ T-cell cross-priming [27]. Selective deletion of IFNAR1 in DCs abrogates tumor rejection in mice and impairs antigen cross-presentation to $CD8^+$ T cells [28]. Clearly, the IFN-DC interaction is essential for effective tumor immunity; however, this activity likely underlies the autoimmune and inflammatory symptoms that accompany IFNα therapy [2]. New strategies are needed to redirect the IFN-mediated immune attack from normal tissues to tumor cells [27].

13.6 IFNα Suppresses Hematopoiesis

IFNα directly suppresses colony formation of normal hematopoietic progenitor cells (HPCs) through IFNAR [29]. The mechanism involves p38 signaling, as inhibition of p38 reverses the antiproliferative response of HPCs to IFNα [30]. The Schlafen (SLFN) genes, such as SLFN2, are possible downstream effectors of this pathway [31]. Another pathway implicated in IFNα-mediated suppression is Mek/Erk MAPK [32]. By activating MAPK-interacting kinase 1 signaling, IFNα stimulates translation of ISGs that lead to HPC suppression [32]. A third antiproliferation signal involves IFNα activation of Crk family members, CrkL and CrkII. These proteins activate the GTPase Rap1, which is known to antagonize the Ras pathway, leading to growth inhibition in HPCs [10]. IFNα may also indirectly suppress HPCs by regulating secretion of growth factors from the bone marrow (BM) microenvironment. In support of this, IFNα treatment decreased levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-1β (IL-1β), and IL-11—known hematopoietic growth factors—in stimulated stromal cultures [33, 34].

Unlike its antiproliferative effects in vitro, IFNα treatment in mice induces transient proliferation of hematopoietic stem cells (HSCs), which is abolished in mice with *Ifnar−/−* HSCs (Fig. 13.3) [35, 36]. Mouse HSCs are typically dormant, but become sensitive to the antiproliferative agent 5-FU upon IFNα treatment [35, 36]. These are cell intrinsic effects as an *Ifnar−/−* stromal environment did not reverse the phenomenon [36]. Further, HSCs lacking STAT1 or the stem cell antigen-1 (Sca-1) do not proliferate in response to IFNα, implicating potential downstream effectors in the pathway [36]. A subsequent study showed that this proliferation is transient, driven by decreased expression of genes supporting quiescence, including *Foxo3a*, *p53*, *p27*, *p57*, and components of the Notch and TGFβ pathways [37]. Importantly, the proliferating HSCs become more susceptible to apoptosis through IFNα-mediated downregulation of pro-survival gene *Mcl1* [37]. By contrast, chronic IFNα exposure leaves a quiescent pool of HSCs that are protected from IFNα-induced apoptosis (Fig. 13.3) [37]. Long-term activation of IFN signaling was also shown to compromise HSC

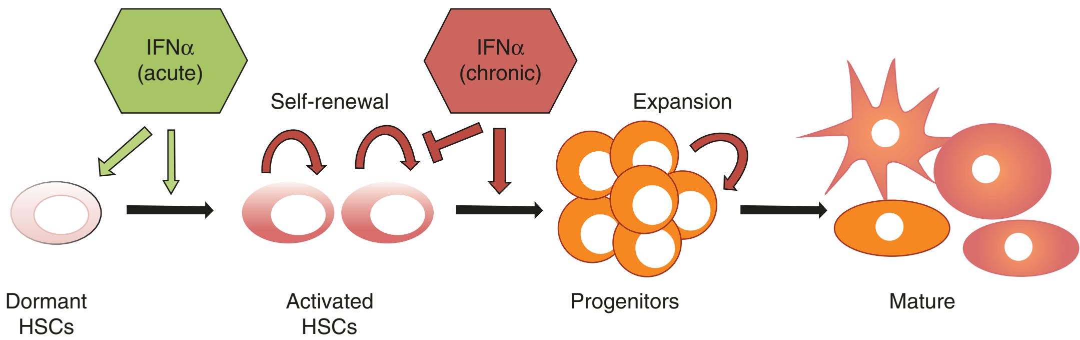


Fig. 13.3 Model showing the activating effects of short-term (acute) IFNα stimulation on dormant/quiescent HSCs and the inhibitory effects of chronic IFNα treatment on HSC self-renewal. Figure and legend adapted from Ref. [36]

function, enabling *Ifnar*$^{-/-}$ cells to outcompete wild-type cells in competitive repopulation assays [36]. Collectively, these findings have clinical significance because, unlike IFNα, imatinib treatment does not kill primitive CML cells [38, 39]. Further, CML early progenitor/stem cells persist in patients who respond to imatinib, and these cells are thought to be responsible for reinitiating disease in relapse cases [40]. By acting on CML stem cells, IFNα may increase the durability of responses with imatinib therapy. Indeed, results from some of the clinical trials testing IFNα plus imatinib combination therapy support this premise, as discussed below. However, as suggested in [37], the therapeutic window for using IFNα to sensitize CML cells to imatinib is unknown and should be further explored.

One of the dose-limiting side effects of IFNα therapy is thrombocytopenia. To explain this phenomenon, in vitro studies have shown IFNα treatment suppresses megakaryocyte (MK) formation, proliferation, and growth [41, 42]. Wang et al. showed that IFNα acts directly on megakaryocytic progenitor cells to suppress JAK/STAT signaling, likely through induction of suppressor of cytokine signaling 1 (SOCS-1) [43]. A subsequent study using in vitro and in vivo models determined that IFNα inhibits late-stage megakaryopoiesis, but not endomitosis, an early event in platelet production [44]. Transcriptional regulation is likely involved as IFNα inhibited expression of transcription factors GATA-1, p45$^{NF\text{-}E2}$, and MafG, which regulate late-stage megakaryopoiesis [44]. The clinical relevance is that this myelosuppressive effect may benefit patients with myeloproliferative neoplasms (MPNs) and high platelet counts, who account for a large number of patients.

13.7 IFNα Mechanism of Action in MPNs

IFNα suppresses the proliferation and growth of MPN CD34+ cells [45, 46]. In BCR-ABL-negative MPNs, *JAK2V617F* is the major mutation and was found to give murine HPCs a proliferative advantage over wild-type (WT) cells [47]. Mice with conditional expression of *JAK2V617F* in HPCs developed an MPN resembling polycythemia vera (PV) [47]. IFNα treatment prevented disease progression in this model by increasing both cycling of WT HSCs and apoptosis of splenic *JAK2V617F* cells. A separate study determined that IFNα induces apoptosis of PV CD34+ cells via activation of p38 [46]. In CML progenitor cells, IFNα induced apoptosis through upregulation of the Fas receptor, which increased the cells' sensitivity to Fas ligand [48]. IFNα treatment also restored proliferation and adhesion functions in CML progenitors through both direct effects and indirect effects on stroma [49]. The direct effects are mediated through IFNAR, whose expression correlates with response to IFNα therapy in CML patients [50]. IFNα may also induce differentiation and exhaustion of CML stem cells by upregulating CCAAT/enhancer binding protein beta (C/EBPβ) via STAT1 and STAT5 activation [51].

Considering *JAK2* and *BCR-ABL1* mutations affect overlapping signaling pathways, it is not surprising that the effects of IFNα on HPCs are similar between CML and other MPNs. However, a recent study suggests *JAK2V617F*- and *BCR-ABL1*-expressing cells have different sensitivities to IFNα, which might be explained by their differential ISG expression patterns and response to STAT2 [52].

Type I IFNs increase the expression of tumor-associated antigens and major histocompatibility complex class I molecules, which has led to an exciting discovery in CML [53]. To identify antigens that could initiate T-cell responses against leukemia, Molldrem et al. screened peptides derived from proteinase 3, a serine protease highly expressed in CML cells [54]. The most promising candidate was PR1, a peptide with high affinity for HLA-A.2.1. CTLs specific for PR1 (PR1-CTLs) eliminated CML progenitors, but not normal marrow cells [54]. Further investigation of PR1's clinical relevance revealed the presence of circulating PR1-CTLs in CML patients who responded to IFNα therapy, but not in nonresponders [20]. PR1-CTLs were also increased in CML patients who received IFNα maintenance after combination therapy with imatinib and IFNα [55] and in CML patients with a complete cytogenetic response (CCyR), indicating no detectable cells with the Philadelphia chromosome (Ph), after IFNα cessation [56]. Furthermore, PR1-CTLs from patients in CCyR off IFNα therapy secreted IFNγ in response to PR1 peptide, whereas PR1-CTLs from relapse patients off therapy lost their ability to secrete IFNγ [56]. These findings suggest that loss of functional PR1-CTLs may contribute to relapse in patients with CML.

Accumulating evidence suggests deregulation of the BM microenvironment plays a major role in the development of myeloid malignancies [57]. IFNα may restore BM regulatory mechanisms, thereby reversing part of the disease process and inducing clinical responses. Pretreatment of cultured stoma, but not progenitors, with IFNα enhanced adhesion of CML progenitors to stroma, a function that is impaired during disease [49]. The effect was mediated in part through production of macrophage inflammatory protein-1α. In the vascular niche, IFNα can directly affect ECs and control the expression of genes including angiogenesis regulators [58]. In the osteoblastic niche, type I IFNs regulate normal bone mass as demonstrated by the reduced trabecular bone mass and increased osteoclast frequency in IFNAR1−/− mice [59]. However, the physiologic mechanism may be specific to IFNβ alone, and the relevance to disease is unclear. IFNα signaling also affects HSC localization in the BM, as its activation (via poly(I:C) injection) mobilized HSCs from periarteriolar niches [60]. By redistributing HSCs presumably to the proliferative niches, IFNα treatment may disrupt niche mechanisms that protect HSCs from chemotherapy or γ-irradiation [60]. Whether direct or stromal mechanisms mediate this effect, and in general, how IFNα therapy affects specific BM stromal components require further study.

13.8 Molecular Markers of Response to IFNα

As mentioned above, STAT proteins mediate canonical type I IFN signaling. One study reported a correlation between response to IFNα and STAT1 expression in peripheral blood mononuclear cells from CML patients [61]. Complete responders expressed STAT1 at diagnosis, whereas resistant cases did not [61]. CML patients responding to IFNα treatment also showed reduced intracellular transcript and protein levels of BCR-ABL1, the molecular driver of CML, compared to levels at diagnosis [62]. This is likely an autocrine effect as IFNα treatment similarly decreased BCR-ABL1 levels in cultured BM mononuclear cells isolated from untreated CML patients [62]. Transcript levels of another important protein in CML, interferon consensus sequence binding protein (ICSBP), correlated with cytogenetic response to IFNα in CML patients [63]. Deletion of ICSBP in mice led to a granulocytic leukemia similar to CML in humans, suggesting a tumor suppressor role for this protein [64]. Mice transplanted with BM cells coexpressing ICSBP and BCR-ABL1 live

longer than mice transplanted with BCR-ABL1 expressing cells alone, indicating a protective role for ICSBP in CML [65]. Another potentially beneficial protein in CML, interferon regulatory factor 4 (IRF4), was expressed at higher levels in T cells of CML patients with good cytogenetic response to IFNα therapy vs poor responders [66]. IRF4 knockout mice exhibit defective cytotoxic responses and develop lymphadenopathies [67]. Other IRF family members IRF1 and IRF2 are antagonistic transcription factors that control IFN gene expression. The ratio of IRF1:IRF2 expression in CML leukocytes correlated with cytogenetic and molecular responses to IFNα therapy [68]. This observation makes sense considering IRF1 activates transcription of IFN and IFN-inducible genes, whereas IRF2 represses the action of IRF1. With further validation, these markers may be useful in monitoring or even predicting response to IFNα therapy.

13.9 Experience with IFNα before TKIs

Since 1981, IFNα was used extensively for the treatment of CML. IFNα was initially used in a partially purified form, until it was cloned in 1980, allowing mass production of recombinant forms alpha 2a (Hoffmann-La Roche, Basel, Switzerland) and alpha 2b (Merck, formerly Schering-Plough, Whitehouse Station, NJ, USA). The first clinical study of IFNα, published in 1979, tested its efficacy in multiple myeloma patients [69]. Studies in CML, hairy-cell leukemia, Ph- MPNs, hypereosinophilic syndromes, and systemic mastocytosis followed. The early trials of IFNα (see Tables 13.2 and 13.3) in CML demonstrated that a subset of patients achieved sustained cytogenetic remissions with a reduction in BCR-ABL1 transcripts. Previous CML therapies such as busulfan and hydroxyurea had never achieved responses of this magnitude. However, IFNα was not approved as standard frontline therapy until 1995, when several randomized studies showed a survival advantage of IFNα over conventional chemotherapy [70]. A meta-analysis of seven randomized studies, with data from 1554 patients, reported 5-year survival rates of 57% with IFNα and 42% with chemotherapy [71]. In 1996, the American Society of Hematology assembled an expert panel on CML to evaluate treatment with standard chemotherapy, IFNα, and bone marrow transplantation [72]. The panel concluded that treatment with IFNα as a single agent or in combination with cytarabine

Table 13.2 Single-agent trials of IFNα: a historical overview

Trial	IFNα dose	IFNα form	n	CHR rate, %	Median survival (months)
Talpaz, McCredie et al. 1983 [197]	9 MU	Partially pure	7	71	
Talpaz, Kantarjian et al. 1987 [198]	3–9 MU	Partially pure	51	71	
Alimena, Morra et al. 1990 [199]	2–5 MU/m^2	rIFNα-2b	105	59	
Talpaz, Kantarjian et al. 1991 [200]	3–9 MU (partially pure) or 5 MU/m^2 (rIFNα-2a)	Partially pure or rIFNα-2a	96	73	62
Niederle, Kloke et al. 1993 [201]	4 MU/m^2	rIFNα-2b	48	46	
Ozer, George et al. 1993 [202]	5 MU/m^2	rIFNα-2b	107	22	66
Thaler, Gastl et al. 1993 [203]	3.5 MU	rIFNα-2c	80	39	
Hehlmann, Heimpel et al. 1994 [204]	5 MU/m^2	rIFNα-2a or rIFNα-2b	133	31	66
Italian Cooperative Study Group on Chronic Myeloid Leukemia 1994 [205]	3–9 MU	rIFNα-2a	218	45 (complete and partial)	72
Allan, Richards et al. 1995 [206]	3–12 MU	Highly purified	293	68	61
Ohnishi, Ohno et al. 1995 [207]	3–9 MU	rIFNα-2a	80	39	

CHR complete hematologic remission, *rIFNα* recombinant interferon alpha
Table adapted from [208]

Table 13.3 Combination trials of IFNα: a historical overview

Trial	Treatment regimen	IFNα form	n	CHR rate	Survival
Kantarjian, Talpaz et al. 1991 [209]	Induction: Daunorubicin + cytarabine + vincristine + prednisone Maintenance: IFNα 3–5 MU/m² daily vs Matched historical control (IFNα)	Human leukocyte IFNα	32 64	NA	Projected 6-year survival rate from start of therapy: 58% 58%
Kantarjian, Keating et al. 1992 [210]	IFNα 5 MU/m² daily + low-dose cytarabine every 2 weeks until remission, then 1 week/month for maintenance vs Historical control (IFNα)	NA	40 39	55% 28% ($P = 0.02$)	3-year rate: 75% 48% ($P < 0.01$)
Hehlmann, Berger et al. 2003 [211]	IFNα 5 MU/m² daily + hydroxyurea vs Hydroxyurea	rIFNα-2a	226 308	59% 32%	Median survival: 64 months 53 months ($P = 0.0063$)
Kantarjian, O'Brien et al. 1999 [212]	IFNα 5 MU/m² daily + low-dose cytarabine daily vs IFNα + intermittent low-dose cytarabine vs IFNα without cytarabine	NA	140 46 274	92% 84% 80% ($P = 0.01$)	~70% for all groups
Arthur, Ma et al. 1993 [213]	IFNα 9 MU daily + intermittent low-dose cytarabine	rIFNα-2a	30	93%	NA
Lindauer, Domkin et al. 1999 [214]	IFNα 5 MU daily + intermittent low-dose cytarabine	rIFNα-2b	65	60%	3-year rate: 77% 5-year rate: 55%
Guilhot, Chastang et al. 1997 [215]	Hydroxyurea + IFNα 5 MU daily + intermittent low-dose cytarabine vs Hydroxyurea +IFNα daily	rIFNα-2b	360 361	66% 55% ($P = 0.003$)	3-year rate: 86% 79% ($P = 0.02$)
Baccarani, Rosti et al. 2002 [216]	Hydroxyurea + IFNα 3–6 MU daily + intermittent low-dose cytarabine vs Hydroxyurea + IFNα daily	rIFNα-2a	275 263	62% 55% (ns)	5-year rate: 68% 65% (ns)

CHR complete hematologic remission, *NA* not available, *NS* not significant, *rIFNα* recombinant interferon alpha
Table adapted from [208]

improves survival, especially for CML patients in early chronic phase (CP) with low-risk features. Ultimately, the forum recommended the use of IFNα for patients with CML in chronic phase (CML-CP), as long as the risks and benefits were clearly conveyed to the patient.

To extend the half-life and reduce the immunogenicity of IFNα, a polyethylene glycol molecule was attached to it. The resulting pegylated IFNα (PegIFNα) was originally available in two commercial forms, PegIFNα-2a (Pegasys®) and PegIFNα-2b (PegIntron®), and could be injected less often than unpegylated IFNα. A phase I trial of PegIFNα-2b found that it was well tolerated and effective in CML patients with prior resistance or intolerance to IFNα. Dose-limiting toxicity was observed at 7.5–9 μg/kg and included severe fatigue, neurotoxicity, liver function abnormalities, and myelosuppression [73]. A subsequent phase II trial compared PegIFNα-2a, 450 μg once weekly, with IFNα-2a, nine million International Units (MIU) once daily, in IFNα-naïve patients with CML [74]. At 12 months, complete hematological responses (CHRs) and

major cytogenetic responses (MCyRs) were significantly higher in the PegIFNα-2a group compared with the IFNα-2a group. Survival rates favored PegIFNα-2a, and safety profiles were similar between treatment groups. An earlier phase III study randomized 344 newly diagnosed CML patients to PegIFNα-2b, 6 μg/kg/week, or IFNα2b, 5 MIU/m^2/day [75]. The study did not demonstrate statistical noninferiority of PegIFNα-2b, although a disproportionate number of patients with clinical anemia were randomized to receive PegIFNα-2b, potentially biasing the results [75]. No clinical trials have formally compared PegIFNα-2a with PegIFNα-2b in CML [70]; however, studies in hepatitis C patients did not find a difference in efficacy or safety between the two drugs [76–78]. Ropeginterferon alfa-2b (ropeg; Besremi®; PharmaEssentia) is a novel monopegylated interferon with a longer half-life and less frequent dose interval than PegIFNα; it is initially given every 2 weeks and can be tapered over time. Based on findings from a phase III study in PV [79], the European Medicine Agency approved ropeg for the treatment of PV without symptomatic splenomegaly on February 21, 2019, and approval from the US Food and Drug Administration (FDA) is underway [80].

13.10 Toxicities

Side effects from IFNα treatment lead to discontinuation rates of approximately 20–30% in clinical trials, even when PegIFNα is used at lower doses [81]. Acute side effects to IFNα therapy commonly present as flu-like symptoms including anorexia, fever, chills, myalgias, and headaches; these are not typically dose limiting and usually resolve in a few days. Chronic side effects include fatigue, weight loss, myalgias/arthralgias, depression, elevated liver enzymes, and immune-mediated complications. Cases of cardiac dysfunction, including dysrhythmias and congestive heart failure, are rare but require immediate discontinuation of IFNα. Chronic fatigue and neurotoxicity, such as depression and cognitive impairment, are common dose-limiting side effects and typically worsen with continued treatment [82]. Patients with a history of psychiatric disorders should be prescribed IFNα with caution. Since these toxicities have hindered compliance with therapy, three joint prospective studies examined whether a lower dose of IFNα at 3 MU/m^2 five times a week would be as effective as the standard dose of 5 MU/m^2 daily [83]. The studies found that overall survival and response rates did not dramatically differ between groups [83]. Some patients may develop autoimmune disorders on IFNα, such as autoimmune hemolytic anemia/thrombocytopenia, collagen vascular disorders, hypothyroidism, polyarthritis, dermatomyositis, and glomerulonephritis [81, 84–86]. Interestingly, these conditions were associated with better responses to therapy, potentially reflecting a heightened immune response against malignant cells [81, 84].

13.11 IFNα in Pregnancy

The FDAα classified IFNα therapy during pregnancy as risk category C, but clinical experience suggests IFNα is safe in the second and third trimesters [87–89]. It does not cross the placental barrier due to its large molecular size and does not inhibit DNA synthesis; thus, its effects on the fetus are minimal. A review of 63 case reports concluded that IFNα therapy does not significantly increase the risk of congenital malformation, miscarriage, stillbirth, or preterm delivery above the general population [89]. On the other hand, PegIFNα is not considered safe during pregnancy because polyethylene glycol can accumulate and cause harmful effects [88]. TKIs are also contraindicated as studies have shown that TKI therapy during pregnancy was associated with higher incidence of developmental abnormalities and poor obstetric outcomes [90, 91]. Thus, women with CML who want to become pregnant should be advised to discontinue TKI therapy while being informed of the risk of suboptimal response or relapse if treatment is interrupted [87, 88]. The recommended treatment options during pregnancy include observation, leukopheresis for management of cytoses, and interferon therapy in the second and third trimesters, depending on the patient's prior response to TKI therapy [92, 88, 93, 94].

13.12 Durable Responses and Unmaintained Remissions with IFNα Therapy

IFNα can induce stable remissions in some patients with CML. In a study of 512 CML patients treated between 1981 and 1995 with IFNα-based therapies at the MD Anderson Cancer Center, 27% of patients achieved CCyR within a median time of 16 months [95]. Ten years posttreatment, 78% of these responders were still alive. Those who maintained cytogenetic remission for more than 2 years on IFNα stayed in remission for an average of 6 years after discontinuing treatment. Similarly, a European registry of 317 CML patients in CCyR after starting IFNα alone or with hydroxyurea (HU) achieved first CCyR at a median of 19 months [96]. After 10 years, 72% of these patients were alive and 46% were in continuous CCyR. Analysis of prognostic factors in this cohort revealed that high-risk patients lost CCyR more frequently and more rapidly than lower-risk patients, and none survived more than 10 years [96]. Recently, long-term outcomes were analyzed in 121 CML patients who were treated in Italy with IFNα-based therapy between 1986 and 2000 and obtained CCyR [97]. After 20 years, 84% of these patients were alive and in CCyR. Like the other two studies, maintenance of CCyR correlated with long-term survival in CML patients. Altogether, these results suggest CCyR after IFNα therapy predicts long-term survival, and low-risk responders will experience the most benefit from IFNα.

Several cases of continuous cytogenetic remission after cessation of IFNα therapy have been reported [98, 99, 96, 95, 100, 101]. Sustained response after IFNα discontinuation was first noted in an early study of seven patients in CCyR with IFNα [100]. Later, Mahon et al. described 15 patients who stopped IFNα after achieving CCyR and had similar survival rates and time of CCyR loss compared with the 41 study patients who had continued IFNα therapy [98]. At last follow-up (median of 36 months after IFNα discontinuation), seven of the 15 patients had not relapsed. The European registry (mentioned above) included 36 patients in CCyR who discontinued IFNα due to toxicity [96]. Of these patients, four progressed to accelerated or blastic phase and 15 were alive and in continuous CCyR at last follow-up. Of the eight additional patients who discontinued IFNα due to attainment of stable CCyR, only one died, and this was due to transplant. Analysis of patients treated with IFNα-based therapy at the MD Anderson Cancer Center revealed that 39 CML patients maintained their CCyR at last follow-up despite being off therapy for a median of 50 months [95].

Molecular responses, a measure of BCR-ABL1 transcript levels by qPCR, have been found to predict outcomes better and earlier than cytogenetic responses. These values are expressed on the international scale (IS) as a log reduction from the standardized baseline. A 3-log reduction in BCR-ABL1 (≤0.1%) is defined as major molecular response (MMR), 4-log reduction is MR^4, and 4.5-log reduction is $MR^{4.5}$; a 4-log reduction or deeper is defined as deep molecular remission (DMR). In a study of 23 CML patients who achieved DMR on IFNα and later discontinued therapy, 18 patients were still off therapy at time of the report (median time from IFNα discontinuation 125.5 months), including four in MMR, six patients in MR^4, five in $MR^{4.5}$, and three with a BCR-ABL1 ratio between 0.5 and 0.1 [101]. Event-free survival in this cohort 10 years after therapy suspension was 77.4% [101]. Although these data demonstrate that responses to IFNα can be durable, molecular evidence of disease is found in virtually all CML patients in CCyR treated with IFNα, even those in long-term remission [102, 103]. The same phenomenon has been observed in patients who maintain CCyR after discontinuation of imatinib [104], suggesting that imatinib and IFNα may induce durable responses through a common mechanism (e.g., restoration of the BM niche) in the presence of residual disease. However, these therapies target different molecules and may sustain patient remissions through different mechanisms (e.g., TKIs cause disease debulking while IFNα activates antitumor immunity). The answer is not known and would be extremely useful in determining how to best combine IFNα with TKI

therapy in an effort to increase rates of unmaintained remission.

13.13 Introduction of Imatinib

Frontline therapy with IFNα was replaced in 2001 by imatinib, a molecular therapy that targets the pathogenic BCR-ABL1 protein. Several major scientific discoveries preceded the development of this targeted therapy. In 1973, Dr. Janet Rowley identified the reciprocal translocation between the long arms of chromosomes 9 and 22 (t(9;22)(q34;q11)), resulting in the shortened chromosome 22, or so-called Philadelphia chromosome (Ph) [105]. The transforming sequence of c-abl was then mapped to the Ph [106]. With advances in DNA cloning and sequencing technology, the *bcr-abl1* transcript was characterized in 1985 and was predicted to encode a fusion protein with tyrosine kinase activity [107]. Two subsequent studies introduced the *bcr-abl1* transgene into mice and definitively linked the fusion gene with generation of leukemia [108, 109]. Imatinib was then developed by rational drug design.

The large-scale phase III International Randomized Study of Interferon and STI571 (IRIS) compared imatinib (400 mg daily) head-to-head with IFNα plus low-dose cytarabine (the standard of care at that time) in 1106 patients with newly diagnosed CML-CP [110]. Imatinib was better tolerated and induced higher CHR and CCyR rates than IFNα. The superior responses translated to longer progression-free survival with imatinib treatment. Overall survival differences were never reported because 65.6% of patients on the IFNα arm eventually crossed over to imatinib and only seven patients (1.3%) completed IFNα treatment [111]. Based on these findings, the FDA approved imatinib for the treatment of newly diagnosed patients with CML-CP. Long-term follow-up of IRIS revealed that 272 patients discontinued assigned imatinib therapy due to toxicity (6.9%), suboptimal response/failure (15.9%), or other reasons (26.4%) [111]. Furthermore, a small fraction (6.9%) of patients taking imatinib eventually progressed to accelerated or blast phase [112, 111]. Thus, interest in using IFNα for CML therapy, especially in combination with BCR-ABL TKI therapy, has been renewed.

13.14 Combination Therapy with TKIs and IFNα

An estimated one-quarter to one-third of CML patients on imatinib will become resistant or intolerant to therapy [113]. To improve the durability of responses to imatinib, IFNα has been incorporated into various treatment schedules. The rationale is that since these drugs have different mechanisms of action, combination therapy may lead to synergistic or additive effects (Table 13.4 summarizes studies of IFNα and TKI combination therapy). Two large multicenter studies have evaluated imatinib and IFNα combination therapy. The German CML-Study IV randomized 1551 CML patients to imatinib 400 mg, imatinib plus IFNα (1.5–3 MU thrice weekly), imatinib plus Ara-C, imatinib after IFNα failure, or imatinib 800 mg [114, 115]. More patients receiving tolerability-adapted imatinib 800 mg reached MMR compared with the other study arms ($P = 0.003$). The investigators suggested that the superior remission rates were a result of the strategy applied (high dose early on and maintenance around 600 mg/day according to tolerability). Longer follow-up revealed that patients on high-dose imatinib achieved $MR^{4.5}$ more quickly than patients on the other study arms, except those receiving imatinib plus IFNα [115]. After 10 years, six out of 430 patients were still taking IFNα [116]. After a median of 34 months, 407 out of 1538 total patients (26.5%) had switched from imatinib to another TKI, mostly dasatinib or nilotinib, due to intolerance or resistance. Survival analysis at 10 years showed no difference between treatment arms [116]. A multivariate analysis showed risk score, major-route chromosomal aberrations, comorbidities, smoking, and treatment center influenced survival, but initial treatment selection did not. In addition, patients who reached molecular response milestones at 3, 6, and 12 months had a higher survival rate than those who did not [116].

Table 13.4 Combination trials of IFNα and TKIs

Trial	Phase of study	TKI and dose	n	Reported benefit/response to IFNα + TKI (TKI alone vs IFNα + TKI)
CML-Study IV [114, 115]	IV	Imatinib 400 or 800 mg/day	1551	None
SPIRIT [117, 118]	III	Imatinib 400 or 600 mg/day	636	Better molecular response rate than imatinib alone
Nordic study [119]	II	Imatinib 400 mg/day	112	Better molecular response rate than imatinib alone
MD Anderson [120]	II	Imatinib 800 mg/day	94	None
Italian Cooperative Study [121–123]	II	Imatinib	76	Better molecular response rate early on than imatinib alone
NCT01872442NCT01392170 (FILMC) [133]	II	Dasatinib 100 mg/day	81	$MR^{4.5}$ 12 mo = 30%
NCT02201459NCT00573378 (PETALS) [127, 131]	III	Nilotinib 300 mg 2x/day	200	MR^{4} 36 mo = 70.2% vs 71.13% $MR^{4.5}$ 36 mo = 37.2% vs 49.5% MR^{5} 36 mo = 33% vs 42.3% Cumulative $MR^{4.5}$ by 36 mo = 44% vs 54.6%
NCT01294618 (NiloPeg) [130]	III	Nilotinib 600 mg 2x/day/day	41	$MR^{4.5}$ 12 mo = 17%, good molecular responses
NCT01657604 (TIGER) [129, 217]	III	Nilotinib 300 mg 2x/day	692	NMR^{4} 18 mo = 39.6% vs 49% $MR^{4.5}$ 18 mo = 23.1% vs 32.6%
NCT02001818 (PINNACLE) [218, 132]	II	Nilotinib 300 mg 2x/day	60	$MR^{4.5}$ 12 mo = 43.3% $MR^{4.5}$ 24 mo = 50%
NCT01725204 (NordCML007) [126]	II	Dasatinib 100 mg/day	35	MR^{4} 12 mo = 46% $MR^{4.5}$ 12 mo = 27%

The French STI571 Prospective Randomized Trial (SPIRIT) randomized 636 CML patients to imatinib 400 mg, imatinib 600 mg, imatinib plus Ara-C, or imatinib plus PegIFNα (90 μg/week) [117]. In contrast to the German CML-Study IV, this study demonstrated significantly faster and better molecular response rates with imatinib plus PegIFNα-2a compared with the other study treatments at 12, 18, and 24 months [117]. The duration of combination therapy was found to be important as patients receiving therapy for more than 12 months had better molecular responses than those who were treated for less than 4 months. However, PegIFNα-2a was not well tolerated, so the dose was lowered to 45 μg/week, which reduced hematological toxicity and lengthened delivery of PegIFNα-2a [118]. No difference in MMR or MR^{4} at 12 months was observed in patients receiving dose-reduced PegIFNa2a compared with those taking the original dose [118]. The second part of the trial has been focusing on whether the earlier and faster response rates with this combination translate into better survival.

Three smaller phase II studies have evaluated combination therapy with imatinib plus IFNα. The Nordic group compared the combination of PegIFNα-2b 50 μg/week and imatinib 400 mg/day with imatinib 400 mg/day alone in patients with low- or intermediate-risk CML (n = 112) [119]. Significantly more MMRs occurred on the combination arm (82%) compared with the monotherapy arm (54%) at 12 months. More than half of the patients in the combination arm discontinued PegIFNα-2b due to toxicity. Nevertheless, patients who completed at least 12 weeks of combination therapy had the same MMR rate as those who completed more than 9 months of therapy, demonstrating that even a short course of IFNα plus imatinib could be beneficial. The second

study ($n = 94$) randomized 94 patients with early CML-CP to high-dose imatinib (800 mg/day) combined with PegIFNα-2b (0.5 μg/kg/week) and GM-CSF or high-dose imatinib alone [120]. Unlike the Nordic and SPIRIT trials, this study did not find an advantage with the combination; however, a high number of patients in the combination arm did not start PegIFNα-2b or discontinued it by 12 months, which may have compromised the potential benefit of IFNα. The third study by the Italian Cooperative Study Group explored optimal dosing of PegIFNα-2b in combination with imatinib. The starting doses of PegIFNα-2b (50, 100, and 150 μg/week) were likely too high in combination with imatinib, as 63% of patients receiving combination therapy experienced grade 3 or 4 neutropenia and 52% experienced grade 3 or 4 non-hematologic adverse events [121, 122]. The high toxicity rate contributed to low patient compliance to PegIFNα-2b therapy. A retrospective analysis showed that patients receiving the combination achieved better CCyR and MMR rates early on, but the advantage was lost over time [123, 122].

The conclusion of these studies is that adding IFNα to imatinib therapy may increase the rate of deep responses at earlier time points, but the undesirable side effects of IFNα can create problems in delivering therapy. Pegylated forms of IFNα at lower doses seem to improve adherence to treatment without reducing efficacy, and even limited courses may prove beneficial. In support of this, a single-institution study of 100 patients with CML who discontinued TKI therapy found that previous IFNα therapy was associated with a lower rate of $MR^{4.5}$ loss compared with patients who never received IFNα (22% vs 41%; $p = 0.03$) [124]. Similarly, a small study of IFNα added to TKI and K562/GM-CSF vaccine therapy suggested that IFNα exposure allowed some patients to discontinue all treatment sooner than in previously reported discontinuation trials [125]. Thus, prior IFNα therapy appears to benefit patients who take TKIs; however, we still do not know at what point and for how long IFNα should be added to TKI therapy.

Studies investigating the combination of IFNα with second-generation TKIs are ongoing and the emerging data is encouraging, as summarized in Table 13.4. These newer studies often assess deep molecular remissions over time on therapy, which have been shown to predict survival and ability to discontinue therapy [115, 126]. Several of these studies have examined the timing at which IFNα should be combined with nilotinib. The French phase III PETALS study assessed the combination of nilotinib plus PegIFNα using the following treatment strategy: (1) priming with PegIFNα-2a alone (±HU) 30 μg/week for 30 days vs nilotinib 300 mg BID alone; (2) combination therapy using nilotinib plus PegIFNα 30 μg/week for 2 weeks, upgraded to 45 μg/week if tolerated for up to 2 years vs nilotinib alone; and 3) nilotinib alone for 4 more years [127]. At 36 months, the rates of MMR were 83% for nilotinib alone vs 86.6% nilotinib plus PegIFNα ($p = 0.31$), MR^4 were 70.2% vs 71.13% ($p = 0.50$), $MR^{4.5}$ were 37.2% vs 49.5% ($p = 0.05$), and MR^5 33% vs 42.3% ($p = 0.12$). The overall cumulative incidence of $MR^{4.5}$ was 44% for nilotinib alone vs 54.6% for nilotinib plus PegIFNα, almost reaching significance ($p = 0.05$). Interestingly, more patients on nilotinib alone (8/51;11.8%) developed mutations in the ABL1 kinase domain after 12 months compared to patients on combination therapy (3/45; 6.6%) [128]. These mutations are associated with worst survival outcomes [128]. Another phase III trial of nilotinib plus PegIFNα-2b, known as the TIGER (CML V; NCT01657604) study, is investigating whether IFNα should be administered concomitantly with nilotinib or as maintenance therapy once MMR has been achieved in CML patients [129] using the following treatment strategy: (1) nilotinib-based induction therapy (nilotinib 300 mg BID + PegIFNα-2b 30–50 μg/week vs nilotinib alone) for at least 2 years, (2) maintenance therapy with PegIFNα-2b (vs nilotinib alone arm) upon achieving MMR, and 3) treatment discontinuation after at least 3 years of treatment and achievement of MR^4 for at least 1 year. Of the initial 692 randomized patients, 477 patients have completed the induction phase and reached the maintenance phase, and 199 completed the maintenance phase and have discontinued all therapy [129]. At 18 months, the rates of MR^4 were 39.6% vs 49.0% ($p < 0.022$) and $MR^{4.5}$ were 23.1% vs 32.6% ($p < 0.0097$) for nilotinib vs nilotinib +

PegIFNα. For patients who discontinued nilotinib and received PegIFNα maintenance therapy, 28% had molecular recurrence after 18 months. For patients who discontinued all therapy, 63 out of 199 (31.7%) experienced a molecular relapse. Relapse-free survival by 18 months after treatment discontinuation was 61% in the total cohort. The French phase II trial of nilotinib plus PegIFNα, known as NiloPeg, combined nilotinib 300 mg BID with PegIFNα-2a 45 μg/week after priming with PegIFNα-2a 90 μg/week alone for a month in 41 newly diagnosed CML patients. The rate of MR^4 was 51%, $MR^{4.5}$ was 17%, and MR^5 was 7% [130, 131]. In the phase II Australian PINNACLE study of 60 newly diagnosed CML patients, PegIFNα-2b 30 μg/week was added to nilotinib 300 mg BID after 3 months [132]. MMR and $MR^{4.5}$ rates at 12 months were 78.3% and 43.3%, respectively. In 40 evaluable patients at 24 months, $MR^{4.5}$ was 50%. Only 21 out of 52 patients (35%) received >85% of their assigned PegIFNα-2b dose; however, most received the full TKI dose.

Fewer studies have assessed the combination of PegIFNα with dasatinib, and no phase III randomized trials have been conducted yet. In the NordCML007 trial (NCT01725204) of 40 patients with newly diagnosed CML, addition of PegIFNα-2b to dasatinib therapy led to MMR in 89% at 18 months and led to MR^4 in 46% and $MR^{4.5}$ in 27% of patients at 12 months [126]. PegIFNα-2b was started at 15 μg/week and increased to 25 μg/week; 84% of the total 40 patients remained on PegIFNα-2b at month 12. A similar trial has been conducted by the French CML Group (FILMC) [133]. After 3 months on dasatinib 100 mg/day, PegIFNα was started at 30 μg/week in 61 newly diagnosed CML patients. The rate of $MR^{4.5}$ at 12 months was 30%.

Another therapeutic combination with biological rationale in CML is IFNα plus granulocyte-macrophage colony-stimulating factor (GM-CSF). Low concentrations of myeloid growth factors, such as GM-CSF, induced terminal differentiation of CML progenitor cells while promoting growth of normal progenitors in vitro [134]. GM-CSF also augmented both IFNα-mediated differentiation of cultured CML progenitors and the antileukemic activity of IFNα [135, 136]. A phase II study evaluated the combination of IFNα with GM-CSF in 58 CML patients [137]. Responses compared favorably with historical studies of IFNα alone, although only 45 patients completed 6 months of combination therapy. Further, imatinib became available during this study period, which partly explains why 69% of patients discontinued IFNα within 3 years. Still, six patients were off all CML therapy at the time of the report (15 months–12 years post-therapy), and three of these patients only received IFNα plus GM-CSF and no TKIs [137]. A previous study added GM-CSF to IFNα therapy in 15 CML patients who had not achieved an optimal cytogenetic response to IFNα [138]. GM-CSF did not cause additional toxicity, and four patients achieved a significant cytogenetic response. As mentioned above, the addition of PegIFNα-2b and GM-CSF to high-dose imatinib did not lead to better patient outcomes compared with imatinib alone [120]. The poor adherence to PegIFNα-2b may have compromised the potential benefit of combination therapy. Thus, IFNα plus GM-CSF may have a future role in CML therapy, but further studies of the timing, dosage, and possible combination with TKIs are needed.

Finally, IFNα and arsenic trioxide (ATO) is an interesting combination that has demonstrated antileukemic effects in preclinical models. In vitro, the combination of ATO and IFNα synergized to inhibit proliferation and induce apoptosis of CML cell lines [139], including imatinib-resistant cells [140]. These effects were associated with induction of autophagy and inhibition of the hedgehog pathway [140]. The combination also reduced the clonogenic activity of primary CML cells [139]. Finally, combined IFNα and arsenic treatment in vivo prolonged the survival of both a murine transduction/transplantation CML model and *T315I*-CML murine model and severely impaired engraftment into untreated secondary recipients [139, 140]. These results suggest IFNα/ATO impairs the function of leukemia-initiating cells both in wild-type *BCR-ABL1* and *T315I* mutant CML. Thus, this combination is worth further investigation in CML patients, particularly those with TKI-resistant disease.

13.15 IFNα Maintenance Therapy

Most CML patients who discontinue imatinib treatment will eventually relapse [104]. For CML patients who develop intolerance or resistance to imatinib, maintenance therapy with IFNα may allow patients to discontinue imatinib and maintain or reestablish remission. A small pilot study tested this premise in 20 CML-CP patients who discontinued imatinib after a median of 2.4 years on imatinib/IFNα combination therapy [55]. IFNα (recombinant or pegylated) was continued as maintenance therapy at really low doses (e.g., 135 μg PegIFNα-2a once every 3–12 weeks) [141] and led to sustained remission in 15 of the patients. Proteinase 3 mRNA levels and frequencies of PR1-CTLs increased during the maintenance period, suggesting that a specific CTL response contributed to this effect. A later 8-year follow-up reported relapse-free survival in 73% (8/11) and 84% (5/6) of patients who discontinued imatinib in MMR and $MR^4/MR^{4.5}$, respectively [141]. Ten patients discontinued IFNα after a median of 4.5 years, and nine of them maintained treatment-free remission (six in MR^5 and three in $MR^{4.5}$). The four patients who still take IFNα are in stable molecular remission [141]. To minimize toxicity from long-term IFNα use, a later study administered PegIFNα 9 months before and 3 months after imatinib discontinuation [142]. This regimen improved the remission status of five of the 11 patients over a median follow-up of 47 months. These studies support further exploration of the role of IFNα in consolidation or maintenance therapy after TKI induction. In addition, IFNα may have a role in therapy after bone marrow transplant in the case of TKI intolerance [143].

13.16 IFNα Activity in Ph- MPNs

IFNα has been used in Ph- MPNs for the last three decades; however, not until 2005 when the *JAK2V617F* mutation was discovered in the majority of Ph- MPN patients have studies been able to investigate the effects of IFNα on molecular disease burden. Phase II trials with IFNα reported complete hematologic responses in 75–95% of patients with PV or essential thrombocythemia (ET) and 15–20% complete molecular responses, as defined by an inability to detect *JAK2V617F* [144–148]. Considering that conventional drugs HU and anagrelide have made little to no impact on molecular disease, the responses with IFNα are encouraging. Also, similar to CML, long-term treatment with IFNα has allowed several patients with PV to discontinue therapy and sustain DMR for up to 36 months, accompanied by normalization of bone marrow histology [81, 148, 149, 150, 151]. A phase III randomized trial PROUD-PV directly compared monopegylated IFNα ropeg (starting at 100 μg every 2 weeks) to HU, the standard cytoreductive treatment for PV, starting at 500 mg/day, in 257 early-stage PV patients [79]. After 1 year, patients could opt to enter the extension part of the trial, CONTINUATION-PV. At the 3-year timepoint, ropeg treatment was associated with significantly better and longer hematological responses and reduced mutant *JAK2* allele burden. The two treatment groups showed different response kinetics: responses to HU peaked at 6 months and gradually declined thereafter, whereas ropeg responses increased over time [79]. Tolerability was comparable between treatments, with treatment-related serious adverse events occurring in three (2%) of 127 patients in the ropeg group and five (4%) of 127 patients in the HU group. Another phase III study of PegIFNα-2a vs HU, the Myeloproliferative Disorders Research Consortium 112 trial, randomized 168 high-risk ET/PV patients to either treatment for up to a year; those who reached partial or complete responses continued on study treatment for a maximum of 6 years [152]. Unlike the CONTINUATION study, complete response rates at 12 and 24 months were similar between groups. In addition, PegIFNα-2a was associated with a higher rate of grade 3/4 toxicity [152]. Clinical studies of ropeg in ET will be opening soon in the USA.

The responses to IFNα in myelofibrosis (MF) patients have not been as impressive as those in the other MPNs. The results of phase II clinical trials with IFNα in MF are difficult to interpret as studies have used differing formulations, dosing regimens, and response assessments, and

sample sizes were usually small [153–157]. A trial of PegIFNα-2a in 62 MF patients found a 64% response rate in anemic patients, with 38.5% achieving transfusion independence [156]. Additionally, constitutional symptoms resolved in 82% and splenomegaly was reduced in 46.5% [156]. The future of IFNα in Ph- MPNs probably lies in combination therapies with conventional (HU or anagrelide) or targeted (JAK1–2 inhibitors, HDACi, and chromatin-modifying) agents [69]. Accordingly, a more recent study of combination therapy with PegIFNα-2a and ruxolitinib reported complete or partial remission in 39% of low- and intermediate-1-risk patients (n = 18) [157]. In patients with advanced and transforming disease, triple therapy with PegIFNα, DNA hypomethylators, and ruxolitinib may improve current outcomes [81]. Many of these combinations have biological rationale and could potentially be administered at lower doses, mitigating the side effects from each drug [69].

13.17 Conclusion

IFNα therapy is undergoing a revival in Ph- MPNs, including PV and ET [4]. Its role in CML therapy is more complicated because other drugs have shown high efficacy, including imatinib. However, resistance and intolerance to imatinib and other BCR-ABL TKIs is still a problem, and in most cases these treatments are not curative. Studies on long-term survival suggest the patient's disease factors have a larger impact on survival than the initial treatment type [116]. IFNα has a broad range of biological effects, including induction of apoptosis, immune cell activation, inhibition of angiogenesis, and cell cycle arrest. This activity was hypothesized to complement the mechanism of action of BCR-ABL TKIs, providing rationale for combination therapy in CML patients. Clinical studies thus far support this niche for IFNα, and ongoing research will likely clarify how to best use IFNα in CML therapy.

Conflict of Interest MT has chaired a satellite symposium for Merck and has received drugs from Merck for clinical studies. The remaining authors declare no conflict of interest.

References

1. Kotredes KP, Gamero AM. Interferons as inducers of apoptosis in malignant cells. J Interferon Cytokine Res. 2013;33(4):162–70. https://doi.org/10.1089/jir.2012.0110.
2. Trinchieri G. Type I interferon: friend or foe? J Exp Med. 2010;207(10):2053–63. https://doi.org/10.1084/jem.20101664.
3. Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat Rev Immunol. 2005;5(5):375–86. https://doi.org/10.1038/nri1604.
4. Kiladjian JJ, Mesa RA, Hoffman R. The renaissance of interferon therapy for the treatment of myeloid malignancies. Blood. 2011;117(18):4706–15. https://doi.org/10.1182/blood-2010-08-258772.
5. Fuchs SY. Hope and fear for interferon: the receptor-centric outlook on the future of interferon therapy. J Interferon Cytokine Res. 2013;33(4):211–25. https://doi.org/10.1089/jir.2012.0117.
6. Der SD, Zhou A, Williams BR, Silverman RH. Identification of genes differentially regulated by interferon α, β, or γ using using oligonucleotide arrays. Proc Natl Acad Sci U S A. 1998;95(26):15623–8.
7. de Veer MJ, Holko M, Frevel M, Walker E, Der S, Paranjape JM, Silverman RH, Williams BR. Functional classification of interferon-stimulated genes identified using microarrays. J Leukoc Biol. 2001;69(6):912–20.
8. Chawla-Sarkar M, Lindner DJ, Liu YF, Williams BR, Sen GC, Silverman RH, Borden EC. Apoptosis and interferons: role of interferon-stimulated genes as mediators of apoptosis. Apoptosis. 2003;8(3):237–49.
9. Maher SG, Romero-Weaver AL, Scarzello AJ, Gamero AM. Interferon: cellular executioner or white knight? Curr Med Chem. 2007;14(12):1279–89.
10. Bekisz J, Baron S, Balinsky C, Morrow A, Zoon KC. Antiproliferative properties of type I and type II interferon. Pharmaceuticals. 2010;3(4):994–1015. https://doi.org/10.3390/ph3040994.
11. Stein BL, Tiu RV. Biological rationale and clinical use of interferon in the classical BCR-ABL-negative myeloproliferative neoplasms. J Interferon Cytokine Res. 2013;33(4):145–53. https://doi.org/10.1089/jir.2012.0120.
12. Gutterman JU. Cytokine therapeutics: lessons from interferon alpha. Proc Natl Acad Sci U S A. 1994;91(4):1198–205.
13. von Marschall Z, Scholz A, Cramer T, Schafer G, Schirner M, Oberg K, Wiedenmann B, Hocker M, Rosewicz S. Effects of interferon alpha on vascular endothelial growth factor gene transcription and tumor angiogenesis. J Natl Cancer Inst. 2003;95(6):437–48.
14. Oliveira IC, Sciavolino PJ, Lee TH, Vilcek J. Downregulation of interleukin 8 gene expression in human fibroblasts: unique mechanism of transcriptional inhibition by interferon. Proc Natl Acad Sci U S A. 1992;89(19):9049–53.

15. Slaton JW, Perrotte P, Inoue K, Dinney CP, Fidler IJ. Interferon-alpha-mediated down-regulation of angiogenesis-related genes and therapy of bladder cancer are dependent on optimization of biological dose and schedule. Clin Cancer Res. 1999;5(10):2726–34.
16. Legros L, Guilhot J, Huault S, Mahon FX, Preudhomme C, Guilhot F, Hueber AO, French CMLG. Interferon decreases VEGF levels in patients with chronic myeloid leukemia treated with imatinib. Leuk Res. 2014;38(6):662–5. https://doi.org/10.1016/j.leukres.2014.01.010.
17. Indraccolo S. Interferon-alpha as angiogenesis inhibitor: learning from tumor models. Autoimmunity. 2010;43(3):244–7. https://doi.org/10.3109/08916930903510963.
18. Andrews DF 3rd, Singer JW, Collins SJ. Effect of recombinant α-interferon on the expression of the *bcr-abl* fusion gene in human chronic myelogenous human leukemia cell lines. Cancer Res. 1987;47(24 Pt 1):6629–32.
19. Yanagisawa K, Yamauchi H, Kaneko M, Kohno H, Hasegawa H, Fujita S. Suppression of cell proliferation and the expression of a *bcr-abl* fusion gene and apoptotic cell death in a new human chronic myelogenous leukemia cell line, KT-1, by interferon-α. Blood. 1998;91(2):641–8.
20. Molldrem JJ, Lee PP, Wang C, Felio K, Kantarjian HM, Champlin RE, Davis MM. Evidence that specific T lymphocytes may participate in the elimination of chronic myelogenous leukemia. Nat Med. 2000;6(9):1018–23.
21. Tough DF, Borrow P, Sprent J. Induction of bystander T cell proliferation by viruses and type I interferon in vivo. Science. 1996;272(5270):1947–50.
22. Hervas-Stubbs S, Riezu-Boj JI, Gonzalez I, Mancheno U, Dubrot J, Azpilicueta A, Gabari I, Palazon A, Aranguren A, Ruiz J, Prieto J, Larrea E, Melero I. Effects of IFN-alpha as a signal-3 cytokine on human naive and antigen-experienced CD8(+) T cells. Eur J Immunol. 2010;40(12):3389–402. https://doi.org/10.1002/eji.201040664.
23. Lee CK, Rao DT, Gertner R, Gimeno R, Frey AB, Levy DE. Distinct requirements for IFNs and STAT1 in NK cell function. J Immunol. 2000;165(7):3571–7.
24. Swann JB, Hayakawa Y, Zerafa N, Sheehan KC, Scott B, Schreiber RD, Hertzog P, Smyth MJ. Type I IFN contributes to NK cell homeostasis, activation, and antitumor function. J Immunol. 2007;178(12):7540–9.
25. Hughes A, Clarson J, White DL, Yeung D, Hughes TP, Yong ASM. Nilotinib/interferon-α combination rapidly enhances leukaemia-associated antigen-specific cytotoxic T-lymphocyte immune responses, limits natural killer cell maturation and triggers B cell remodelling. Blood. 2017;130(Supplement 1):1581. https://doi.org/10.1182/blood.V130.Suppl_1.1581.1581.
26. Alves R, McArdle SEB, Vadakekolathu J, Goncalves AC, Freitas-Tavares P, Pereira A, Almeida AM, Sarmento-Ribeiro AB, Rutella S. Flow cytometry and targeted immune transcriptomics identify distinct profiles in patients with chronic myeloid leukemia receiving tyrosine kinase inhibitors with or without interferon-alpha. J Transl Med. 2020;18(1):2. https://doi.org/10.1186/s12967-019-02194-x.
27. Rizza P, Moretti F, Belardelli F. Recent advances on the immunomodulatory effects of IFN-alpha: implications for cancer immunotherapy and autoimmunity. Autoimmunity. 2010;43(3):204–9. https://doi.org/10.3109/08916930903510880.
28. Diamond MS, Kinder M, Matsushita H, Mashayekhi M, Dunn GP, Archambault JM, Lee H, Arthur CD, White JM, Kalinke U, Murphy KM, Schreiber RD. Type I interferon is selectively required by dendritic cells for immune rejection of tumors. J Exp Med. 2011;208(10):1989–2003. https://doi.org/10.1084/jem.20101158.
29. Giron-Michel J, Weill D, Bailly G, Legras S, Nardeux PC, Azzarone B, Tovey MG, Eid P. Direct signal transduction via functional interferon-alphabeta receptors in CD34+ hematopoietic stem cells. Leukemia. 2002;16(6):1135–42. https://doi.org/10.1038/sj.leu.2402492.
30. Verma A, Deb DK, Sassano A, Uddin S, Varga J, Wickrema A, Platanias LC. Activation of the p38 mitogen-activated protein kinase mediates the suppressive effects of type I interferons and transforming growth factor-beta on normal hematopoiesis. J Biol Chem. 2002;277(10):7726–35. https://doi.org/10.1074/jbc.M106640200.
31. Katsoulidis E, Carayol N, Woodard J, Konieczna I, Majchrzak-Kita B, Jordan A, Sassano A, Eklund EA, Fish EN, Platanias LC. Role of Schlafen 2 (SLFN2) in the generation of interferon alpha-induced growth inhibitory responses. J Biol Chem. 2009;284(37):25051–64. https://doi.org/10.1074/jbc.M109.030445.
32. Joshi S, Kaur S, Redig AJ, Goldsborough K, David K, Ueda T, Watanabe-Fukunaga R, Baker DP, Fish EN, Fukunaga R, Platanias LC. Type I interferon (IFN)-dependent activation of Mnk1 and its role in the generation of growth inhibitory responses. Proc Natl Acad Sci U S A. 2009;106(29):12097–102. https://doi.org/10.1073/pnas.0900562106.
33. Aman MJ, Keller U, Derigs G, Mohamadzadeh M, Huber C, Peschel C. Regulation of cytokine expression by interferon-alpha in human bone marrow stromal cells: inhibition of hematopoietic growth factors and induction of interleukin-1 receptor antagonist. Blood. 1994;84(12):4142–50.
34. Aman MJ, Bug G, Aulitzky WE, Huber C, Peschel C. Inhibition of interleukin-11 by interferon-alpha in human bone marrow stromal cells. Exp Hematol. 1996;24(8):863–7.
35. Sato T, Onai N, Yoshihara H, Arai F, Suda T, Ohteki T. Interferon regulatory factor-2 protects quiescent hematopoietic stem cells from type I interferon-dependent exhaustion. Nat Med. 2009;15(6):696–700. https://doi.org/10.1038/nm.1973.

36. Essers MA, Offner S, Blanco-Bose WE, Waibler Z, Kalinke U, Duchosal MA, Trumpp A. IFNα activates dormant haematopoietic stem cells in vivo. Nature. 2009;458(7240):904–8. https://doi.org/10.1038/nature07815.
37. Pietras EM, Lakshminarasimhan R, Techner JM, Fong S, Flach J, Binnewies M, Passegue E. Re-entry into quiescence protects hematopoietic stem cells from the killing effect of chronic exposure to type I interferons. J Exp Med. 2014;211(2):245–62. https://doi.org/10.1084/jem.20131043.
38. Graham SM, Jørgensen HG, Allan E, Pearson C, Alcorn MJ, Richmond L, Holyoake TL. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. Blood. 2002;99(1):319–25.
39. Copland M, Hamilton A, Elrick LJ, Baird JW, Allan EK, Jordanides N, Barow M, Mountford JC, Holyoake TL. Dasatinib (BMS-354825) targets an earlier progenitor population than imatinib in primary CML but does not eliminate the quiescent fraction. Blood. 2006;107(11):4532–9.
40. Bhatia R, Holtz M, Niu N, Gray R, Snyder DS, Sawyers CL, Arber DA, Slovak ML, Forman SJ. Persistence of malignant hematopoietic progenitors in chronic myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. Blood. 2003;101(12):4701–7.
41. Mazur EM, Richtsmeier WJ, South K. Alpha-interferon: differential suppression of colony growth from human erythroid, myeloid, and megakaryocytic hematopoietic progenitor cells. J Interf Res. 1986;6(3):199–206.
42. Ganser A, Carlo-Stella C, Greher J, Volkers B, Hoelzer D. Effect of recombinant interferons alpha and gamma on human bone marrow-derived megakaryocytic progenitor cells. Blood. 1987;70(4):1173–9.
43. Wang Q, Miyakawa Y, Fox N, Kaushansky K. Interferon-alpha directly represses megakaryopoiesis by inhibiting thrombopoietin-induced signaling through induction of SOCS-1. Blood. 2000;96(6):2093–9.
44. Yamane A, Nakamura T, Suzuki H, Ito M, Ohnishi Y, Ikeda Y, Miyakawa Y. Interferon-alpha 2b-induced thrombocytopenia is caused by inhibition of platelet production but not proliferation and endomitosis in human megakaryocytes. Blood. 2008;112(3):542–50. https://doi.org/10.1182/blood-2007-12-125906.
45. Mayer IA, Verma A, Grumbach IM, Uddin S, Lekmine F, Ravandi F, Majchrzak B, Fujita S, Fish EN, Platanias LC. The p38 MAPK pathway mediates the growth inhibitory effects of interferon-alpha in BCR-ABL-expressing cells. J Biol Chem. 2001;276(30):28570–7.
46. Lu M, Zhang W, Li Y, Berenzon D, Wang X, Wang J, Mascarenhas J, Xu M, Hoffman R. Interferon-alpha targets JAK2V617F-positive hematopoietic progenitor cells and acts through the p38 MAPK pathway. Exp Hematol. 2010;38(6):472–80. https://doi.org/10.1016/j.exphem.2010.03.005.
47. Hasan S, Lacout C, Marty C, Cuingnet M, Solary E, Vainchenker W, Villeval JL. JAK2V617F expression in mice amplifies early hematopoietic cells and gives them a competitive advantage that is hampered by IFNalpha. Blood. 2013;122(8):1464–77. https://doi.org/10.1182/blood-2013-04-498956.
48. Selleri C, Sato T, Del Vecchio L, Luciano L, Barrett AJ, Rotoli B, Young NS, Maciejewski JP. Involvement of Fas-mediated apoptosis in the inhibitory effects of interferon-α in chronic myelogenous leukemia. Blood. 1997;89(3):957–64.
49. Bhatia R, Verfaillie CM. The effect of interferon-alpha on beta-1 integrin mediated adhesion and growth regulation in chronic myelogenous leukemia. Leuk Lymphoma. 1998;28(3–4):241–54.
50. Ito K, Tanaka H, Ito T, Sultana TA, Kyo T, Imanaka F, Ohmoto Y, Kimura A. Initial expression of interferon alpha receptor 2 (IFNAR2) on CD34-positive cells and its down-regulation correlate with clinical response to interferon therapy in chronic myelogenous leukemia. Eur J Haematol. 2004;73(3):191–205. https://doi.org/10.1111/j.1600-0609.2004.00275.x.
51. Yokota A, Hirai H, Sato R, Adachi H, Sato F, Hayashi Y, Sato A, Kamio N, Miura Y, Nakano M, Tenen DG, Kimura S, Tashiro K, Maekawa T. C/EBPbeta is a critical mediator of IFN-alpha-induced exhaustion of chronic myeloid leukemia stem cells. Blood Adv. 2019;3(3):476–88. https://doi.org/10.1182/bloodadvances.2018020503.
52. Schubert C, Allhoff M, Tillmann S, Maie T, Costa IG, Lipka DB, Schemionek M, Feldberg K, Baumeister J, Brummendorf TH, Chatain N, Koschmieder S. Differential roles of STAT1 and STAT2 in the sensitivity of JAK2V617F- vs. BCR-ABL-positive cells to interferon alpha. J Hematol Oncol. 2019;12(1):36. https://doi.org/10.1186/s13045-019-0722-9.
53. Biron CA. Interferons alpha and beta as immune regulators--a new look. Immunity. 2001;14(6):661–4.
54. Molldrem J, Dermime S, Parker K, Jiang YZ, Mavroudis D, Hensel N, Fukushima P, Barrett AJ. Targeted T-cell therapy for human leukemia: cytotoxic T lymphocytes specific for a peptide derived from proteinase 3 preferentially lyse human myeloid leukemia cells. Blood. 1996;88(7):2450–7.
55. Burchert A, Muller MC, Kostrewa P, Erben P, Bostel T, Liebler S, Hehlmann R, Neubauer A, Hochhaus A. Sustained molecular response with interferon alfa maintenance after induction therapy with imatinib plus interferon alfa in patients with chronic myeloid leukemia. J Clin Oncol. 2010;28(8):1429–35. https://doi.org/10.1200/JCO.2009.25.5075.
56. Kanodia S, Wieder E, Lu S, Talpaz M, Alatrash G, Clise-Dwyer K, Molldrem JJ. PR1-specific T cells are associated with unmaintained cytogenetic remission of chronic myelogenous leukemia after interferon withdrawal. PLoS One. 2010;5(7):e11770. https://doi.org/10.1371/journal.pone.0011770.

57. Schepers K, Pietras EM, Reynaud D, Flach J, Binnewies M, Garg T, Wagers AJ, Hsiao EC, Passegue E. Myeloproliferative neoplasia remodels the endosteal bone marrow niche into a self-reinforcing leukemic niche. Cell Stem Cell. 2013;13(3):285–99. https://doi.org/10.1016/j.stem.2013.06.009.
58. Indraccolo S, Pfeffer U, Minuzzo S, Esposito G, Roni V, Mandruzzato S, Ferrari N, Anfosso L, Dell'Eva R, Noonan DM, Chieco-Bianchi L, Albini A, Amadori A. Identification of genes selectively regulated by IFNs in endothelial cells. J Immunol. 2007;178(2):1122–35.
59. Takayanagi H, Kim S, Matsuo K, Suzuki H, Suzuki T, Sato K, Yokochi T, Oda H, Nakamura K, Ida N, Wagner EF, Taniguchi T. RANKL maintains bone homeostasis through c-Fos-dependent induction of interferon-beta. Nature. 2002;416(6882):744–9. https://doi.org/10.1038/416744a.
60. Kunisaki Y, Bruns I, Scheiermann C, Ahmed J, Pinho S, Zhang D, Mizoguchi T, Wei Q, Lucas D, Ito K, Mar JC, Bergman A, Frenette PS. Arteriolar niches maintain haematopoietic stem cell quiescence. Nature. 2013;502(7473):637–43. https://doi.org/10.1038/nature12612.
61. Landolfo S, Guarini A, Riera L, Gariglio M, Gribaudo G, Cignetti A, Cordone I, Montefusco E, Mandelli F, Foa R. Chronic myeloid leukemia cells resistant to interferon-alpha lack STAT1 expression. Hematol J. 2000;1(1):7–14. https://doi.org/10.1038/sj/thj/6200004.
62. Pane F, Mostarda I, Selleri C, Salzano R, Raiola AM, Luciano L, Saglio G, Rotoli B, Salvatore F. BCR/ABL mRNA and the P210(BCR/ABL) protein are downmodulated by interferon-alpha in chronic myeloid leukemia patients. Blood. 1999;94(7):2200–7.
63. Schmidt M, Hochhaus A, Nitsche A, Hehlmann R, Neubauer A. Expression of nuclear transcription factor interferon consensus sequence binding protein in chronic myeloid leukemia correlates with pretreatment risk features and cytogenetic response to interferon-alpha. Blood. 2001;97(11):3648–50.
64. Holtschke T, Lohler J, Kanno Y, Fehr T, Giese N, Rosenbauer F, Lou J, Knobeloch KP, Gabriele L, Waring JF, Bachmann MF, Zinkernagel RM, Morse HC 3rd, Ozato K, Horak I. Immunodeficiency and chronic myelogenous leukemia-like syndrome in mice with a targeted mutation of the ICSBP gene. Cell. 1996;87(2):307–17.
65. Hao SX, Ren R. Expression of interferon consensus sequence binding protein (ICSBP) is downregulated in Bcr-Abl-induced murine chronic myelogenous leukemia-like disease, and forced coexpression of ICSBP inhibits Bcr-Abl-induced myeloproliferative disorder. Mol Cell Biol. 2000;20(4):1149–61.
66. Schmidt M, Hochhaus A, Konig-Merediz SA, Brendel C, Proba J, Hoppe GJ, Wittig B, Ehninger G, Hehlmann R, Neubauer A. Expression of interferon regulatory factor 4 in chronic myeloid leukemia: correlation with response to interferon alfa therapy. J Clin Oncol. 2000;18(19):3331–8.
67. Mittrucker HW, Matsuyama T, Grossman A, Kundig TM, Potter J, Shahinian A, Wakeham A, Patterson B, Ohashi PS, Mak TW. Requirement for the transcription factor LSIRF/IRF4 for mature B and T lymphocyte function. Science. 1997;275(5299):540–3.
68. Hochhaus A, Yan XH, Willer A, Hehlmann R, Gordon MY, Goldman JM, Melo JV. Expression of interferon regulatory factor (IRF) genes and response to interferon-alpha in chronic myeloid leukaemia. Leukemia. 1997;11(7):933–9.
69. Hasselbalch HC. A new era for IFN-alpha in the treatment of Philadelphia-negative chronic myeloproliferative neoplasms. Expert Rev Hematol. 2011;4(6):637–55. https://doi.org/10.1586/EHM.11.63.
70. Simonsson B, Hjorth-Hansen H, Bjerrum OW, Porkka K. Interferon alpha for treatment of chronic myeloid leukemia. Curr Drug Targets. 2011;12(3):420–8.
71. Chronic Myeloid Leukemia Trialists' Collaborative Group. Interferon alfa versus chemotherapy for chronic myeloid leukemia: a meta-analysis of seven randomized trials. J Natl Cancer Inst. 1997;89(21):1616–20.
72. Silver RT, Woolf SH, Hehlmann R, Appelbaum FR, Anderson J, Bennett C, Goldman JM, Guilhot F, Kantarjian HM, Lichtin AE, Talpaz M, Tura S. An evidence-based analysis of the effect of busulfan, hydroxyurea, interferon, and allogeneic bone marrow transplantation in treating the chronic phase of chronic myeloid leukemia: developed for the American Society of Hematology. Blood. 1999;94(5):1517–36.
73. Talpaz M, O'Brien S, Rose E, Gupta S, Shan J, Cortes J, Giles FJ, Faderl S, Kantarjian HM. Phase 1 study of polyethylene glycol formulation of interferon α-2B (Schering 54031) in Philadelphia chromosome-positive chronic myelogenous leukemia. Blood. 2001;98(6):1708–13.
74. Lipton JH, Khoroshko N, Golenkov A, Abdulkadyrov K, Nair K, Raghunadharao D, Brummendorf T, Yoo K, Bergstrom B. Phase II, randomized, multicenter, comparative study of peginterferon–α–2a (40 kD) (Pegasys®) versus interferon α-2a (Roferon®-A) in patients with treatment-naïve, chronic-phase chronic myelogenous leukemia. Leuk Lymphoma. 2007;48(3):497–505. https://doi.org/10.1080/10428190601175393. 773533619 [pii]
75. Michallet M, Maloisel F, Delain M, Hellmann A, Rosas A, Silver RT, Tendler C, Group PE-ICS. Pegylated recombinant interferon alpha-2b vs recombinant interferon alpha-2b for the initial treatment of chronic-phase chronic myelogenous leukemia: a phase III study. Leukemia. 2004;18(2):309–15.
76. Berenguer J, Gonzalez-Garcia J, Lopez-Aldeguer J, Von-Wichmann MA, Quereda C, Hernando A, Sanz J, Tural C, Ortega E, Mallolas J, Santos I, Miralles P, Montes ML, Bellon JM, Esteban H,

cohort GHH. Pegylated interferon {alpha}2a plus ribavirin versus pegylated interferon {alpha}2b plus ribavirin for the treatment of chronic hepatitis C in HIV-infected patients. J Antimicrob Chemother. 2009;63(6):1256–63. https://doi.org/10.1093/jac/dkp106.
77. Laguno M, Cifuentes C, Murillas J, Veloso S, Larrousse M, Payeras A, Bonet L, Vidal F, Milinkovic A, Bassa A, Villalonga C, Perez I, Tural C, Martinez-Rebollar M, Calvo M, Blanco JL, Martinez E, Sanchez-Tapias JM, Gatell JM, Mallolas J. Randomized trial comparing pegylated interferon alpha-2b versus pegylated interferon alpha-2a, both plus ribavirin, to treat chronic hepatitis C in human immunodeficiency virus patients. Hepatology. 2009;49(1):22–31. https://doi.org/10.1002/hep.22598.
78. Scotto G, Fazio V, Fornabaio C, Tartaglia A, Di Tullio R, Saracino A, Angarano G. Early and sustained virological response in non-responders with chronic hepatitis C: a randomized open-label study of pegylated interferon-alpha-2a versus pegylated interferon-alpha-2b. Drugs. 2008;68(6):791–801.
79. Gisslinger H, Klade C, Georgiev P, Krochmalczyk D, Gercheva-Kyuchukova L, Egyed M, Rossiev V, Dulicek P, Illes A, Pylypenko H, Sivcheva L, Mayer J, Yablokova V, Krejcy K, Grohmann-Izay B, Hasselbalch HC, Kralovics R, Kiladjian JJ, Group P-PS. Ropeginterferon alfa-2b versus standard therapy for polycythaemia vera (PROUD-PV and CONTINUATION-PV): a randomised, non-inferiority, phase 3 trial and its extension study. Lancet Haematol. 2020;7(3):e196–208. https://doi.org/10.1016/S2352-3026(19)30236-4.
80. Tremblay D, Mascarenhas J. Novel therapies in polycythemia vera. Curr Hematol Malig Rep. 2020; https://doi.org/10.1007/s11899-020-00564-7.
81. Hasselbalch HC, Holmstrom MO. Perspectives on interferon-alpha in the treatment of polycythemia vera and related myeloproliferative neoplasms: minimal residual disease and cure? Semin Immunopathol. 2019;41(1):5–19. https://doi.org/10.1007/s00281-018-0700-2.
82. Jonasch E, Haluska FG. Interferon in oncological practice: review of interferon biology, clinical applications, and toxicities. Oncologist. 2001;6(1):34–55. https://doi.org/10.1634/theoncologist.6-1-34.
83. Kluin-Nelemans HC, Buck G, le Cessie S, Richards S, Beverloo HB, Falkenburg JH, Littlewood T, Muus P, Bareford D, van der Lelie H, Green AR, Roozendaal KJ, Milne AE, Chapman CS, Shepherd P, Mrc, groups H. Randomized comparison of low-dose versus high-dose interferon-alfa in chronic myeloid leukemia: prospective collaboration of 3 joint trials by the MRC and HOVON groups. Blood. 2004;103(12):4408–15. https://doi.org/10.1182/blood-2003-10-3605.
84. Steegmann JL, Requena MJ, Martin-Regueira P, De La Camara R, Casado F, Salvanes FR, Fernandez Ranada JM. High incidence of autoimmune alterations in chronic myeloid leukemia patients treated with interferon-alpha. Am J Hematol. 2003;72(3):170–6. https://doi.org/10.1002/ajh.10282.
85. Tothova E, Kafkova A, Stecova N, Fricova M, Guman T, Svorcova E. Immune-mediated complications during interferon alpha therapy in chronic myelogenous leukemia. Neoplasma. 2002;49(2):91–4.
86. Herishanu Y, Trestman S, Kirgner I, Rachmani R, Naparstek E. Autoimmune thrombocytopenia in chronic myeloid leukemia treated with interferon-alpha: differential diagnosis and possible pathogenesis. Leuk Lymphoma. 2003;44(12):2103–8. https://doi.org/10.1080/1042819031000123447.
87. Luskin MR. Chronic myeloid leukemia and pregnancy: patient and partner perspectives. Expert Rev Hematol. 2018;11(8):597–9. https://doi.org/10.1080/17474086.2018.1500889.
88. Palani R, Milojkovic D, Apperley JF. Managing pregnancy in chronic myeloid leukaemia. Ann Hematol. 2015;94(Suppl 2):S167–76. https://doi.org/10.1007/s00277-015-2317-z.
89. Yazdani Brojeni P, Matok I, Garcia Bournissen F, Koren G. A systematic review of the fetal safety of interferon alpha. Reprod Toxicol. 2012;33(3):265–8. https://doi.org/10.1016/j.reprotox.2011.11.003.
90. Pye SM, Cortes J, Ault P, Hatfield A, Kantarjian H, Pilot R, Rosti G, Apperley JF. The effects of imatinib on pregnancy outcome. Blood. 2008;111(12):5505–8. https://doi.org/10.1182/blood-2007-10-114900.
91. Law AD, Dong Hwan Kim D, Lipton JH. Pregnancy: part of life in chronic myelogenous leukemia. Leuk Lymphoma. 2017;58(2):280–7. https://doi.org/10.1080/10428194.2016.1201571.
92. Lasica M, Willcox A, Burbury K, Ross DM, Branford S, Butler J, Filshie R, Januszewicz H, Joske D, Mills A, Simpson D, Tam C, Taylor K, Watson AM, Wolf M, Grigg A. The effect of tyrosine kinase inhibitor interruption and interferon use on pregnancy outcomes and long-term disease control in chronic myeloid leukemia. Leuk Lymphoma. 2019;60(7):1796–802. https://doi.org/10.1080/10428194.2018.1551533.
93. Milojkovic D, Apperley JF. How I treat leukemia during pregnancy. Blood. 2014;123(7):974–84. https://doi.org/10.1182/blood-2013-08-283580.
94. Berman E. Pregnancy in patients with chronic myeloid leukemia. J Natl Compr Cancer Netw. 2018;16(5S):660–2. https://doi.org/10.6004/jnccn.2018.0035.
95. Kantarjian HM, O'Brien S, Cortes JE, Shan J, Giles FJ, Rios MB, Faderl SH, Wierda WG, Ferrajoli A, Verstovsek S, Keating MJ, Freireich EJ, Talpaz M. Complete cytogenetic and molecular responses to interferon-alpha-based therapy for chronic myelogenous leukemia are associated with excellent long-term prognosis. Cancer. 2003;97(4):1033–41.
96. Bonifazi F, de Vivo A, Rosti G, Guilhot F, Guilhot J, Trabacchi E, Hehlmann R, Hochhaus A, Shepherd PC, Steegmann JL, Kluin-Nelemans HC, Thaler J, Simonsson B, Louwagie A, Reiffers J, Mahon FX,

Montefusco E, Alimena G, Hasford J, Richards S, Saglio G, Testoni N, Martinelli G, Tura S, Baccarani M. Chronic myeloid leukemia and interferon-α: a study of complete cytogenetic responders. Blood. 2001;98(10):3074–81.
97. Malagola M, Breccia M, Skert C, Cancelli V, Soverini S, Iacobucci I, Cattina F, Liberati AM, Tiribelli M, Annunziata M, Trabacchi E, De Vivo A, Castagnetti F, Martinelli G, Fogli M, Stagno F, Pica G, Iurlo A, Pregno P, Abruzzese E, Pardini S, Bocchia M, Russo S, Pierri I, Lunghi M, Barulli S, Merante S, Mandelli F, Alimena G, Rosti G, Baccarani M, Russo D. Long term outcome of Ph+ CML patients achieving complete cytogenetic remission with interferon based therapy moving from interferon to imatinib era. Am J Hematol. 2014;89(2):119–24. https://doi.org/10.1002/ajh.23593.
98. Mahon FX, Delbrel X, Cony-Makhoul P, Faberes C, Boiron JM, Barthe C, Bilhou-Nabera C, Pigneux A, Marit G, Reiffers J. Follow-up of complete cytogenetic remission in patients with chronic myeloid leukemia after cessation of interferon alfa. J Clin Oncol. 2002;20(1):214–20.
99. Veneri D, Tecchio C, De Matteis G, Paviati E, Benati M, Franchini M, Pizzolo G. Long-term persistence of molecular response after discontinuation of interferon-alpha in two patients with chronic myeloid leukaemia. Blood Transfus = Trasfusione del sangue. 2012;10(2):233–4.
100. Talpaz M, Estrov Z, Kantarjian H, Ku S, Foteh A, Kurzrock R. Persistence of dormant leukemic progenitors during interferon-induced remission in chronic myelogenous leukemia. Analysis by polymerase chain reaction of individual colonies. J Clin Invest. 1994;94(4):1383–9.
101. Latagliata R, Romano A, Mancini M, Breccia M, Carmosino I, Vozella F, Montagna C, Volpicelli P, De Angelis F, Petrucci L, Serrao A, Molica M, Salaroli A, Diverio D, Alimena G. Discontinuation of alpha-interferon treatment in patients with chronic myeloid leukemia in long-lasting complete molecular response. Leuk Lymphoma. 2016;57(1):99–102. https://doi.org/10.3109/10428194.2015.1043548.
102. Hochhaus A, Reiter A, Saussele S, Reichert A, Emig M, Kaeda J, Schultheis B, Berger U, Shepherd PC, Allan NC, Hehlmann R, Goldman JM, Cross NC. Molecular heterogeneity in complete cytogenetic responders after interferon-α therapy for chronic myelogenous leukemia: low levels of minimal residual disease are associated with continuing remission. Blood. 2000;95(1):62–6.
103. Chomel JC, Brizard F, Veinstein A, Rivet J, Sadoun A, Kitzis A, Guilhot F, Brizard A. Persistence of BCR-ABL genomic rearrangement in chronic myeloid leukemia patients in complete and sustained cytogenetic remission after interferon-alpha therapy or allogeneic bone marrow transplantation. Blood. 2000;95(2):404–8.
104. Mahon FX, Rea D, Guilhot J, Guilhot F, Huguet F, Nicolini F, Legros L, Charbonnier A, Guerci A, Varet B, Etienne G, Reiffers J, Rousselot P. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre stop Imatinib (STIM) trial. Lancet Oncol. 2010;11(11):1029–35. https://doi.org/10.1016/S1470-2045(10)70233-3.
105. Rowley JD. Letter: a new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature. 1973;243(5405):290–3.
106. de Klein A, van Kessel AG, Grosveld G, Bartram CR, Hagemeijer A, Bootsma D, Spurr NK, Heisterkamp N, Groffen J, Stephenson JR. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature. 1982;300(5894):765–7.
107. Shtivelman E, Lifshitz B, Gale RP, Canaani E. Fused transcript of abl and bcr genes in chronic myelogenous leukaemia. Nature. 1985;315(6020):550–4.
108. Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science. 1990;247(4944):824–30.
109. Heisterkamp N, Jenster G, ten Hoeve J, Zovich D, Pattengale PK, Groffen J. Acute leukaemia in bcr/abl transgenic mice. Nature. 1990;344(6263):251–3.
110. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, Cornelissen JJ, Fischer T, Hochhaus A, Hughes T, Lechner K, Nielsen JL, Rousselot P, Reiffers J, Saglio G, Shepherd J, Simonsson B, Gratwohl A, Goldman JM, Kantarjian H, Taylor K, Verhoef G, Bolton AE, Capdeville R, Druker BJ, Investigators I. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2003;348(11):994–1004.
111. Hochhaus A, Larson RA, Guilhot F, Radich JP, Branford S, Hughes TP, Baccarani M, Deininger MW, Cervantes F, Fujihara S, Ortmann CE, Menssen HD, Kantarjian H, O'Brien SG, Druker BJ, Investigators I. Long-term outcomes of Imatinib treatment for chronic myeloid leukemia. N Engl J Med. 2017;376(10):917–27. https://doi.org/10.1056/NEJMoa1609324.
112. Deininger M, O'Brien SG, Guilhot F, Goldman JM, Hochhaus A, Hughes TP, Radich JP, Hatfield AK, Mone M, Filian J, Reynolds J, Gathmann I, Larson RA, Druker BJ. International randomized study of interferon vs STI571 (IRIS) 8-year follow up: sustained survival and low risk for progression or events in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP) treated with imatinib. Blood. 2009;114:22. (abstract [1126])
113. Hochhaus A, O'Brien SG, Guilhot F, Druker BJ, Branford S, Foroni L, Goldman JM, Muller MC, Radich JP, Rudoltz M, Mone M, Gathmann I, Hughes TP, Larson RA, Investigators I. Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. Leukemia. 2009;23(6):1054–61. https://doi.org/10.1038/leu.2009.38.

114. Hehlmann R, Lauseker M, Jung-Munkwitz S, Leitner A, Muller MC, Pletsch N, Proetel U, Haferlach C, Schlegelberger B, Balleisen L, Hanel M, Pfirrmann M, Krause SW, Nerl C, Pralle H, Gratwohl A, Hossfeld DK, Hasford J, Hochhaus A, Saussele S. Tolerability-adapted imatinib 800 mg/d versus 400 mg/d versus 400 mg/d plus interferon-alpha in newly diagnosed chronic myeloid leukemia. J Clin Oncol. 2011;29(12):1634–42. https://doi.org/10.1200/JCO.2010.32.0598.
115. Hehlmann R, Muller MC, Lauseker M, Hanfstein B, Fabarius A, Schreiber A, Proetel U, Pletsch N, Pfirrmann M, Haferlach C, Schnittger S, Einsele H, Dengler J, Falge C, Kanz L, Neubauer A, Kneba M, Stegelmann F, Pfreundschuh M, Waller CF, Spiekermann K, Baerlocher GM, Ehninger G, Heim D, Heimpel H, Nerl C, Krause SW, Hossfeld DK, Kolb HJ, Hasford J, Saussele S, Hochhaus A. Deep molecular response is reached by the majority of patients treated with imatinib, predicts survival, and is achieved more quickly by optimized high-dose imatinib: results from the randomized CML-study IV. J Clin Oncol. 2014;32(5):415–23. https://doi.org/10.1200/JCO.2013.49.9020.
116. Hehlmann R, Lauseker M, Saussele S, Pfirrmann M, Krause S, Kolb HJ, Neubauer A, Hossfeld DK, Nerl C, Gratwohl A, Baerlocher GM, Heim D, Brummendorf TH, Fabarius A, Haferlach C, Schlegelberger B, Muller MC, Jeromin S, Proetel U, Kohlbrenner K, Voskanyan A, Rinaldetti S, Seifarth W, Spiess B, Balleisen L, Goebeler MC, Hanel M, Ho A, Dengler J, Falge C, Kanz L, Kremers S, Burchert A, Kneba M, Stegelmann F, Kohne CA, Lindemann HW, Waller CF, Pfreundschuh M, Spiekermann K, Berdel WE, Muller L, Edinger M, Mayer J, Beelen DW, Bentz M, Link H, Hertenstein B, Fuchs R, Wernli M, Schlegel F, Schlag R, de Wit M, Trumper L, Hebart H, Hahn M, Thomalla J, Scheid C, Schafhausen P, Verbeek W, Eckart MJ, Gassmann W, Pezzutto A, Schenk M, Brossart P, Geer T, Bildat S, Schafer E, Hochhaus A, Hasford J. Assessment of imatinib as first-line treatment of chronic myeloid leukemia: 10-year survival results of the randomized CML study IV and impact of non-CML determinants. Leukemia. 2017;31(11):2398–406. https://doi.org/10.1038/leu.2017.253.
117. Preudhomme C, Guilhot J, Nicolini FE, Guerci-Bresler A, Rigal-Huguet F, Maloisel F, Coiteux V, Gardembas M, Berthou C, Vekhoff A, Rea D, Jourdan E, Allard C, Delmer A, Rousselot P, Legros L, Berger M, Corm S, Etienne G, Roche-Lestienne C, Eclache V, Mahon FX, Guilhot F. Imatinib plus peginterferon alfa-2a in chronic myeloid leukemia. N Engl J Med. 2010;363(26):2511–21. https://doi.org/10.1056/NEJMoa1004095.
118. Johnson-Ansah H, Guilhot J, Rousselot P, Rea D, Legros L, Rigal-Huguet F, Nicolini FE, Mahon FX, Preudhomme C, Guilhot F. Tolerability and efficacy of pegylated interferon-alpha-2a in combination with imatinib for patients with chronic-phase chronic myeloid leukemia. Cancer. 2013;119(24):4284–9. https://doi.org/10.1002/cncr.28328.
119. Simonsson B, Gedde-Dahl T, Markevärn B, Remes K, Stentoft J, Almqvist A, Bjoreman M, Flogegard M, Koskenveesa P, Lindblom A, Malm C, Mustjoki S, Myhr-Eriksson K, Ohm L, Rasanen A, Sinisalo M, Sjalander A, Stromberg U, Weiss Bjerrum O, Ehrencrona H, Gruber F, Kairisto V, Olsson K, Sandin F, Nagler A, Lanng Nielsen J, Hjorth-Hansen H, Porkka K. Combination of pegylated IFN-α2b with imatinib increases molecular response rates in patients with low- or intermediate-risk chronic myeloid leukemia. Blood. 2011;118:3228–35. https://doi.org/10.1182/blood-2011-02-336685.
120. Cortes J, Quintas-Cardama A, Jones D, Ravandi F, Garcia-Manero G, Verstovsek S, Koller C, Hiteshew J, Shan J, O'Brien S, Kantarjian H. Immune modulation of minimal residual disease in early chronic phase chronic myelogenous leukemia: a randomized trial of frontline high-dose imatinib mesylate with or without pegylated interferon alpha-2b and granulocyte-macrophage colony-stimulating factor. Cancer. 2011;117(3):572–80. https://doi.org/10.1002/cncr.25438.
121. Palandri F, Iacobucci I, Castagnetti F, Testoni N, Poerio A, Amabile M, Breccia M, Intermesoli T, Iuliano F, Rege-Cambrin G, Tiribelli M, Miglino M, Pane F, Saglio G, Martinelli G, Rosti G, Baccarani M. Front-line treatment of Philadelphia positive chronic myeloid leukemia with imatinib and interferon-α: 5-year outcome. Haematologica. 2008;93(5):770–4.
122. Baccarani M, Martinelli G, Rosti G, Trabacchi E, Testoni N, Bassi S, Amabile M, Soverini S, Castagnetti F, Cilloni D, Izzo B, de Vivo A, Messa E, Bonifazi F, Poerio A, Luatti S, Giugliano E, Alberti D, Fincato G, Russo D, Pane F, Saglio G. Imatinib and pegylated human recombinant interferon-α2b in early chronic-phase chronic myeloid leukemia. Blood. 2004;104(13):4245–51.
123. Palandri F, Castagnetti F, Iacobucci I, Martinelli G, Amabile M, Gugliotta G, Poerio A, Testoni N, Breccia M, Bocchia M, Crugnola M, Rege-Cambrin G, Martino B, Pierri I, Radaelli F, Specchia G, Pane F, Saglio G, Rosti G, Baccarani M. The response to imatinib and interferon-α is more rapid than the response to imatinib alone: a retrospective analysis of 495 Philadelphia-positive chronic myeloid leukemia patients in early chronic phase. Haematologica. 2010;95(8):1415–9.
124. Chamoun K, Kantarjian H, Atallah R, Gonzalez GN, Issa GC, Rios MB, Garcia-Manero G, Borthakur G, Ravandi F, Jain N, Daver N, Konopleva M, DiNardo CD, Kadia T, Pemmaraju N, Jabbour E, Cortes J. Tyrosine kinase inhibitor discontinuation in patients with chronic myeloid leukemia: a single-institution experience. J Hematol Oncol. 2019;12(1):1. https://doi.org/10.1186/s13045-018-0686-1.
125. Webster JA, Ferguson A, Gocke C, Jones RJ, Levitsky H, Smith BD. A randomized phase II trial of interferon (IFN)/GM-CSF versus K562/GM-CSF

vaccination in chronic phase CML patients on front-line tyrosine kinase inhibitor (TKI) therapy: potential of IFN to enhance molecular remissions. Blood. 2016;128(22):3088. https://doi.org/10.1182/blood.V128.22.3088.3088.
126. Hjorth-Hansen H, Stentoft J, Richter J, Koskenvesa P, Hoglund M, Dreimane A, Porkka K, Gedde-Dahl T, Gjertsen BT, Gruber FX, Stenke L, Eriksson KM, Markevarn B, Lubking A, Vestergaard H, Udby L, Bjerrum OW, Persson I, Mustjoki S, Olsson-Stromberg U. Safety and efficacy of the combination of pegylated interferon-alpha2b and dasatinib in newly diagnosed chronic-phase chronic myeloid leukemia patients. Leukemia. 2016;30(9):1853–60. https://doi.org/10.1038/leu.2016.121.
127. Nicolini FE, Etienne G, Huguet F, Guerci-Bresler A, Charbonnier A, Escoffre-Barbe M, Dubruille V, Johnson-Ansah H, Legros L, Coiteux V, Cony-Makhoul P, Lenain P, Roy L, Rousselot P, Guyotat D, Ianotto J-C, Gardembas M, Deconinck E, Larosa F, Caillot D, Turlure P, Courby S, Quittet P, Hermet E, Ame S, Lapusan S, Deloire A, Morisset S, Etienne M, Rea D, Dulucq S, Mahon F-X. The combination of Nilotinib + Pegylated IFN Alpha 2a provides somewhat higher cumulative incidence rates of MR4.5 at M36 versus nilotinib alone in newly diagnosed CP CML patients. Updated Results of the Petals Phase III National Study. Blood. 2019;134(Supplement_1):494. https://doi.org/10.1182/blood-2019-123674.
128. de Lavallade H, Jackson S, Kizilors A, Etienne G, Huguet F, Guerci-Bresler A, Rea D, Chollet C, Morisset S, Robbesyn F, Mahon F-X, Dulucq S, Nicolini FE. Prospective evaluation of ABL kinase domain mutational analysis by next-generation-sequencing in newly diagnosed CP CML patients undergoing first-line treatment with Nilotinib alone or Nilotinib + Pegylated interferon-α2a in a prospective phase III trial. Blood. 2019;134(Supplement_1):664. https://doi.org/10.1182/blood-2019-125108.
129. Hochhaus A, Burchert A, Saussele S, Baerlocher GM, Brümmendorf TH, La Rosée P, Heim D, Krause SW, le Coutre PD, Niederwieser D, Lange T, Fabarius A, Hänel M, Stegelmann F, Mayer J, Gil A, Himsel D, Hasford J, Hehlmann R, Ernst T, Fabisch C, Pfirrmann M. Nilotinib vs nilotinib plus pegylated interferon α (peg-IFN) induction and Nilotinib or peg-IFN maintenance therapy for newly diagnosed BCR-ABL1 positive chronic myeloid Leukemia patients in chronic phase (TIGER study): The addition of peg-IFN is associated with higher rates of deep molecular response. Blood. 2019;134(Supplement_1):495. https://doi.org/10.1182/blood-2019-130043.
130. Nicolini FE, Etienne G, Dubruille V, Roy L, Huguet F, Legros L, Giraudier S, Coiteux V, Guerci-Bresler A, Lenain P, Cony-Makhoul P, Gardembas M, Hermet E, Rousselot P, Ame S, Gagnieu MC, Pivot C, Hayette S, Maguer-Satta V, Etienne M, Dulucq S, Rea D, Mahon FX. Nilotinib and peginterferon alfa-2a for newly diagnosed chronic-phase chronic myeloid leukaemia (NiloPeg): a multicentre, non-randomised, open-label phase 2 study. Lancet Haematol. 2015;2(1):e37–46. https://doi.org/10.1016/S2352-3026(14)00027-1.
131. Nicolini FE, Etienne G, Huguet F, Guerci-Bresler A, Charbonnier A, Escoffre-Barbe M, Dubruille V, Johnson-Ansah H, Legros L, Coiteux V, Cony-Makhoul P, Lenain P, Roy L, Rousselot P, Guyotat D, Ianotto J-C, Gardembas M, Larosa F, Caillot D, Turlure P, Courby S, Quittet P, Hermet E, Ame S, Lapusan S, Schwiertz V, Morisset S, Etienne M, Rea D, Dulucq S, Mahon F-X. Nilotinib versus Nilotinib combined to Pegylated-interferon alfa 2a in first-line chronic phase chronic myelogenous Leukemia patients. Interim analysis of a phase III trial. Blood. 2017;130(Supplement 1):899. https://doi.org/10.1182/blood.V130.Suppl_1.899.899.
132. Yeung DT, Grigg AP, Shanmuganathan N, Cunningham I, Shortt J, Rowling P, Reynolds J, Cushion R, Harrup RA, Ross DM, Kipp D, Mills AK, Arthur CK, Schwarer AP, Jackson K, Viiala N, Weinkove R, Yong ASM, White DL, Branford S, Hughes TP, ALLG OBot. Combination of Nilotinib and Pegylated Interferon Alfa-2b Results in High Molecular Response Rates in Chronic Phase CML: Interim Results of the ALLG CML 11 Pinnacle Study. Blood. 2018;132(Supplement 1):459. https://doi.org/10.1182/blood-2018-99-110569.
133. Roy L, Chomel J-C, Guilhot J, Guerci-Bresler A, Escoffre-Barbe M, Giraudier S, Charbonnier A, Dubruille V, Huguet F, Johnson-Ansah H, Lenain P, Amé S, Etienne G, Nicolini FE, Rea D, Cony-Makhoul P, Courby S, Ianotto J-C, Legros L, Delain M, Coiteux V, Hermet E, Gardembas M, Molimard M, Cayuela J-M, Thibaud M, Duranton S, Mahon F-X, Rousselot P, Guilhot F. Combination of dasatinib and peg-interferon alpha 2b in chronic phase chronic myeloid leukemia (CP-CML) first line: preliminary results of a phase II trial, from the French Intergroup of CML (fi-LMC). Blood. 2015;126(23):134. https://doi.org/10.1182/blood.V126.23.134.134.
134. Bedi A, Griffin CA, Barber JP, Vala MS, Hawkins AL, Sharkis SJ, Zehnbauer BA, Jones RJ. Growth factor-mediated terminal differentiation of chronic myeloid leukemia. Cancer Res. 1994;54(21):5535–8.
135. Angstreich GR, Matsui W, Huff CA, Vala MS, Barber J, Hawkins AL, Griffin CA, Smith BD, Jones RJ. Effects of imatinib and interferon on primitive chronic myeloid leukaemia progenitors. Br J Haematol. 2005;130(3):373–81.
136. Paquette RL, Hsu N, Said J, Mohammed M, Rao NP, Shih G, Schiller G, Sawyers C, Glaspy JA. Interferon-α induces dendritic cell differentiation of CML mononuclear cells in vitro and in vivo. Leukemia. 2002;16(8):1484–9.
137. Zeidner JF, Gladstone DE, Zahurak M, Matsui WH, Gocke C, Jones RJ, Smith BD. Granulocyte-macrophage colony stimulating factor (GM-CSF)

enhances the clinical responses to interferon-alpha (IFN) in newly diagnosed chronic myeloid leukemia (CML). Leuk Res. 2014;38(8):886–90. https://doi.org/10.1016/j.leukres.2014.05.012.

138. Cortes J, Kantarjian H, O'Brien S, Kurzrock R, Keating M, Talpaz M. GM-CSF can improve the cytogenetic response obtained with interferon-alpha therapy in patients with chronic myelogenous leukemia. Leukemia. 1998;12(6):860–4.
139. El Eit RM, Iskandarani AN, Saliba JL, Jabbour MN, Mahfouz RA, Bitar NM, Ayoubi HR, Zaatari GS, Mahon FX, De The HB, Bazarbachi AA, Nasr RR. Effective targeting of chronic myeloid leukemia initiating activity with the combination of arsenic trioxide and interferon alpha. Int J Cancer. 2014;134(4):988–96. https://doi.org/10.1002/ijc.28427.
140. El Eit R, Itani AR, Nassar F, Rasbieh N, Jabbour M, Santina A, Zaatari G, Mahon FX, Bazarbachi A, Nasr R. Antitumor efficacy of arsenic/interferon in preclinical models of chronic myeloid leukemia resistant to tyrosine kinase inhibitors. Cancer. 2019;125(16):2818–28. https://doi.org/10.1002/cncr.32130.
141. Burchert A, Saussele S, Eigendorff E, Muller MC, Sohlbach K, Inselmann S, Schutz C, Metzelder SK, Ziermann J, Kostrewa P, Hoffmann J, Hehlmann R, Neubauer A, Hochhaus A. Interferon alpha 2 maintenance therapy may enable high rates of treatment discontinuation in chronic myeloid leukemia. Leukemia. 2015;29(6):1331–5. https://doi.org/10.1038/leu.2015.45.
142. Hardan I, Stanevsky A, Volchek Y, Tohami T, Amariglio N, Trakhtenbrot L, Koren-Michowitz M, Shimoni A, Nagler A. Treatment with interferon alpha prior to discontinuation of imatinib in patients with chronic myeloid leukemia. Cytokine. 2012;57(2):290–3.
143. Bezerra ED, Flowers ME, Onstad LE, Chielens D, Radich J, Higano CS. A phase 2 study of alpha interferon for molecularly measurable residual disease in chronic myeloid leukemia after allogeneic hematopoietic cell transplantation. Leuk Lymphoma. 2019;60(11):2754–61. https://doi.org/10.1080/10428194.2019.1605508.
144. Kiladjian JJ, Cassinat B, Chevret S, Turlure P, Cambier N, Roussel M, Bellucci S, Grandchamp B, Chomienne C, Fenaux P. Pegylated interferon-alfa-2a induces complete hematologic and molecular responses with low toxicity in polycythemia vera. Blood. 2008;112(8):3065–72. https://doi.org/10.1182/blood-2008-03-143537.
145. Kiladjian JJ, Cassinat B, Turlure P, Cambier N, Roussel M, Bellucci S, Menot ML, Massonnet G, Dutel JL, Ghomari K, Rousselot P, Grange MJ, Chait Y, Vainchenker W, Parquet N, Abdelkader-Aljassem L, Bernard JF, Rain JD, Chevret S, Chomienne C, Fenaux P. High molecular response rate of polycythemia vera patients treated with pegylated interferon alpha-2a. Blood. 2006;108(6):2037–40. https://doi.org/10.1182/blood-2006-03-009860.
146. Quintas-Cardama A, Kantarjian H, Manshouri T, Luthra R, Estrov Z, Pierce S, Richie MA, Borthakur G, Konopleva M, Cortes J, Verstovsek S. Pegylated interferon alfa-2a yields high rates of hematologic and molecular response in patients with advanced essential thrombocythemia and polycythemia vera. J Clin Oncol. 2009;27(32):5418–24. https://doi.org/10.1200/JCO.2009.23.6075.
147. Quintas-Cardama A, Abdel-Wahab O, Manshouri T, Kilpivaara O, Cortes J, Roupie AL, Zhang SJ, Harris D, Estrov Z, Kantarjian H, Levine RL, Verstovsek S. Molecular analysis of patients with polycythemia vera or essential thrombocythemia receiving pegylated interferon alpha-2a. Blood. 2013;122(6):893–901. https://doi.org/10.1182/blood-2012-07-442012.
148. Stauffer Larsen T, Iversen KF, Hansen E, Mathiasen AB, Marcher C, Frederiksen M, Larsen H, Helleberg I, Riley CH, Bjerrum OW, Ronnov-Jessen D, Moller MB, de Stricker K, Vestergaard H, Hasselbalch HC. Long term molecular responses in a cohort of Danish patients with essential thrombocythemia, polycythemia vera and myelofibrosis treated with recombinant interferon alpha. Leuk Res. 2013;37(9):1041–5. https://doi.org/10.1016/j.leukres.2013.06.012.
149. Larsen TS, Bjerrum OW, Pallisgaard N, Andersen MT, Moller MB, Hasselbalch HC. Sustained major molecular response on interferon alpha-2b in two patients with polycythemia vera. Ann Hematol. 2008;87(10):847–50. https://doi.org/10.1007/s00277-008-0498-4.
150. Larsen TS, Moller MB, de Stricker K, Norgaard P, Samuelsson J, Marcher C, Andersen MT, Bjerrum OW, Hasselbalch HC. Minimal residual disease and normalization of the bone marrow after long-term treatment with alpha-interferon2b in polycythemia vera. A report on molecular response patterns in seven patients in sustained complete hematological remission. Hematology. 2009;14(6):331–4. https://doi.org/10.1179/102453309X12473408860587.
151. Utke Rank C, Weis Bjerrum O, Larsen TS, Kjaer L, de Stricker K, Riley CH, Hasselbalch HC. Minimal residual disease after long-term interferon-alpha2 treatment: a report on hematological, molecular and histomorphological response patterns in 10 patients with essential thrombocythemia and polycythemia vera. Leuk Lymphoma. 2016;57(2):348–54. https://doi.org/10.3109/10428194.2015.1049171.
152. Mascarenhas J, Kosiorek HE, Prchal JT, Rambaldi A, Berenzon D, Yacoub A, Harrison CN, McMullin MF, Vannucchi AM, Ewing J, O'Connell CL, Kiladjian J-J, Mead AJ, Winton EF, Leibowitz DS, De Stefano V, Arcasoy MO, Kessler CM, Catchatourian R, Rondelli D, Silver RT, Bacigalupo A, Nagler A, Kremyanskaya M, Sandy L, Salama ME, Najfeld V, Tripodi J, Weinberg RS, Price L, Goldberg JD, Rampal RK, Mesa RA, Dueck AC, Hoffman R. Results of the myeloproliferative neoplasms - research consortium (MPN-RC)

112 randomized trial of Pegylated interferon alfa-2a (PEG) versus hydroxyurea (HU) therapy for the treatment of high risk Polycythemia Vera (PV) and high risk essential thrombocythemia (ET). Blood. 2018;132(Supplement 1):577. https://doi.org/10.1182/blood-2018-99-111946.
153. Silver RT, Vandris K, Goldman JJ. Recombinant interferon-alpha may retard progression of early primary myelofibrosis: a preliminary report. Blood. 2011;117(24):6669–72. https://doi.org/10.1182/blood-2010-11-320069.
154. Gowin K, Thapaliya P, Samuelson J, Harrison C, Radia D, Andreasson B, Mascarenhas J, Rambaldi A, Barbui T, Rea CJ, Camoriano J, Gentry A, Kiladjian JJ, O'Connell C, Mesa R. Experience with pegylated interferon alpha-2a in advanced myeloproliferative neoplasms in an international cohort of 118 patients. Haematologica. 2012;97(10):1570–3. https://doi.org/10.3324/haematol.2011.061390.
155. Ianotto JC, Kiladjian JJ, Demory JL, Roy L, Boyer F, Rey J, Dupriez B, Berthou C, Abgrall JF. PEG-IFN-alpha-2a therapy in patients with myelofibrosis: a study of the French Groupe d'Etudes des Myelofibroses (GEM) and France Intergroupe des syndromes Myeloproliferatifs (FIM). Br J Haematol. 2009;146(2):223–5. https://doi.org/10.1111/j.1365-2141.2009.07745.x.
156. Ianotto JC, Boyer-Perrard F, Gyan E, Laribi K, Cony-Makhoul P, Demory JL, De Renzis B, Dosquet C, Rey J, Roy L, Dupriez B, Knoops L, Legros L, Malou M, Hutin P, Ranta D, Schoenwald M, Andreoli A, Abgrall JF, Kiladjian JJ. Efficacy and safety of pegylated-interferon alpha-2a in myelofibrosis: a study by the FIM and GEM French cooperative groups. Br J Haematol. 2013;162(6):783–91. https://doi.org/10.1111/bjh.12459.
157. Mikkelsen SU, Kjaer L, Bjorn ME, Knudsen TA, Sorensen AL, Andersen CBL, Bjerrum OW, Brochmann N, Fassi DE, Kruse TA, Larsen TS, Mourits-Andersen HT, Nielsen CH, Pallisgaard N, Thomassen M, Skov V, Hasselbalch HC. Safety and efficacy of combination therapy of interferon-alpha2 and ruxolitinib in polycythemia vera and myelofibrosis. Cancer Med. 2018;7(8):3571–81. https://doi.org/10.1002/cam4.1619.
158. Fish EN, Platanias LC. Interferon receptor signaling in malignancy: a network of cellular pathways defining biological outcomes. Mol Cancer Res. 2014;12(12):1691–703. https://doi.org/10.1158/1541-7786.MCR-14-0450.
159. Hervas-Stubbs S, Perez-Gracia JL, Rouzaut A, Sanmamed MF, Le Bon A, Melero I. Direct effects of type I interferons on cells of the immune system. Clin Cancer Res. 2011;17(9):2619–27. https://doi.org/10.1158/1078-0432.CCR-10-1114.
160. Bio-Techne (2018) Type I interferon signaling pathways. Available via R&D Systems. https://www.rndsystems.com/pathways/type-i-interferon-signaling-pathways.
161. Liu Y, George CX, Patterson JB, Samuel CE. Functionally distinct double-stranded RNA-binding domains associated with alternative splice site variants of the interferon-inducible double-stranded RNA-specific adenosine deaminase. J Biol Chem. 1997;272(7):4419–28.
162. Cheon H, Borden EC, Stark GR. Interferons and their stimulated genes in the tumor microenvironment. Semin Oncol. 2014;41(2):156–73. https://doi.org/10.1053/j.seminoncol.2014.02.002.
163. Guenzi E, Topolt K, Lubeseder-Martellato C, Jorg A, Naschberger E, Benelli R, Albini A, Sturzl M. The guanylate binding protein-1 GTPase controls the invasive and angiogenic capability of endothelial cells through inhibition of MMP-1 expression. EMBO J. 2003;22(15):3772–82. https://doi.org/10.1093/emboj/cdg382.
164. Raffaella R, Gioia D, De Andrea M, Cappello P, Giovarelli M, Marconi P, Manservigi R, Gariglio M, Landolfo S. The interferon-inducible IFI16 gene inhibits tube morphogenesis and proliferation of primary, but not HPV16 E6/E7-immortalized human endothelial cells. Exp Cell Res. 2004;293(2):331–45.
165. Martensen PM, Sogaard TM, Gjermandsen IM, Buttenschon HN, Rossing AB, Bonnevie-Nielsen V, Rosada C, Simonsen JL, Justesen J. The interferon alpha induced protein ISG12 is localized to the nuclear membrane. Eur J Biochem. 2001;268(22):5947–54.
166. Cheon H, Holvey-Bates EG, Schoggins JW, Forster S, Hertzog P, Imanaka N, Rice CM, Jackson MW, Junk DJ, Stark GR. IFNbeta-dependent increases in STAT1, STAT2, and IRF9 mediate resistance to viruses and DNA damage. EMBO J. 2013;32(20):2751–63. https://doi.org/10.1038/emboj.2013.203.
167. Sarkar SN, Sen GC. Novel functions of proteins encoded by viral stress-inducible genes. Pharmacol Ther. 2004;103(3):245–59. https://doi.org/10.1016/j.pharmthera.2004.07.007.
168. Wang C, Pflugheber J, Sumpter R Jr, Sodora DL, Hui D, Sen GC, Gale M Jr. Alpha interferon induces distinct translational control programs to suppress hepatitis C virus RNA replication. J Virol. 2003;77(7):3898–912.
169. Lai KC, Liu CJ, Chang KW, Lee TC. Depleting IFIT2 mediates atypical PKC signaling to enhance the migration and metastatic activity of oral squamous cell carcinoma cells. Oncogene. 2013;32(32):3686–97. https://doi.org/10.1038/onc.2012.384.
170. Lucas M, Schachterle W, Oberle K, Aichele P, Diefenbach A. Dendritic cells prime natural killer cells by trans-presenting interleukin 15. Immunity. 2007;26(4):503–17. https://doi.org/10.1016/j.immuni.2007.03.006.
171. Honda K, Taniguchi T. IRFs: master regulators of signalling by toll-like receptors and cytosolic pattern-recognition receptors. Nat Rev Immunol. 2006;6(9):644–58. https://doi.org/10.1038/nri1900.

172. Khoo JJ, Forster S, Mansell A. Toll-like receptors as interferon-regulated genes and their role in disease. J Interferon Cytokine Res. 2011;31(1):13–25. https://doi.org/10.1089/jir.2010.0095.
173. Ritchie KJ, Hahn CS, Kim KI, Yan M, Rosario D, Li L, de la Torre JC, Zhang DE. Role of ISG15 protease UBP43 (USP18) in innate immunity to viral infection. Nat Med. 2004;10(12):1374–8. https://doi.org/10.1038/nm1133.
174. Potu H, Sgorbissa A, Brancolini C. Identification of USP18 as an important regulator of the susceptibility to IFN-alpha and drug-induced apoptosis. Cancer Res. 2010;70(2):655–65. https://doi.org/10.1158/0008-5472.CAN-09-1942.
175. D'Cunha J, Knight E Jr, Haas AL, Truitt RL, Borden EC. Immunoregulatory properties of ISG15, an interferon-induced cytokine. Proc Natl Acad Sci U S A. 1996;93(1):211–5.
176. Schiavoni G, Mattei F, Gabriele L. Type I interferons as stimulators of DC-mediated cross-priming: impact on anti-tumor response. Front Immunol. 2013;4:483. https://doi.org/10.3389/fimmu.2013.00483.
177. Cresswell P. Intracellular surveillance: controlling the assembly of MHC class I-peptide complexes. Traffic. 2000;1(4):301–5.
178. Mushinski JF, Nguyen P, Stevens LM, Khanna C, Lee S, Chung EJ, Lee MJ, Kim YS, Linehan WM, Horisberger MA, Trepel JB. Inhibition of tumor cell motility by the interferon-inducible GTPase MxA. J Biol Chem. 2009;284(22):15206–14. https://doi.org/10.1074/jbc.M806324200.
179. Zhou A, Paranjape J, Brown TL, Nie H, Naik S, Dong B, Chang A, Trapp B, Fairchild R, Colmenares C, Silverman RH. Interferon action and apoptosis are defective in mice devoid of 2′,5′-oligoadenylate-dependent RNase L. EMBO J. 1997;16(21):6355–63. https://doi.org/10.1093/emboj/16.21.6355.
180. Malathi K, Dong B, Gale M Jr, Silverman RH. Small self-RNA generated by RNase L amplifies antiviral innate immunity. Nature. 2007;448(7155):816–9. https://doi.org/10.1038/nature06042.
181. Castelli JC, Hassel BA, Wood KA, Li XL, Amemiya K, Dalakas MC, Torrence PF, Youle RJ. A study of the interferon antiviral mechanism: apoptosis activation by the 2-5A system. J Exp Med. 1997;186(6):967–72.
182. Meurs E, Chong K, Galabru J, Thomas NS, Kerr IM, Williams BR, Hovanessian AG. Molecular cloning and characterization of the human double-stranded RNA-activated protein kinase induced by interferon. Cell. 1990;62(2):379–90.
183. Williams BR. PKR; a sentinel kinase for cellular stress. Oncogene. 1999;18(45):6112–20. https://doi.org/10.1038/sj.onc.1203127.
184. Zhou Q, Zhao J, Al-Zoghaibi F, Zhou A, Wiedmer T, Silverman RH, Sims PJ. Transcriptional control of the human plasma membrane phospholipid scramblase 1 gene is mediated by interferon-alpha. Blood. 2000;95(8):2593–9.
185. Silverman RH, Halloum A, Zhou A, Dong B, Al-Zoghaibi F, Kushner D, Zhou Q, Zhao J, Wiedmer T, Sims PJ. Suppression of ovarian carcinoma cell growth in vivo by the interferon-inducible plasma membrane protein, phospholipid scramblase 1. Cancer Res. 2002;62(2):397–402.
186. Cheng X, Liu Y, Chu H, Kao HY. Promyelocytic leukemia protein (PML) regulates endothelial cell network formation and migration in response to tumor necrosis factor alpha (TNFalpha) and interferon alpha (IFNalpha). J Biol Chem. 2012;287(28):23356–67. https://doi.org/10.1074/jbc.M112.340505.
187. Johnsen A, France J, Sy MS, Harding CV. Down-regulation of the transporter for antigen presentation, proteasome subunits, and class I major histocompatibility complex in tumor cell lines. Cancer Res. 1998;58(16):3660–7.
188. Lattanzi L, Rozera C, Marescotti D, D'Agostino G, Santodonato L, Cellini S, Belardelli F, Gavioli R, Ferrantini M. IFN-alpha boosts epitope cross-presentation by dendritic cells via modulation of proteasome activity. Immunobiology. 2011;216(5):537–47. https://doi.org/10.1016/j.imbio.2010.10.003.
189. Huyton T, Gottmann W, Bade-Doding C, Paine A, Blasczyk R. The T/NK cell co-stimulatory molecule SECTM1 is an IFN "early response gene" that is negatively regulated by LPS in human monocytic cells. Biochim Biophys Acta. 2011;1810(12):1294–301. https://doi.org/10.1016/j.bbagen.2011.06.020.
190. Wang T, Huang C, Lopez-Coral A, Slentz-Kesler KA, Xiao M, Wherry EJ, Kaufman RE. K12/SECTM1, an interferon-gamma regulated molecule, synergizes with CD28 to costimulate human T cell proliferation. J Leukoc Biol. 2012;91(3):449–59. https://doi.org/10.1189/jlb.1011498.
191. Katsoulidis E, Mavrommatis E, Woodard J, Shields MA, Sassano A, Carayol N, Sawicki KT, Munshi HG, Platanias LC. Role of interferon {alpha} (IFN{alpha})-inducible Schlafen-5 in regulation of anchorage-independent growth and invasion of malignant melanoma cells. J Biol Chem. 2010;285(51):40333–41. https://doi.org/10.1074/jbc.M110.151076.
192. El Hage F, Durgeau A, Mami-Chouaib F. TAP expression level in tumor cells defines the nature and processing of MHC class I peptides for recognition by tumor-specific cytotoxic T lymphocytes. Ann N Y Acad Sci. 2013;1283:75–80. https://doi.org/10.1111/j.1749-6632.2012.06777.x.
193. Kayagaki N, Yamaguchi N, Nakayama M, Eto H, Okumura K, Yagita H. Type I interferons (IFNs) regulate tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression on human T cells: a novel mechanism for the antitumor effects of type I IFNs. J Exp Med. 1999;189(9):1451–60.
194. Chen Q, Gong B, Mahmoud-Ahmed AS, Zhou A, Hsi ED, Hussein M, Almasan A. Apo2L/TRAIL and Bcl-2-related proteins regulate type I interferon-induced apoptosis in multiple myeloma. Blood. 2001;98(7):2183–92.

195. Leaman DW, Chawla-Sarkar M, Vyas K, Reheman M, Tamai K, Toji S, Borden EC. Identification of X-linked inhibitor of apoptosis-associated factor-1 as an interferon-stimulated gene that augments TRAIL Apo2L-induced apoptosis. J Biol Chem. 2002;277(32):28504–11. https://doi.org/10.1074/jbc.M204851200.
196. Borden EC, Sen GC, Uze G, Silverman RH, Ransohoff RM, Foster GR, Stark GR. Interferons at age 50: past, current and future impact on biomedicine. Nat Rev Drug Discov. 2007;6(12):975–90. https://doi.org/10.1038/nrd2422.
197. Talpaz M, McCredie KB, Mavligit GM, Gutterman JU. Leukocyte interferon-induced myeloid cytoreduction in chronic myelogenous leukemia. Blood. 1983;62(3):689–92.
198. Talpaz M, Kantarjian HM, McCredie KB, Keating MJ, Trujillo J, Gutterman J. Clinical investigation of human alpha interferon in chronic myelogenous leukemia. Blood. 1987;69(5):1280–8.
199. Alimena G, Morra E, Lazzarino M, Liberati AM, Montefusco E, Inverardi D, Bernasconi P, Mancini M, Donti E, Grignani F. Interferon alpha-2b as therapy for patients with Ph'-positive chronic myelogenous leukemia. Eur J Haematol Suppl. 1990;52:25–8.
200. Talpaz M, Kantarjian H, Kurzrock R, Trujillo JM, Gutterman JU. Interferon-alpha produces sustained cytogenetic responses in chronic myelogenous leukemia. Philadelphia chromosome-positive patients. Ann Intern Med. 1991;114(7):532–8.
201. Niederle N, Kloke O, Wandl UB, Becher R, Moritz T, Opalka B. Long-term treatment of chronic myelogenous leukemia with different interferons: results from three studies. Leuk Lymphoma. 1993;9(1–2):111–9.
202. Ozer H, George SL, Schiffer CA, Rao K, Rao PN, Wurster-Hill DH, Arthur DD, Powell B, Gottlieb A, Peterson BA, Rai K, Testa JR, LeBeau M, Tantravahi R, Bloomfield CD. Prolonged subcutaneous administration of recombinant alpha 2b interferon in patients with previously untreated Philadelphia chromosome-positive chronic-phase chronic myelogenous leukemia: effect on remission duration and survival: cancer and Leukemia group B study 8583. Blood. 1993;82(10):2975–84.
203. Thaler J, Gastl G, Fluckinger T, Niederwieser D, Huber H, Seewann H, Silly H, Lang A, Abbrederis C, Gadner H. Treatment of chronic myelogenous leukemia with interferon alfa-2c: response rate and toxicity in a phase II multicenter study. Semin Hematol. 1993;30(3 Suppl 3):17–9.
204. Hehlmann R, Heimpel H, Hasford J, Kolb HJ, Pralle H, Hossfeld DK, Queisser W, Loffler H, Hochhaus A, Heinze B. Randomized comparison of interferon-alpha with busulfan and hydroxyurea in chronic myelogenous leukemia. Blood. 1994;84(12):4064–77.
205. Italian Cooperative Study Group on Chronic Myeloid Leukemia. Interferon alfa-2a as compared with conventional chemotherapy for the treatment of chronic myeloid leukemia. N Engl J Med. 1994;330(12):820–5.
206. Allan NC, Richards SM, Shepherd PC, on behalf of the UK Medical Research Council's Working Parties for Therapeutic Trials in Adult Leukaemia. UK Medical Research Council randomised, multicentre trial of interferon-alpha n1 for chronic myeloid leukaemia: improved survival irrespective of cytogenetic response. Lancet. 1995;345(8962):1392–7.
207. Ohnishi K, Ohno R, Tomonaga M, Kamada N, Onozawa K, Kuramoto A, Dohy H, Mizoguchi H, Miyawaki S, Tsubaki K. A randomized trial comparing interferon-alpha with busulfan for newly diagnosed chronic myelogenous leukemia in chronic phase. Blood. 1995;86(3):906–16.
208. Talpaz M, Hehlmann R, Quintas-Cardama A, Mercer J, Cortes J. Re-emergence of interferon-alpha in the treatment of chronic myeloid leukemia. Leukemia. 2013;27(4):803–12. https://doi.org/10.1038/leu.2012.313.
209. Kantarjian HM, Talpaz M, Keating MJ, Estey EH, O'Brien S, Beran M, McCredie KB, Gutterman J, Freireich EJ. Intensive chemotherapy induction followed by interferon-alpha maintenance in patients with Philadelphia chromosome-positive chronic myelogenous leukemia. Cancer. 1991;68(6):1201–7.
210. Kantarjian HM, Keating MJ, Estey EH, O'Brien S, Pierce S, Beran M, Koller C, Feldman E, Talpaz M. Treatment of advanced stages of Philadelphia chromosome-positive chronic myelogenous leukemia with interferon-alpha and low-dose cytarabine. J Clin Oncol. 1992;10(5):772–8.
211. Hehlmann R, Berger U, Pfirrmann M, Hochhaus A, Metzgeroth G, Maywald O, Hasford J, Reiter A, Hossfeld DK, Kolb HJ, Loffler H, Pralle H, Queisser W, Griesshammer M, Nerl C, Kuse R, Tobler A, Eimermacher H, Tichelli A, Aul C, Wilhelm M, Fischer JT, Perker M, Scheid C, Schenk M, Weiss J, Meier CR, Kremers S, Labedzki L, Schmeiser T, Lohrmann HP, Heimpel H. Randomized comparison of interferon α and hydroxyurea with hydroxyurea monotherapy in chronic myeloid leukemia (CML-study II): prolongation of survival by the combination of interferon α and hydroxyurea. Leukemia. 2003;17(8):1529–37. https://doi.org/10.1038/sj.leu.2403006.
212. Kantarjian HM, O'Brien S, Smith TL, Rios MB, Cortes J, Beran M, Koller C, Giles FJ, Andreeff M, Kornblau S, Giralt S, Keating MJ, Talpaz M. Treatment of Philadelphia chromosome-positive early chronic phase chronic myelogenous leukemia with daily doses of interferon alpha and low-dose cytarabine. J Clin Oncol. 1999;17(1):284–92.
213. Arthur CK, Ma DD. Combined interferon alfa-2a and cytosine arabinoside as first-line treatment for chronic myeloid leukemia. Acta Haematol. 1993;89:15–21.
214. Lindauer M, Domkin D, Döhner H, Kolb HJ, Neubauer A, Huhn D, Kreiter H, Koch B, Huber C, Aulitzky W, Fischer T. Efficacy and toxic-

ity of IFN-α2b combined with cytarabine in chronic myelogenous leukaemia. Br J Haematol. 1999;106(4):1013–9.

215. Guilhot F, Chastang C, Michallet M, Guerci A, Harousseau JL, Maloisel F, Bouabdallah R, Guyotat D, Cheron N, Nicolini F, Abgrall JF, Tanzer J. Interferon alfa-2b combined with cytarabine versus interferon alone in chronic myelogenous leukemia. N Engl J Med. 1997;337(4):223–9.
216. Baccarani M, Rosti G, de Vivo A, Bonifazi F, Russo D, Martinelli G, Testoni N, Amabile M, Fiacchini M, Montefusco E, Saglio G, Tura S, Italian Cooperative Study Group on Myeloid L. A randomized study of interferon-α versus interferon-α and low-dose arabinosyl cytosine in chronic myeloid leukemia. Blood. 2002;99(5):1527–35.
217. Hochhaus A, Saussele S, Baerlocher GM, Brümmendorf TH, Burchert A, La Rosée P, Hasford J, Hehlmann R, Heim D, Krause SW, le Coutre P, Niederwieser D, Mayer J, Lange T, Haenel M, Stegelmann F, Gil A, Ernst T, Fabisch C, Pfirrmann M. Nilotinib vs Nilotinib plus pegylated interferon-alpha2b induction and nilotinib or pegylated interferon-alpha2b maintenance therapy for newly diagnosed BCR-ABL+ chronic myeloid leukemia patients in chronic phase: interim analysis of the Tiger (CML V)-study. Blood. 2018;132(Supplement 1):460. https://doi.org/10.1182/blood-2018-99-112119.
218. Yeung DT, Shanmuganathan N, Grigg A, Cunningham I, Shortt J, Rowling P, Reynolds J, Harrup RA, Ross DM, Kipp D, Mills AK, Arthur CK, Schwarer AP, Jackson K, Viiala N, Weinkove R, Yong ASM, White DL, Branford S, Hughes TP, ALLG OBot. Combination of nilotinib and pegylated interferon Alfa-2B results in high rates of MR4.5 at 24 months—primary analysis of the ALLG CML 11 Pinnacle study. Blood. 2019;134(Supplement_1):2926. https://doi.org/10.1182/blood-2019-125740.

Check for updates

14 Managing Pregnancy in Chronic Myeloid Leukaemia

Elisabetta Abruzzese and Jane F. Apperley

14.1 Introduction

The median age of CML at diagnosis varies from 55–65 years in industrialised countries to 10–15 years earlier in developing countries [1]. Consequently many female patients will be of childbearing age at diagnosis. This, together with the improvement in survival over recent years, secondary to drug availability and accurate molecular monitoring [2–4], results in many patients seeking guidance as to the possibility and advisability of pregnancy.

Although the possibility of discontinuing treatment and remaining in remission, aptly named treatment-free remission (TFR), has become a new goal of CML practice for some patients [5–7], the current consensus is that TKI therapy will continue lifelong with regular molecular monitoring so most patients attempting TFR will usually have been diagnosed at least 5 years earlier. For all but the youngest at diagnosis, most women who become pregnant will be on treatment at or shortly before conception.

In CML the therapeutic benefit of TKI relies on inhibition of the SH1 domain of Abl1; it is important to remember that tyrosine kinases are key mediators of signalling pathways involved in cellular regulation of proliferation, differentiation, metabolism, angiogenesis and survival. None of them is specific for the Bcr-Abl1 protein. All have varying degrees of 'off-target' inhibition of other tyrosine kinases such as c-kit and the platelet-derived growth factor receptors (PDGFR) (Table 14.1), and these effects may interfere with reproductive organ function and embryo-foetal development. At the time of product development, all the TKIs were investigated for such effects in animal studies, but perhaps more relevant clinical

Table 14.1 Inhibition of off-target tyrosine kinases as measured by the IC50 (Phos IC50) in nanomoles (nM): IC50 is the drug concentration that will inhibit phosphorylation of a given substrate by 50%

	Phos IC50 (nM)			
Drug	BCR-ABL1	KIT	PDGFR	SRC
Imatinib	100–1000	10–100	10–100	1000–10,000
Bosutinib	10–100	>10,000	1000–10,000	1–10
Dasatinib	1–10	10–100	1–10	<1
Nilotinib	10–100	100–1000	10–100	1000–10,000
Ponatinib	<1	10–100	1–10	1–10

E. Abruzzese (✉)
Hematology, S. Eugenio Hospital, ASL Roma2, Tor Vergata University, Rome, Italy
e-mail: elisabetta.abruzzese@uniroma2.it

J. F. Apperley
Centre for Haematology, Imperial College London, London, UK

Department of Clinical Haematology, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, UK

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
R. Hehlmann (ed.), *Chronic Myeloid Leukemia*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-71913-5_14

data have surfaced in the form of case reports or small series in the medical literature.

14.2 Animal Studies

Preclinical studies of fertility and embryotoxicity in male or female mice, rats and rabbit reported in the TKI investigator brochures for all drugs suggest that the drugs are not gonadotoxic, but can be teratogenic.

Following drug exposure in laboratory animals at levels equal or higher than that expected with standard dosing in humans, there was evidence of impairment of male and female reproductive organs with imatinib [8] and dasatinib [9], but not with nilotinib [10], although the fertility data on nilotinib are based on a single animal study. In contrast, all five currently licensed TKIs are associated with significant maternal and embryo-foetal toxicity in animal studies. The US Food and Drug Administration (FDA) therefore assigned the TKIs to pregnancy category D which indicates 'there is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience or studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks'.

14.2.1 Imatinib

In preclinical studies there was evidence of a reduction in testicular weight and sperm motility when male rats were exposed to imatinib 70 days prior to mating. Despite this, fertility was preserved in the male, and there was no overall impact on spermatogenesis [8].

Ovarian function was preserved in imatinib-exposed female rats. Female rats experienced post-implantation loss when given imatinib at doses ≥45 mg/kg, although there were no foetal losses at lower doses (less than 30 mg/kg). There was evidence of serious malformations involving the brain, gastrointestinal tract and bone when imatinib was given at doses >100 mg/kg to female rats during organogenesis (equivalent to dose in adults of 800 mg/day based on body surface area). This resulted in foetal loss in all animals [8]. When lactating female rats were exposed to imatinib at a dose of 100 mg/kg (approximately 800 mg/day in adults), imatinib and its metabolites were extensively secreted in milk. The concentration of imatinib in milk was threefold higher than the concentration in plasma (http://www.accessdata.fda.gov/drugsatfda_docs/label/2002/21335se8-Gleevec_lbl.pdf).

14.2.2 Dasatinib

The results from repeat-dose toxicity studies of dasatinib in multiple animal species indicate the potential for impairment of male and female reproductive function. Some of the gonadotoxic effects demonstrated in males include reduced size and secretion of immature prostate, seminal vesicle and testes. In preclinical studies, dasatinib caused uterine inflammation and mineralisation in female monkeys and cystic ovaries and ovarian hypertrophy in female rodents. Embryo-foetal toxicities were seen in rats and rabbits at plasma concentrations below that achieved in humans receiving therapeutic doses of dasatinib. The lowest doses of dasatinib studied resulted in embryo-foetal toxicities (rat 2.5 mg/kg/day and rabbit 0.5 mg/kg/day). These doses produced maternal AUC (area under the curve) of 105 ng/h/mL (0.3-fold the human AUC in females at a dose of 70 mg twice daily) and 44 ng/h/mL (0.1-fold the human AUC) in rats and rabbits, respectively. Embryo-foetal defects included skeletal malformations at multiple sites (scapula, humerus, femur, radius, ribs, clavicle), reduced ossification (sternum, vertebrae, pelvis and hyoid body), oedema and microhepatia [9]. It is not known whether dasatinib is secreted in human milk, but dasatinib was excreted into milk in female rats [11].

14.2.3 Nilotinib

There were no adverse effects on sperm count or motility in male rats exposed to nilotinib. Fertility was unaffected in male and female rats. The peak experimental dose reached an exposure of approximately five times that expected

in humans receiving the recommended dose. In the preclinical studies, nilotinib induced embryo-foetal toxicity at doses that also exhibited maternal toxicity. There was evidence of increased post-implantation loss in both the fertility study, which included treatment of both males and females, and in the embryo toxicity study, which only involved treatment of females.

In the embryo-foetal toxicity studies, low foetal birth weights and increased skeletal changes in rats and skeletal variations in rabbits were observed. The amount of exposure to nilotinib in females was generally less than or equal to that in humans at 400 mg b.i.d. in the pre- and postnatal study. Oral administration of nilotinib to female rats from day 6 of gestation to day 21 or 22 postpartum resulted in maternal side effects (reduced food consumption and decreased body weight) and led to longer gestational periods when given at a dose of 60 mg/kg. This dose was also associated with decreased pup body weight and changes in some physical developmental parameters. It is not known whether nilotinib is secreted in human milk. However results from animal studies have demonstrated that nilotinib is secreted in their milk [10].

14.2.4 Bosutinib

Animal studies have shown fertility impairment in both male and female rats and foetal toxicity in rabbits following exposure levels lower than human exposure at the recommended human dose of 500 mg/day. The foetal abnormalities included fused vertebrae, visceral abnormalities and approximately 6% decrease in foetal body weight. There is evidence that bosutinib crosses the placenta in pregnant rats resulting in foetal exposure to bosutinib and/or its metabolites. In lactating rats, transfer of bosutinib through the milk to nursing litters was confirmed [12].

14.2.5 Radotinib

Radotinib is a second-generation TKI approved for use in South Korea that is structurally similar to nilotinib sharing the same spectrum of action. This drug has not been reviewed by the FDA or the EMA, but it needs to be mentioned for completeness. Data relating to animal toxicity studies are not available for this review.

14.2.6 Ponatinib

In the preclinical studies of ponatinib, a dose of 3 mg/kg/day (equivalent to the AUC in patients receiving the recommended dose of 45 mg/day) resulted in embryo-foetal toxicity with low birth weight, multiple organ and vessel abnormalities, skeletal malformations and reduced ossification. Embryo-foetal malformations were also observed at the lower dose of 1 mg/kg/day (approximately 24% of the AUC in patients receiving the recommended dose) which resulted in similar foetal soft tissue and skeletal abnormalities. It is unknown whether ponatinib is secreted in human milk [13].

Although animal models are not always good predictors of the human situation, there is sufficient evidence from these animal studies to justify concern regarding the use of TKI during pregnancy. The uncertainties surrounding the effects of TKI on male and female reproductive function further emphasise the need to address fertility issues and options prior to treatment initiation.

14.3 Pregnancy Outcomes on Tyrosine Kinase Inhibitors

14.3.1 Male Patients

There are several case reports and series of varying sizes that suggest that the offspring of men taking imatinib at the time of conception are not at increased risk of developing congenital malformations. Ault and colleagues published an observational study of 19 pregnancies (involving ten females and eight males) in which either the male or female partner was receiving TKI therapy during early pregnancy and/or at the time of conception. Of the eight male patients who conceived, one patient conceived twice whilst on imatinib therapy. At the time of conception, these men had

been on imatinib therapy for a median duration of 18 months (range, 4 weeks to 48 months). These pregnancies resulted in the birth of eight healthy offspring and one spontaneous abortion. One of the eight offspring was born with a mild rotation of the small intestine necessitating surgical intervention following birth with no long-term complications. To date, over 200 pregnancies have been reported in the partners of men receiving imatinib therapy [14–19]. There has been no suggestion of complications occurring with conception, pregnancy, delivery or any increase in congenital abnormalities in the offspring. Hence, men with CML who wish to start a family can safely remain on imatinib therapy without treatment interruption.

Data relating to the safety of the second-generation TKI in men who wish to parent children are more limited. Cortes et al. [20] originally reported nine male patients on dasatinib whose partners became pregnant whilst on treatment, and normal offspring were reported for seven cases, with the outcome of the other cases unknown. These men remained on dasatinib therapy during and after the pregnancies. In this small cohort, there was one case of maternal pre-eclampsia; however, a healthy baby was delivered without any complications at 37 weeks. More recently, using information derived from the Bristol-Myers Squibb (BMS) pharmacovigilance database, Cortes et al. reported on 69 pregnancies in the partners of men taking dasatinib at the time of conception. Detailed information was available for only 33 of these pregnancies. Thirty (91%) pregnancies resulted in full-term deliveries of normal infants. Two pregnancies ended in spontaneous abortions and one in a full-term infant with syndactyly [21].

There have been three reports on the use of nilotinib in partners of men with CML. Zhou et al. [19] recently reported on the successful pregnancy outcome in the partner of a man receiving nilotinib at a dose of 800 mg daily for 31.5 months at the time of conception. The second case was reported by Abruzzese et al. in which a 33-year-old male conceived on nilotinib after 40 days of exposure, resulting in the delivery of a healthy offspring [22]. A recent report of 49 male patients receiving imatinib (34), dasatinib (6) or nilotinib (9) at conception showed no evidence of an adverse outcome [23]. TKIs did not appear to affect fertility or pregnancy outcome in the partners of male CML patients, suggesting that therapy interruption is unnecessary; of the 55 infants born, the authors reported one premature birth, two low birth weight infants and one occurrence of hypospadias.

Cortes et al. have recently published data derived from the Pfizer safety database for bosutinib through until February 28, 2018. Seventeen pregnancies were reported in the partners of men taking bosutinib with the outcome known in 14. There were nine normal live births and four elective terminations and one spontaneous abortion thought to be unrelated to the drug. In the induced abortion the foetus was said to have growth problems, but there was no confirmation of congenital abnormalities and no further information was available at the time of the report [24].

To date there are no published reports of men conceiving whilst on ponatinib. Two cases were reported anecdotally with unremarkable outcome (Abruzzese E, personal communication).

Currently available data would suggest that men wishing to father whilst on treatment do not seem to need to discontinue treatment. The possibility of an increased risk of abnormalities cannot be completely excluded, particularly for the newer drugs such as nilotinib, bosutinib and ponatinib where information is still limited. It seems that dasatinib is as safe as imatinib, and it is entirely possible that the other second- and third-generation TKIs will also be safe, but no guarantee can be given at present. At diagnosis there are no reliable means of predicting whether any individual patient will require second- or third-generation TKIs, or even allogeneic stem cell transplantation, and for this reason semen cryopreservation at diagnosis should be discussed with the patient.

14.3.2 Female Patients

Results are less favourable for children born to women exposed to imatinib during pregnancy. In

the report of Ault et al. [25], ten female patients became pregnant whilst receiving imatinib with one patient having a twin pregnancy. These women had been taking imatinib for a median of 8 months (range, 1–52 months) at the time of pregnancy for CML in chronic ($n = 9$) or accelerated phase ($n = 1$). Imatinib was discontinued immediately on confirmation of pregnancy. The median time of exposure to imatinib from conception to treatment discontinuation was 4 weeks (range, 4–9 weeks). Some of the patients were managed with hydroxycarbamide during the second or third trimesters ($n = 3$), leukapheresis ($n = 1$) or interferon alpha (IFN-α) ($n = 1$). Two patients experienced spontaneous abortion following discontinuation of imatinib and another underwent elective termination. The other seven pregnancies progressed to term and resulted in the birth of eight babies (including the twins). One of the newborns had hypospadias which was surgically corrected without complications. The other seven babies were all healthy with normal growth and development on long-term follow-up. In those who interrupted treatment, five out of nine women in complete haematologic response (CHR) at the time of treatment interruption lost their haematological response, and six women experienced a rise in Philadelphia-positive metaphases. After restarting imatinib, eight women achieved cytogenetic response (complete cytogenetic response, CCyR, in three women) at a median of 18 months following treatment resumption.

This was followed by a large international retrospective study of 180 women exposed to imatinib during pregnancy [26]. In this cohort, over 70% of women were exposed to imatinib in the first trimester only. The outcome of 125 of the pregnancies was reported. Sixty-three pregnancies (50%) resulted in normal live births, 18 (14.4%) ended in spontaneous abortion (slightly higher than the spontaneous abortion rate in normal population at 12%) [27] and 35 women underwent elective termination of pregnancy (three following identification of foetal abnormalities) (Table 14.2). Congenital malformations occurred in 12 of these pregnancies (eight live births, one stillbirth and three elective terminations). The congenital malformations observed were relatively unusual, and overlapping abnormalities were discovered in offspring of women exposed to imatinib during pregnancy. These include premature closure of skull sutures (craniosynostosis), hypoplastic lungs, omphalocele, duplex kidney, absent kidney, shoulder anomaly, exomphalos, renal agenesis, hemivertebrae and scoliosis. Exomphalos was found to occur in three of the 125 cases with known outcome. Since the expected incidence of exomphalos in the normal population is approximately 1 in 3000–4000 births, it seems likely that there is a causative association with imatinib exposure. Furthermore, similar bony defects including exencephaly, encephaloceles and deformities of the skull bones were all observed in the animal studies [8]. There is a possibility that these congenital malformations result from the inhibition of 'off-target' tyrosine kinases. From animal models it was found that mice homozygous for null mutations in platelet-derived growth factor receptor-alpha (PDGFR-α) displayed a combination of birth defects, including facial clefting, severe spina bifida occulta, cardiac defects, omphalocele, renal and urogenital anomalies and vertebral and rib fusion defects [28–30]. Additional data from animal studies suggest that PDGFR-α also plays a role in lung maturation, and inhibition of PDGFR-α may lead to lung hypoplasia [31].

Table 14.2 Outcome of pregnancies in women who received imatinib during their pregnancy

Pregnancy outcome for women on imatinib	Number	Percentage of those with known outcome ($n = 125$)	Percentage of total number ($n = 180$)
Live births	63	50	35
Elective termination	35	28	19.5
Spontaneous abortion	18	14.4	10
Foetal abnormality	12	9.6	6.7

A review of the outcome of 167 of a total of 210 pregnancies in women exposed to imatinib was reported by Abruzzese et al. These women

were all exposed to imatinib during period of organogenesis (>5 weeks gestation). Of the 167 pregnancies, 128 (77%) resulted in delivery of normal offspring, whilst 24 pregnancies (14%) ended in spontaneous abortion. Serious congenital malformations were seen in 15 of 167 pregnancies (9%). Since this was a combination of retrospective case series and the authors' own experience, we cannot exclude some level of double reporting, but the overall results mirror previous reports. There is a body of evidence suggesting an increased risk of malformations in patients treated with imatinib during organogenesis (first trimester). An updated literature search for imatinib and teratogenesis revealed one further case of omphalocele and one of craniosynostosis in ten patients continuing therapy throughout pregnancy [32].

In recent years multicentric databases such as the GIMEMA (Italian Group for Adult Hematologic Diseases) and ELN (European Leukemia Network) registries were established to collect the outcomes of pregnancies in patients with CML. The GIMEMA data collect retrospective and prospective information regarding conception and pregnancies from male (n = 83) and female (N = 54) patients from Italian centres [14]. The ELN database is the largest archive of pregnancy in women with CML: it now includes retrospective and prospective data on 305 pregnancies from 17 countries. Most patients were treated with imatinib at pregnancy (70% in both databases). Whilst in the GIMEMA database treatment was in most cases stopped at the first positive pregnancy test, in the ELN there were 14 pregnancies in which imatinib was used during the first trimester (until 12–17 weeks) with ten patients never stopping TKI therapy. All 14 pregnancies resulted in normal infants [33].

Imatinib is highly bound to plasma proteins and has a high molecular weight that can limit placental transfer [34, 35]. It is possible that imatinib can be used safely after placental formation (>16–18 weeks gestation), and this may be considered in situations where effective treatment cannot be withheld [36–40].

Approximately 50 cases of pregnancy have been reported in woman treated with nilotinib. Santorsola et al. reported a successful pregnancy in a patient in whom nilotinib was stopped prior to conception and replaced by IFN-α [41]. Two case reports on the outcome after nilotinib exposure in early pregnancy [22, 42] described the birth of healthy offspring without any complications. However, Etienne et al. described a 38-year-old female who became pregnant on nilotinib which was discontinued upon confirmation of pregnancy and replaced by IFN-α. Unfortunately, ultrasound scan at 3 months of gestation revealed a large omphalocele and the pregnancy was terminated [43]. Forty-five cases of nilotinib exposure during pregnancy have been reported in the nilotinib investigator's brochure, with only one case of foetal malformation. They also described nilotinib exposure during a twin pregnancy, with one twin developing congenital transposition of great vessels resulting in death; the second twin had a benign heart murmur. Placental transfer was investigated by Chelysheva et al. who tested ten patients on imatinib (n = 7) or nilotinib (n = 3) at delivery. Maternal plasma, placental and cord blood concentrations showed a low foetal/maternal and a high placental/maternal ratio [37–39] suggesting limited transfer across the placenta.

A more serious scenario was described with dasatinib. Initially Cortes et al. [20] reported on the outcome of pregnancies in women who conceived whilst receiving treatment with dasatinib. Three women underwent elective termination of pregnancy, two had spontaneous abortions and three others delivered healthy babies. Two further case studies reported normal pregnancy outcomes following dasatinib exposure at 100 and 140 mg/day, during the first trimester of pregnancy. Dasatinib was discontinued following the confirmation of pregnancy [44–47]. However Berveiller et al. [48] described a tragic foetal outcome following transplacental transfer of dasatinib in a 23-year-old woman who was diagnosed with chronic-phase CML at 7 weeks gestation and treated with dasatinib. Obstetric monitoring revealed hydrops fetalis associated with severe cytopaenia in the foetus and resulted in an elective termination of pregnancy at 16 weeks gestation. Dasatinib drug levels were measured and were found to be 4 ng/ml in maternal plasma

(compatible with therapeutic level), 3 ng/ml in foetal plasma and 2 ng/ml in the amniotic fluid. Foetal chromosome analysis was normal. This report documented the transplacental transfer of dasatinib in addition to leucopaenia, thrombocytopaenia, ascites, pleural effusions and oedema in the foetus, all side effects known to be associated with dasatinib.

Scrutiny of the BMS pharmacovigilance database identified 78 pregnancies in women taking dasatinib, with full details available for 46 patients (59%) (Table 14.2) [21]. Forty-one of 46 women (89%) were taking dasatinib at the time of conception, and in 32/41 (78%) dasatinib was stopped on confirmation of pregnancy in the first trimester. There were 20 live births (Table 14.3). Four women had problematic pregnancies with four premature deliveries, three occurrences of intrauterine growth retardation and one placental abruption. Seven infants had congenital abnormalities, two identified after birth, two after spontaneous abortion and three after elective termination. One of these cases included the pregnancy reported by Berveiller et al. [48] (see above) which ended in elective termination; the other elective termination also reported a foetus with hydrops fetalis but with additional central nervous system abnormalities including a parieto-occipital encephalocele and premature closure of the cranial vault sutures. Full details were unavailable for a further three infants. Of the two live births, one child delivered at 36 weeks had renal tract abnormalities, and the other, born at 28 weeks after dasatinib had been started at 17 weeks gestation, had hydrops fetalis and died within 24 hours. Because of the occurrence of hydrops fetalis when the dasatinib was started in the second trimester, even if the association was coincidental, dasatinib cannot be recommended for use at any time during pregnancy.

Recently, 16 cases of maternal exposure to bosutinib were identified through the Pfizer safety database which contains reports from patients, healthcare professionals, registries and published literature. Six of these resulted in live births, with five of six patients stopping treatment during pregnancy. There were two elective terminations (one induced because of a molar degeneration) and two spontaneous abortions (one after a suspected ectopic pregnancy: outcomes of the remaining six pregnancies were unavailable at the time of the report [24].

Only one case of a woman becoming pregnant on radotinib has been reported to date. A 19-year-old woman conceived 1 year after being diagnosed with CML and treated with radotinib. The pregnancy was detected at 7 weeks and 6 days and the patient elected for termination. Three weeks later, after 10 weeks and 5 days of amenorrhoea she stopped radotinib; the pregnancy was unremarkable with a female infant born at 39 weeks. Examination revealed low-set ears and nose-forehead angle abnormalities, further laryngomalacia and omega-shaped epiglottis deemed responsible for breathing problems during lactation. The infant improved after 10 days and regular growth was reported in the follow-up [49].

There are no reported cases of female patients becoming pregnant on ponatinib. In one of our institutions, a patient treated with ponatinib and IFN-α after nilotinib and dasatinib failure just gave birth naturally at term to a healthy baby boy (weight

Table 14.3 Outcome of pregnancies in women who received dasatinib during their pregnancy

Pregnancy outcome for women on dasatinib	Number	Percentage of those with known outcome ($n = 46$)	Percentage of total number ($n = 78$)
Live births	20	43	25
Normal pregnancy and normal live infant	15	32	19
Abnormal pregnancy	4	9	5
Elective termination	18	39	23
Spontaneous abortion	8	17	10
Foetal abnormality	7 (2 after live birth and 5 after termination)	15	9

3.670 kg; 52 cm long). She stopped treatment at the first positive pregnancy test and has maintained molecular remission so far. The pregnancy has developed normally. She started breastfeeding.

In general the use of any TKI should be avoided during pregnancy. Evidence to date, albeit limited, suggests that neither imatinib nor nilotinib crosses significantly the placenta and could be considered after the 16th week of gestation whilst dasatinib should not be used at any time. The current recommendation is that women wishing to conceive should stop treatment with TKI prior to conception or at first positive pregnancy test and preferably remain off TKI therapy throughout their pregnancy [50].

However, this strategy is not always possible for a variety of reasons, and alternative management approaches are needed for women with unplanned pregnancies and those diagnosed with CML during their pregnancy.

14.4 Pregnancy in CML after the Start of TKI Treatment

Patients established on TKI therapy may want to discuss the advisability of stopping treatment to consider pregnancy irrespective of the degree of their molecular response. Although the optimal situation is when the patient would, independent of the desire to conceive, be eligible for a trial of treatment discontinuation, this is often not the case. There are some very understandable reasons why patients want to have children when they themselves have not yet reached stable deep responses, including women approaching an age where fertility is declining, societal and cultural pressures and where they have been on TKI for some considerable time but have not reached, nor appear likely to reach, MR4. It is possible to support these women through attempts at conception, but it remains important to discuss with them their risk of disease progression, whether this is simply a rise in the RT-qPCR, loss of cytogenetic or haematological remission or transformation to more advanced-phase CML.

The most valuable data are derived from a number of stopping treatment studies, either in the setting of prospective clinical trials in patients with deep and durable responses ([6, 7, 51]) or retrospective observational studies of patients who discontinued treatment due to drug intolerance or for financial reasons [52]. Approximately 40–50% of those who achieve molecular negativity (as measured by RT-qPCR, sustained for at least 1–2 years) can remain off TKI therapy indefinitely without loss of MMR. It is reassuring that patients who do relapse eventually regain their previous excellent disease responses upon reintroduction of TKI. Although data are currently limited regarding the outcome of restarting therapy after discontinuation for pregnancy, it seems that individuals who have achieved deep molecular responses are more likely to regain these responses upon restarting TKI than those who were only in haematological or cytogenetic response at the time of treatment discontinuation (Table 14.4) [52, 53].

In recent years, international treatment guidelines for CML have provided recommendations for attempting discontinuation of treatment with TKI outside clinical trials based on the biological characteristics of CML, time from TKI therapy, presence of prior TKI resistance and the depth and duration of response [54].

Using the lessons learned from the trials for TFR, the ideal setting for stopping a TKI in order to seek for conception would be in a woman who has achieved sustained deep molecular response (DMR) (≥MR4) for at least 2 years and would have been eligible to stop irrespective of pregnancy. The expectation is that their course will mirror that of non-pregnant patients in that some 50% will be able to remain off treatment indefinitely and that those who lose their molecular response, usually defined as loss of MR3, will do so within the first 6 months from stopping [55, 56]. There are several possible outcomes:

- By 6 months the patient is pregnant and has not lost MR3. She should continue off treatment and on regular molecular monitoring.
- By 6 months the patient is not pregnant and has not lost MR3. She should continue off treatment and on regular molecular monitoring and can continue to try to conceive.

Table 14.4 Best responses of patients restarting imatinib after discontinuation after pregnancy

Publication	Patient	Duration of imatinib treatment prepregnancy (month)	Status pre-imatinib cessation	Duration of imatinib treatment post-pregnancy (month)	Best response after restarting imatinib
Ault et al. [25]	1	1	CHR	NG	CCyR
	2	3	CHR	NG	CCyR
	3	4	No CHR	NG	Progression to BP
	4	5	MCyR	NG	CCyR
	5	7	MCyR	NG	MCyR
	6	9	MCyR	NG	CHR
	7	24	CHR	NG	MCyR
	8	36	CHR	NG	CHR
	9	48	CHR	NG	NK
	10	52	CCyR	NG	MCyR
Kuwabara et al. [53]	1	7	MCyR	50	MCyR
	2	19	CCyR	29	CHR
	3	21	CCyR	26	CCyR
	4	42	CCyR	18	Loss of CHR
	5	9	MMR	30	MR4.5
	6	14	MMR	90	MMR
	7	50	MMR	14	MMR

- By 6 months the patient is pregnant but has lost MR3. In most circumstances, when starting from a DMR, it is unlikely that the patients will experience a rapid pace of their relapse such that they lose CCyR or even CHR before delivery. Most patients can remain off treatment until after delivery but may be advised to restart treatment promptly thereafter and not breastfeed for a prolonged period of time.
- By 6 months the patient is not pregnant but has lost MR3. She should restart treatment with the same, or a more potent, TKI and reestablish a DMR. It may be possible to stop again in the future and reattempt both TFR and conception. If this is not an acceptable approach for the patient, then it may be appropriate to consider referral to the in vitro fertilisation (IVF) department to consider oocyte, ovarian or embryo cryopreservation. The patient could then restart treatment and stop at a later date for IVF when her DMR has been achieved and is stable.

Unfortunately many patients are unable to fulfil the criteria for a trial of discontinuation. In such situations, the woman and her partner should be counselled as to the risk of losing response and also of disease progression (particularly if her response to TKI is limited to cytogenetic or haematological remission). If after appropriate discussion the patient is still determined to attempt pregnancy in less than ideal circumstances, personalised approaches should be used in order to consider the parents' wishes and the safety of both mother and baby. For these patients consideration should be given as to how long a patient might remain off TKI therapy before becoming pregnant since this period of time has to be added to the 9 months of gestation, or at least to the first 5–12 weeks of organ formation. The time to conceive naturally varies and depends on age, with a mean of 15 weeks in a healthy fertile population [57]. Based on highly variable lead-in time and required time to avoid treatment, an alternative is to stop the TKI at first pregnancy test (typically 4–5 weeks of pregnancy), or a more 'conservative' intermittent treatment with TKI taken days 1–14 days of menstrual cycle, stop at ovulation and restart as cycle restarts if pregnancy did not succeed [58]. Among the described cases there

are virtually no data to recommend this strategy of intermittent dosing before conception, in terms of efficacy or safety, and it is not recommended by any TKI manufacturer. However, for some women where the desire and/or pressure to have children outweighs the risk to her own health, this represents a compromise solution. Intermittent dosing with TKI has occasionally been used in an attempt to reduce the severity of side effects and potentially improve compliance: continued efficacy has been demonstrated, but the approach is still not widely used [59, 60]. This is also a population of patients in which referral for IVF procedures is appropriate.

All patients who stop their TKI to become pregnant should be cautioned that their tumour load may rise off treatment. The frequency of molecular monitoring is controversial and should be discussed with the individual patient. The loss of MMR is not an indication to reintroduce treatment and too frequent monitoring may lead to unnecessary anxiety. Moreover, the rate of rise in RT-qPCR can be quite variable, although the majority of patients who had to restart therapy in the treatment stopping studies experienced molecular relapse within the first 6 months. The kinetics of the rise in transcript levels was studied in a subgroup of patients of the GIMEMA database [14], and the rise in BCR-ABL1 transcript levels during TKI cessation for pregnancy identified two populations. In one there was a rapid increase with a mean doubling time of 5.8 days, and in the other this was much longer at 182 days, irrespective of their molecular status pre-cessation. These data were confirmed by others [37–39, 61] who also showed a higher than expected rate of MMR retention and response stability and hinting that the kinetics of regrowth of residual CML during pregnancy in female patients may be different than that observed in non-pregnant TFR patients. Thus, the rate of change in the RT-qPCR is informative and can be useful to predict those women likely to require treatment later in the pregnancy at the time of loss of CCyR or CHR. We currently recommend RT-qPCR at baseline and thereafter at 6–8 weekly intervals, with more frequent monitoring if the transcript level appears to be increasing rapidly.

The principles outlined above also apply to women who become pregnant unexpectedly whilst on treatment for TKI. If the woman wishes to proceed with the pregnancy, the TKI should be stopped immediately and subsequent management will depend on the depth of response already achieved.

14.5 Treating CML in Pregnancy

In women who have discontinued therapy after achieving deep and durable molecular response, treatment may not be required at all for the duration of the pregnancy. On the contrary, relapse of disease is much more likely if the patient is not in a deep response at the time of treatment discontinuation, and some form of treatment might become necessary during the pregnancy. Options for therapy include IFN-α, leukapheresis, hydroxycarbamide and TKIs, together with supportive therapy such as aspirin or low molecular weight heparin (LMWH) (Table 14.5).

IFN-α is an option when stopping TKI therapy in women with suboptimal responses. It is an entirely reasonable strategy although there is no evidence that it is capable of sustaining or achieving MMR. Serial RT-qPCR monitoring was not available when patients were routinely treated with IFN-α, and achievement of CCyR occurred in only 10–15%, so deep molecular responses were unlikely. In addition there are numerous side effects associated with IFN-α which may impact on quality of life. The most appropriate management might be simply pragmatic, with avoidance of unnecessary medication especially in the first trimester of pregnancy. With regular RT-qPCR monitoring, the rate of increase in transcript levels can be followed, and IFN-α could be introduced at a time when loss of CCyR has either occurred or could be predicted to occur in the near future. In women who come off their TKI without having achieved MMR or even CCyR, it is justifiable to consider introducing IFN-α much sooner [57].

IFN-α inhibits cell proliferation through its effect on protein synthesis, RNA breakdown and possibly by immunomodulation: it does not

Table 14.5 Therapy management during pregnancy in CML patients considering pregnancy stage and tumour burden (adapted from [50])

<table>
<tr><th rowspan="2">Initial BCR-ABL level at pregnancy confirmation</th><th colspan="2">Pregnancy stage</th><th rowspan="2">After delivery</th></tr>
<tr><th>1st trimester until week 15 (early second trimester)</th><th>2nd–third trimester—from week 16 until delivery</th></tr>
<tr><td>DMR
BCR-ABL $\leq 0.01\%$</td><td colspan="2">• No treatment if TFR criteria are met
• Monitor BCR-ABL levels/kinetics every 4–8 weeks</td><td rowspan="2">• Restart the same or a more potent TKI if MMR is lost
• TKI switch if intolerant or MMR not restored within 6–12 months</td></tr>
<tr><td>MMR;
BCR-ABL $\leq 0.1\% > 0.01\%$</td><td colspan="2">• No treatment or IFN consideration
• Monitor CBC, BCR-ABL levels/kinetics every 4–8 weeks</td></tr>
<tr><td>MR2
BCR-ABL $> 0.1 \leq 1\%$</td><td>• Consider IFN
• BCR-ABL level at 15th week</td><td>If confirmed CHR loss
• Imatinib 400 mg
• Nilotinib 400 mg if resistant/intolerant to imatinib
• If TKI restarted monitor CBC monthly and BCR-ABL every 1–3 months as indicated</td><td>• Restart/continue same or a more potent TKI
• TKI switch if intolerant or optimal response lacking within 6–12 months</td></tr>
<tr><td>No MR2 and CHR
BCR-ABL $> 1–10\%$</td><td>• IFN or no treatment if CHR remains
• BCR-ABL level and CBC at 15th week</td><td rowspan="2">• Imatinib 400 mg
• Consider nilotinib 400 mg if resistant/intolerant to imatinib
• IFN if no TKI therapy is available
• Monitor CBC monthly and BCR-ABL every 1–3 months as indicated</td><td rowspan="2">• Continue the same or a more potent TKI, check compliance and treatment tolerance
• TKI switch if intolerant or optimal response lacking within 6–12 months</td></tr>
<tr><td>No CHR
BCR-ABL $> 1–10\%$</td><td>IFN</td></tr>
</table>

CBC complete blood count, *CHR* complete haematologic response, *DMR* deep molecular response, *IFN* interferon, *MMR* major molecular response, *TFR* treatment-free remission, *TKI* tyrosine kinase inhibitor

inhibit DNA synthesis [62]. In view of its high molecular weight (19 kDa), it does not cross the placental barrier. Mutagenicity and teratogenicity have not been observed in animal studies of IFN-α [63]. Two major case reports on the safety of IFN-α in pregnancy [64, 65] reported on the outcome of 40 patients (eight with CML, 27 with essential thrombocythaemia, two with hairy cell leukaemia, one with multiple myeloma and two carriers of hepatitis C) and their offspring. Eight of 40 patients were treated with IFN-α in the first trimester of pregnancy. There were no reports of congenital malformations when IFN-α was given as monotherapy. One foetus whose mother was also exposed to hydroxycarbamide at the time of conception was found to have multiple congenital malformations. Four women experienced premature delivery and six newborns had documented intrauterine growth retardation. There are several case reports of IFN-α treatment during pregnancy in women with CML, and there have been no reports of congenital abnormalities in these infants [41, 66–68]. Based on this experience, IFN-α is considered safe. The pegylated form of IFN-α has been contraindicated in the past throughout pregnancy due to its potentially harmful effects as a result of accumulation of polyethylene glycol, but the concentration of the alcohol seems negligible and is unlikely to cause harm to the foetus [69].

Hydroxycarbamide is a cytotoxic agent which inhibits DNA synthesis. There are several case reports of hydroxycarbamide exposure during pregnancy, including a single-institution experience of 31 women treated with hydroxycarbamide for a variety of haematological disorders including essential thrombocythaemia ($n = 22$), CML ($n = 8$) and sickle cell disease ($n = 1$) [70]. Twenty-two of 31 women were exposed to hydroxycarbamide in their first trimester. There were two intrauter-

ine foetal deaths (both occurred in patients treated with hydroxycarbamide in the first trimester), three infants had minor abnormalities (hip dysplasia, unilateral renal dilatation, pilonidal sinus) and nine pregnancies resulted in premature delivery. Second and third trimester exposure to hydroxycarbamide was associated with an increased risk of pre-eclampsia. Hydroxycarbamide is teratogenic in animals and generally should be avoided, although there have been single case reports of using hydroxycarbamide from the second trimester onwards with no adverse outcomes [71].

The potential teratogenicity of TKI is clear matter of concern, but an alternative approach in patients in whom IFN-α is not tolerated and/or effective and in whom treatment is necessary is the reintroduction of imatinib during the second or third trimester. The congenital abnormalities observed in the offspring of women taking imatinib occurring early in pregnancy during organogenesis have led some to speculate that TKI could be safely introduced thereafter. Russell et al. described two pregnancies exposed to imatinib during the third trimester [35]. The concentration of imatinib and its active metabolite CGP74588 was measured at delivery in maternal blood, placenta and cord blood. Imatinib was found to be present at high concentration in maternal blood and in the placenta; however minimal or no drug was found in the cord blood [35]. A subsequent report by Ali et al. on imatinib exposure in pregnancy from the 21st to 39th week of gestation revealed that imatinib was present at 338 ng/mL in the cord blood and 478 ng/mL in the peripheral blood of the newborn compared to 1562 ng/mL in maternal blood [34]. There were no maternal-foetal complications observed in this case. In a further study the drug concentration was measured in maternal plasma, placenta and cord blood in ten patients treated at delivery with imatinib (n = 7) and nilotinib (N = 3), and this confirmed limited placental transfer. Patients restarted TKI between 12 and 40 weeks of gestation (mean 18 week). All pregnancies ended in live births and no birth defects or developmental problems were identified [37–39]. It is important to note that there is limited evidence to support this approach and that the use of any TKI at any stage of pregnancy is prohibited according to the manufacturers' instructions.

The development of hydrops fetalis in the offspring of two women who commenced dasatinib at 6 and 17 weeks must be a cause for concern regarding the use of this drug at any time during the pregnancy [21, 48]. Little is known about the other second- and third-generation TKI.

Data gathered within the ELN registry are beginning to provide information about the various treatment strategies and their outcome. Seventy-six patients were treated with TKI, four with nilotinib and 72 with imatinib; 14 of these women were treated with imatinib during first trimester, 11 never stopped treatment, whilst three discovered late pregnancy and foetus was exposed until 10–13 weeks. There were no abnormalities reported; however low birth weight was observed in offspring of mothers treated with TKIs [72, 73].

14.6 Breastfeeding

Results from animal studies have demonstrated transfer of TKI into breast milk. Approximately 1.5% of maternal dose of imatinib is secreted into milk, which is equivalent to a dose to the infant of 30% the maternal dose per unit body weight [8]. The same results were confirmed in nursing mothers on TKI [37–39, 74]. Although those concentrations are below the therapeutic dosage, nursing mothers should be advised against breastfeeding whilst they are on treatment. Hydroxycarbamide is also secreted in breast milk and is therefore contraindicated during the breastfeeding period [75]. There is evidence to suggest that IFN-α is secreted into breast milk (despite its high molecular weight), but its lack of oral absorption makes possible breastfeeding whilst on treatment [76].

14.7 Management of the Patient Presenting in Pregnancy

It is not uncommon to diagnose CML during pregnancy. CML constitutes 10% of pregnancy-associated leukaemias and has an annual inci-

dence of one per 100,000 pregnancies [77]. The diagnosis of a potentially fatal malignant disease at a time that for most expectant mothers is of great happiness is undoubtedly devastating and should be managed with empathy and sensitivity, with involvement of a multidisciplinary team. For women presenting in chronic phase, there is no requirement for elective termination, although some women may request this procedure given their personal circumstances and the uncertainty of their future. It is reassuring for both patient and physician to note that pregnancy itself does not appear to affect the natural course of CML [78].

However, women presenting in accelerated or blast phase of CML may require urgent treatment with TKI and/or induction chemotherapy. Median survival following the diagnosis of blast crisis is dismal, estimated between 7 and 11 months. Patients in blast crisis are generally advised to terminate pregnancy in order to commence chemotherapy, unless the pregnancy is close to term and there is no immediate harm to the mother. Induction chemotherapy can then start following delivery. Almost all cytotoxic agents have been shown to be associated with congenital malformations in animal models. The decision to terminate a pregnancy is difficult and requires extensive discussion, counselling for both parents and a multidisciplinary approach involving obstetricians and psychologists. Treatment decisions should be individualised based on the relative risks and benefits of the patient and foetus and taking into account parents' wishes.

For those women in chronic phase with leucocytosis and/or extreme thrombocytosis, leukapheresis is a possible alternative, particularly during the first and second trimesters [79–81], as it has the advantage of rapid reduction in counts without exposing the foetus to potentially teratogenic agents. The disadvantages of leukapheresis are that it is not readily available in all centres and requires good venous access. Regular leukapheresis should be performed in order to keep the white cell count $< 100 \times 10^9$/l and platelets < 500 × 10^9/L. This can be done on alternate days if the white count is $>100 \times 10^9$/l or at regular weekly or fortnightly intervals depending on the stability of blood counts. Leukapheresis can be performed safely with minimal risk to the foetus and mother. There is a theoretical risk of increased haemodynamic instability, but so far, there have not been any reports of adverse outcomes following leukapheresis in pregnancy. If leukapheresis is not available or the frequency required is not practical, then IFN-α is a reasonable alternative. Low molecular weight heparin (LMWH) along with aspirin should be considered in women with persistent thrombocytosis with platelet count >500 × 10^9/L.

In the ELN CML Pregnancy Registry 21% of the patients were diagnosed during pregnancy, and approximately 70 cases of CML diagnosed during pregnancy in the TKI era have been reported. Normal childbirth has been reported in several international case series using different treatment approaches [50]. Many of those patients could be managed with observation only throughout the whole gestation period. Table 14.5 can be used as a reference for treatment.

14.8 Management of Fertility at Diagnosis

At the time of diagnosis, it is not possible to predict the future treatment needs of a newly diagnosed patient. In patients treated with imatinib as first-line therapy, approximately 30% will change treatment to a second-generation drug, due to lack or loss of response and/or intolerance. A second-generation TKI is successful in achieving a durable complete cytogenetic response (CCyR) in 40–50%, and a few failing patients might obtain benefit from an alternative second- or third-generation agent, but around 5–10% will remain resistant to TKI therapy and some will be potential candidates for allogeneic stem cell transplantation. For such reasons the potential impact on fertility should be discussed with every newly diagnosed patient.

Fertility preservation in the form of semen cryopreservation, ovarian or oocyte retrieval and storage as well as embryo cryopreservation should be considered at the time of diagnosis. Patients should be informed of these fertility options and offered consultation with fertility

experts if they are readily accessible. Although embryo cryopreservation and reimplantation provides better outcomes, cryopreservation of unfertilised eggs or ovarian tissue cryopreservation may be options for female patients without stable partners, if these are permitted in that country. The process of embryo cryopreservation usually takes at least 2–4 weeks to complete.

14.9 Conclusion

Recent advances in CML therapy have profoundly improved survival and offer most patients durable molecular response and normal life expectancies. However, the management of CML during pregnancy remains a clinical challenge. Management of CML in pregnancy should be individualised based on the relative risks and benefits to the mother and foetus, focusing on survival of the mother whilst limiting treatment-related toxicity to the developing foetus. Planned and unplanned pregnancies during CML can be managed through close collaboration of a multidisciplinary team (haematology, obstetrics, neonatology and IVF, if appropriate) and the patient who should be informed of the pros and cons of all pregnancy and treatment options.

The advice given to patients will differ according to their disease response particularly with respect to previous or current accelerated phase or blast crisis. Patients who present with chronic-phase disease during pregnancy can safely continue their pregnancy to term and can be successfully managed with IFN-α or leukapheresis if necessary during the first and subsequent trimesters. TKIs (notably imatinib and nilotinib) can be introduced if necessary from the second trimester onwards, as placental transfer is limited. Patients presenting in advanced-phase disease should be counselled with respect to consideration of elective termination of pregnancy in order to commence induction chemotherapy and/or a TKI.

Patients who wish to become pregnant after treatment has initiated can usually be supported to achieve their goals. Those who have attained deep and durable molecular responses can be managed by an approach similar to that of other patients wishing to discontinue treatment in the context of TFR. Management of a patient with high tumour burden (≥MR2) or a CML diagnosis at pregnancy onset is particularly challenging.

With such a door open for younger patients with CML, further progress should with certainty include quality of life in all of aspects, particularly one of the most basic human desires: the ability to conceive and procreate.

Per aspera ad astra

(through difficulties to the stars)

Seneca

Acknowledgements Thanks to all mothers and fathers who followed their dreams regardless of life difficulties, to doctors, nurses and researchers who teamed up to help them succeed.

Authorship Contribution: EA and JFA performed the literature review and wrote the manuscript.

Conflict-of-interest disclosure: Authors declare no conflict of interest with regard to this paper.

References

1. Hoffmann VS, Baccarani M, Hasford J, Lindoerfer D, Burgstaller S, Sertic D, Costeas P, Mayer J, Indrak K, Everaus H, Koskenvesa P, Guilhot J, Schubert-Fritschle G, Castagnetti F, Di Raimondo F, Lejniece S, Griskevicius L, Thielen N, Sacha T, Hellmann A, Turkina AG, Zaritskey A, Bogdanovic A, Sninska Z, Zupan I, Steegmann JL, Simonsson B, Clark RE, Covelli A, Guidi G, Hehlmann R. The EUTOS population-based registry: incidence and clinical characteristics of 2904 CML patients in 20 European countries. Leukemia. 2015;29(6):1336–43.
2. Gambacorti-Passerini C, Antolini L, Mahon FX, Guilhot F, Deininger M, Fava C, Nagler A, Della Casa CM, Morra E, Abruzzese E, D'Emilio A, Stagno F, le Coutre P, Hurtado-Monroy R, Santini V, Martino B, Pane F, Piccin A, Giraldo P, Assouline S, Durosinmi MA, Leeksma O, Pogliani EM, Puttini M, Jang E, Reiffers J, Piazza R, Valsecchi MG, Kim DW. Multicenter independent assessment of outcomes in chronic myeloid leukemia patients treated with imatinib. J Natl Cancer Inst. 2011;103(7):553–61.
3. Hehlmann R, Lauseker M, Saußele S, Pfirrmann M, Krause S, Kolb HJ, Neubauer A, Hossfeld DK, Nerl C, Gratwohl A, Baerlocher GM, Heim D, Brümmendorf TH, Fabarius A, Haferlach C, Schlegelberger B, Müller MC, Jeromin S, Proetel U, Kohlbrenner K, Voskanyan A, Rinaldetti S, Seifarth W, Spieß B, Balleisen L, Goebeler MC, Hänel M, Ho A, Dengler J, Falge C, Kanz

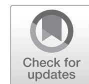
L, Kremers S, Burchert A, Kneba M, Stegelmann F, Köhne CA, Lindemann HW, Waller CF, Pfreundschuh M, Spiekermann K, Berdel WE, Müller L, Edinger M, Mayer J, Beelen DW, Bentz M, Link H, Hertenstein B, Fuchs R, Wernli M, Schlegel F, Schlag R, de Wit M, Trümper L, Hebart H, Hahn M, Thomalla J, Scheid C, Schafhausen P, Verbeek W, Eckart MJ, Gassmann W, Pezzutto A, Schenk M, Brossart P, Geer T, Bildat S, Schäfer E, Hochhaus A, Hasford J. Assessment of imatinib as first-line treatment of chronic myeloid leukemia: 10-year survival results of the randomized CML study IV and impact of non-CML determinants. Leukemia. 2017;31(11):2398–406.
4. Sasaki K, Strom SS, O'Brien S, Jabbour E, Ravandi F, Konopleva M, Borthakur G, Pemmaraju N, Daver N, Jain P, Pierce S, Kantarjian H, Cortes JE. Relative survival in patients with chronic-phase chronic myeloid leukaemia in the tyrosine-kinase inhibitor era: analysis of patient data from six prospective clinical trials. Lancet Haematol. 2015;2:e186–93.
5. Hochhaus A, Baccarani M, Silver RT, Schiffer C, Apperley JF, Cervantes F, Clark RE, Cortes JE, Deininger MW, Guilhot F, Hjorth-Hansen H, Hughes TP, JJWM J, Kantarjian HM, Kim DW, Larson RA, Lipton JH, Mahon FX, Mayer J, Nicolini F, Niederwieser D, Pane F, Radich JP, Rea D, Richter J, Rosti G, Rousselot P, Saglio G, Saußele S, Soverini S, Steegmann JL, Turkina A, Zaritskey A, Hehlmann R. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020;34(4):966–84.
6. Mahon FX, Réa D, Guilhot J, Guilhot F, Huguet F, Nicolini F, Legros L, Charbonnier A, Guerci A, Varet B, Etienne G, Reiffers J, Rousselot P. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre stop Imatinib (STIM) trial. Lancet Oncol. 2010;11:1029–35.
7. Ross DM, Branford S, Seymour JF, Schwarer AP, Arthur C, Yeung DT, Dang P, Goyne JM, Slader C, Filshie RJ, Mills AK, Melo JV, White DL, Grigg AP, Hughes TP. Safety and efficacy of imatinib cessation for CML patients with stable undetectable minimal residual disease: results from the TWISTER study. Blood. 2013;122:515–22.
8. Gleevec. (imatinib) [prescribing information]. East Hanover: Novartis Pharmaceuticals Corporation; 2014.
9. Sprycel. (dasatinib) [prescribing information]. Princeton, NJ: Bristol Myers Squibb Company; 2014.
10. Tasigna. (nilotinib) [prescribing information]. East Hanover, NJ: Novartis Pharmaceuticals Corporation; 2014.
11. He K, Lago MW, Iyer RA, Shyu WC, Humphreys WG, Christopher LJ. Lacteal secretion, fetal and maternal tissue distribution of dasatinib in rats. Drug Metab Dispos. 2008;26:2564–70.
12. Bosulif. (bosutinib) [prescribing information]. New York, NY: Pfizer Inc; 2013.
13. Iclusig. (ponatinib) [prescribing information]. Cambridge, MA: Ariad Pharmaceuticals Inc.; 2013.
14. Abruzzese E, Elena C, Castagnetti F, Gambacorti-Passerini C, Annunziata M, Luciano M, Specchia G, Iurlo A, Capodanno I, Pregno P, Gozzini A, Sica S, Tiribelli S, Galimberti S, Bocchia M, Caracciolo M, Rege Cambrin G, Bergamaschi M, Scortechini AR, Rambaldi A, Turri D, Fozza C, D'adda M, Mastrullo L, Salvucci M, Musolino C, Gaidano G, Gherlinzoni F, d'Emilio A, Fazi P, Baccarani M. Gimema registry of conception/pregnancy in adult Italian patients diagnosed with chronic myeloid Leukemia (CML): report on 166 outcomes. Blood. 2018;132(Supplement 1):43.
15. Breccia M, Cannella L, Montefusco E, Frustaci A, Pacilli M, Alimena G. Male patients with chronic myeloid leukemia treated with Imatinib involved in healthy pregnancies: report of five cases. Leuk Res. 2008;32(3):519–20.
16. Hensley ML, Ford JM. Imatinib treatment: specific issues related to safety, fertility, and pregnancy. Semin Hematol. 2003;40(2 Suppl3):21–5.
17. Iqbal J, Ali Z, Khan AU, Aziz Z. Pregnancy outcomes in patients with chronic myeloid leukemia treated with Imatinib mesylate: short report from a developing country. Leuk Lymphoma. 2014;55(9):2109–13.
18. Ramasamy K, Hayden J, Lim Z, Mufti GJ, Ho AY. Successful pregnancies involving men with chronic myeloid leukaemia on Imatinib therapy. Br J Haematol. 2007;137(4):374–5.
19. Zhou L, You JH, Wu W, Li JM, Shen ZX, Wang AH. Pregnancies in patients with chronic myeloid leukemia treated with tyrosine kinase inhibitor. Leuk Res. 2013;37:1216–21.
20. Cortes J, O'Brien S, Ault P, Borthakur G, Jabbour E, Bradley-Garelik B, Debreczeni K, Yang D, Liu D, Kantarjian H. Pregnancy outcomes among patients with chronic myeloid leukemia treated with dasatinib. Blood. 2008;112. Abstract 3230
21. Cortes JE, Abruzzese E, Chelysheva E, Guha M, Wallis N, Apperley JF. The impact of dasatinib on pregnancy outcomes. Am J Hematol. 2015;90:1111–5.
22. Abruzzese E, Trawinska MM, Perrotti AP, De Fabritiis P. Tyrosine kinase inhibitors and pregnancy. Mediterr J Hematol Infect Dis. 2014;6(1):e2014028.
23. Dou XL, Qin YZ, Shi HX, Lai YY, Hou Y, Huang XJ, Jiang Q. Fertility and disease outcomes in patients with chronic myeloid leukemia. 2019;40(12):980–5.
24. Cortes JE, Gambacorti-Passerini C, Deininger M, Abruzzese E, DeAnnuntis L, Brümmendorf TH. Pregnancy outcomes in patients treated with bosutinib. Int J Hematol Oncol. 2020;9(2): IJH26.
25. Ault P, Kantarjian H, O'Brien S, Faderl S, Beran M, Rios MB, Koller C, Giles F, Keating M, Talpaz M, Cortes J. Pregnancy among patients with chronic myeloid leukemia treated with imatinib. J Clin Oncol. 2006;24:1204–8.

26. Pye SM, Cortes J, Ault P, Hatfied A, Kantarjian H, Pilot R, Rosti G, Apperley J. The effects of Imatinib on pregnancy outcome. Blood. 2008;111:5505–8.
27. Laferla JJ. Spontaneous abortion. Clin Obstet Gynaecol. 1986;13:105–14.
28. Apperley J. Issues of imatinib and pregnancy outcome. J Natl Compr Cancer Netw. 2009;7(10):1–9.
29. Bleyl SB, Moshrefi A, Shaw GM, Saijoh Y, Schoenwolf GC, Pennacchio LA, Slavotinek AM. Candidate genes for congenital diaphragmatic hernia from animal models: sequencing of FOG2 and PDGFR alpha reveals rare variants in diaphragmatic hernia patients. Eur J Hum Genet. 2007;15:950–8.
30. Soriano P. The PDGF alpha receptor is required for neural crest cell development and for normal patterning of the somites. Development. 1997;124:2691–700.
31. Sun T, Jayatilake D, Afink GB, Ataliotis P, Nistér M, Richardson WD, Smith HK. A human YAC transgene rescues craniofacial and neural tube development in PDGFR alpha knockout mice and uncovers a role for PDGFR alpha in prenatal lung growth. Development. 2000;127:4519–29.
32. Madabhavi I, Sarkar M, Modi M, Kadakol N. Pregnancy outcomes in chronic myeloid Leukemia: a single center experience. J Glob Oncol. 2019;5:1–11.
33. Chelysheva E, Turkina A, Rea D, Rousselot P, Nicolini FE, Trawinska MM, Romano A, Malagola M, Cangemi D, Dyagil I, Kotlyarchuk K, Kazakbaeva K, Saliev S, Pavlovsky C, Moiraghi B, Kim D-W, Klamova H, Yassin M, Meliktesyan K, Mikhailov G, Ganeva P, Osorio S, Mauro M, Polushkina E, Shmakov R, Chabaeva J, Kulikov S, Abruzzese E. Pregnancy outcome in female patients with chronic myeloid leukemia worldwide: analysis of 305 cases of the European Leukemia Net registry. HemaSphere. 2019;3(S1):395–6.
34. Ali R, Ozkalemkas F, Kimya Y, Koksal N, Ozkocaman V, Gulten T, Yorulmaz H, Tunali A. Imatinib use during pregnancy and breast feeding: a case report and review of the literature. Arch Gynecol Obstet. 2009;280:169–75.
35. Russell MA, Carpenter MW, Akhtar MS, Lagattuta TF, Egorin MJ. Imatinib mesylate and metabolite concentrations in maternal blood, umbilical cord blood, placenta and breast milk. J Perinatol. 2007;27:241–3.
36. Burwick RM, Kuo K, Brewer D, Druker BJ. Maternal, fetal, and neonatal Imatinib levels with treatment of chronic myeloid Leukemia in pregnancy. Obstet Gynecol. 2017;129(5):831–4.
37. Chelysheva E, Turkina A, Polushkina E, Shmakov R, Zeifman A, Aleshin S, Shokhin I, Guranda D, Oksenjuk O, Mordanov S, Kazakbaeva K, Chilov G. Placental transfer of tyrosine kinase inhibitors used for chronic myeloid leukemia treatment. Leuk Lymphoma. 2018a;59(3):733–8.
38. Chelysheva E, Abruzzese E, Rea D, et al. Chronic myeloid leukemia diagnosed during pregnancy: therapy, outcomes and follow-up. Blood. 2018b;132(Suppl. 1):4255.
39. Chelysheva E, Apperley J, Abruzzese E, Kim DW, Kotlyarchuk K, Shukhov O, Turkina AJ. Kinetics of the leukemic clone in patients with chronic myeloid Leukemia during pregnancy. Blood. 2018c;132(Supplement 1):4254.
40. Yadav U, Solanki SL, Yadav R. Chronic myeloid leukemia with pregnancy: successful management of pregnancy and delivery with hydroxyurea and imatinib continued till delivery. J Cancer Res Ther. 2013;9(3):484–6.
41. Santorsola D, Abruzzese E. Successful management of pregnancy and hepatic toxicity in a CML female patient treated with nilotinib: a case report and a review. Mediterr J Hematol Infect Dis. 2015;7(1):e2015020.
42. Conchon M, Sanabani SS, Bendit I. Two successful pregnancies in a woman with chronic myeloid leukemia exposed to nilotinib during the first trimester of her second pregnancy: case study. J Hematol Oncol. 2009:42.
43. Etienne G, Milpied B, Réa D, Rigal-Huguet F, Tulliez M, Nicolini FE, French Intergroup of CML (Fi-LMC group). Guidelines for the management of nilotinib (Tasigna)-induced side effects in chronic myelogenous leukemia: recommendations of French intergroup of CML (Fi-LMC group). Bull Cancer. 2010;97:997–1009.
44. Bayraktar S, Morency B, Escalon MP. Successful pregnancy in a patient with chronic myeloid leukaemia exposed to dasatinib during the first trimester. BMJ Case Reports Online Publication; 21 Oct 2010 2010.
45. Conchon M, Sanabani SS, Serpa M, Novaes MM, Nardinelli L, Ferreira PB, Dorliac-Lacer PE, Bendit I. Successful pregnancy and delivery in a patient with chronic myeloid leukemia while on dasatinib therapy. Adv Hematol. 2010;2010:136252.
46. Dine G, Levert M, Rehn Y, Ali AN, Brahimi S, Gaillard B, Bocq I, Fumigalli G. Two successful successive pregnancies in a woman with CML treated with dasatinib and temporary peg-interferon. J US China Med Sci. 2013;10:128–33.
47. Kroll T, Ames MB, Pruett JA, Fenske TS. Successful management of pregnancy occurring in a patient with chronic myeloid leukemia on dasatinib. Leuk Lymphoma. 2010;51:1751–3.
48. Berveiller P, Andreoli A, Mir O, Anselem O, Delezoide AL, Sauvageon H, Chapuis N, Tsatsaris V. A dramatic fetal outcome following transplacental transfer of dasatinib. Anti-Cancer Drugs. 2012;23:754–7.
49. Cheon J, Ahn JW, Park KM, Lee G, Jo YS. Teratogenic effect of Radotinib: case report. Anticancer Res. 2016;36(12):6599–601.
50. Abruzzese E, Mauro M, Apperley J, Chelysheva E. Tyrosine kinase inhibitors and pregnancy in chronic myeloid leukemia: opinion, evidence, and recommendations. Therapeutic Advances in Hematology. First published online October 31, 2020 2020.
51. Saussele S, Richter J, Guilhot J, Gruber FX, Hjorth-Hansen H, Almeida A, Janssen JJWM, Mayer J,

Koskenvesa P, Panayiotidis P, Olsson-Strömberg U, Martinez-Lopez J, Rousselot P, Vestergaard H, Ehrencrona H, Kairisto V, Machová Poláková K, Müller MC, Mustjoki S, Berger MG, Fabarius A, Hofmann WK, Hochhaus A, Pfirrmann M, Mahon FX, EURO-SKI investigators. Discontinuation of tyrosine kinase inhibitor therapy in chronic myeloid leukaemia (EURO-SKI): a prespecified interim analysis of a prospective, multicentre, non-randomised, trial. Lancet Oncol. 2018;19:747–57.

52. Goh HG, Kim YJ, Kim DW, Kim HJ, Kim SH, Jang SE, Lee J, Kim D, Kim WS, Park SH, Kweon IY. Previous best responses can be re-achieved by resumption after imatinib discontinuation in patients with chronic myeloid leukaemia: implications for intermittent imatinib therapy. Leuk Lymphoma. 2009;50:944–51.
53. Kuwabara A, Babb A, Ibrahim A, Milojkovic D, Apperley J, Bua M, Reid A, Foroni L, Rezvani K, Goldman J, Marin D. Poor outcome after reintroduction of imatinib in patients with CML who interrupt therapy on account of pregnancy without having achieved an optimal response. Blood. 2010;116:1014–6.
54. Molica M, Noguera NI, Trawinska MM, Martinelli G, Cerchione C, Abruzzese E. Treatment free remission in chronic myeloid leukemia: lights and shadows. Hematol Rep. 2020;12(Suppl 1):8950.
55. Fava C, Rege-Cambrin G, Dogliotti I, Cerrano M, Berchialla P, Dragani M, Rosti G, Castagnetti F, Gugliotta G, Martino B, Gambacorti-Passerini C, Abruzzese E, Elena C, Pregno P, Gozzini A, Capodanno I, Bergamaschi M, Crugnola M, Bocchia M, Galimberti S, Rapezzi D, Iurlo A, Cattaneo D, Latagliata R, Breccia M, Cedrone M, Santoro M, Annunziata M, Levato L, Stagno F, Cavazzini F, Sgherza N, Giai V, Luciano L, Russo S, Musto P, Caocci G, Sorà F, Iuliano F, Lunghi F, Specchia G, Pane F, Ferrero D, Baccarani M, Saglio G. Observational study of chronic myeloid leukemia Italian patients who discontinued tyrosine kinase inhibitors in clinical practice. Haematologica. 2019;104(8):1589–96.
56. Mori S, Vagge E, le Coutre P, Abruzzese E, Martino B, Pungolino E, Elena C, Pierri I, Assouline S, D'Emilio A, Gozzini A, Giraldo P, Stagno F, Iurlo A, Luciani M, De Riso G, Redaelli S, Kim DW, Pirola A, Mezzatesta C, Petroccione A, Lodolo D'Oria A, Crivori P, Piazza R, Gambacorti-Passerini C. Age and dPCR can predict relapse in CML patients who discontinued imatinib: the ISAV study. Am J Hematol. 2015;90(10):910–4.
57. Lasica M, Willcox A, Burbury K, Ross DM, Branford S, Butler J, Filshie R, Januszewicz H, Joske D, Mills A, Simpson D, Tam C, Taylor K, Watson AM, Wolf M, Grigg A. The effect of tyrosine kinase inhibitor interruption and interferon use on pregnancy outcomes and long-term disease control in chronic myeloid leukemia. Leuk Lymphoma. 2019;60(7):1796–802.
58. Abruzzese E, Trawinska MM, de Fabritiis P, Baccarani M. Management of pregnant chronic myeloid leukemia patients. Expert Rev Hematol. 2016;9(8):781–91.
59. La Rosée P, Martiat P, Leitner A, Klag T, Müller MC, Erben P, Schenk T, Saussele S, Hochhaus A. Improved tolerability by a modified intermittent treatment schedule of dasatinib for patients with chronic myeloid leukemia resistant or intolerant to imatinib. Ann Hematol. 2013;92:1345–50.
60. Russo D, Martinelli G, Malagola M, Skert C, Soverini S, Iacobucci I, De Vivo A, Testoni N, Castagnetti F, Gugliotta G, Turri D, Bergamaschi M, Pregno P, Pungolino E, Stagno F, Breccia M, Martino B, Intermesoli T, Fava C, Abruzzese E, Tiribelli M, Bigazzi C, Cesana BM, Rosti G, Baccarani M. Effects and outcome of a policy of intermittent imatinib treatment in elderly patients with chronic myeloid leukemia. Blood. 2013;121(26):5138–44.
61. Lee JO, Kim DW, Abruzzese E, Apperley J, Caldwell L, Mauro MJ. Kinetics of BCR-ABL after TKI interruption during pregnancy in CML: a multinational retrospective analysis. Blood. 2018;132(Supplement 1):4263.
62. Baer MR, Ozer H, Foon KA. Interferon-alpha therapy during pregnancy in chronic myelogenous leukaemia and hairy cell leukaemia. Br J Haematol. 1992;81(2):167–9.
63. Mubarak AA, Kakil IR, Awidi A, Al-Homsi U, Fawzi Z, Kelta M, Al-Hassan A. Normal outcome of pregnancy in chronic myeloid leukemia treated with interferon-a in 1st trimester: report of 3 cases and review of the literature. Am J Hematol. 2002;69:115–8.
64. Hiratsuka M, Minakami H, Koshizuka S, Sato I. Administration of interferon-a during pregnancy: effect on fetus. J Perinat Med. 2000;28:372–6.
65. Vantroyen B, Vanstraelen D: Treatment of essential thrombocythemia in a young woman with interferon alpha-2a before and during pregnancy (unpubl. data) personal communication.
66. Al Bahar S, Pandita R, Nath SV. Pregnancy in chronic myeloid leukemia patients treated with alpha interferon. Int J Gynecol Obstet. 2004;85:281–2.
67. Kuroiwa M, Gondo H, Ashida K, Kamimura T, Miyamoto T, Niho Y, Tsukimori K, Nakano H, Ohga S. Interferon alpha therapy for chronic myeloid leukemia during pregnancy. Am J Hematol. 1998;59:101–2.
68. Regierer AC, Schulz CO, Kuehnhardt D, Flath B, Possinger K. Interferon α – therapy for chronic myeloid leukemia during pregnancy. Am J Hematol. 2006;81:149–56.
69. Abu-Tineh M, Kassem N, Abdulla MA, Ismail OM, Ghasoub R, Aldapt MB, Yassin MA. Outcome of pregnancy in the era of pegylated interferon alpha 2a in females with essential thrombocythemia: an experience from Qatar. Case Rep Oncol. 2020;13(1):336–40.

70. Thauvin Robinet C, Maingueneau C, Robert E, et al. Exposure to hydroxyurea during pregnancy: a case series. Leukemia. 2001;15:1309–11.
71. Fadilah SA, Ahmad-Zailani H, Soon-Keng C, Norlaila M. Successful treatment of chronic myeloid leukemia during pregnancy with hydroxyurea. Leukemia. 2002;16:1202–3.
72. Abruzzese E, de Fabritiis P, Trawinska MM, Niscola P, Apperley JF, Mauro MJ. Back to the future: treatment-free remission and pregnancy in chronic myeloid leukemia. Eur J Haematol. 2019a;102(2):197–9.
73. Abruzzese E, Turkina AJ, Apperley JF, Bondanini F, de Fabritiis P, Kim DW, Dyagil I, Ganeva P, Garcia-Gutiérrez V, Kazakbaeva K, Klamová H, Kotlyarchuk K, Mauro MJ, Milojkovic D, Moriaghi B, Meliktesyan K, Nicolini FE, Polushkina E, Rea D, Rousselot P, Shacham A, Shmakov R, Trawinska MM, Chelysheva EY. Pregnancy management in CML patients: to treat or not to treat? Report of 224 outcomes of the European Leukemia Net (ELN) database. Blood. 2019b;134(Supplement_1):498.
74. Gambacorti-Passerini CB, Tornaghi L, Marangon E, Franceschino A, Pogliani EM, D'Incalci M, Zucchetti M. Imatinib concentrations in human milk. Blood. 2007;109(4):1790.
75. Bristol-Myers Squibb Canada. Hydrea@product monograph. Montreal 2006.
76. Kumar AR, Hale TW, Mock RE. Transfer of interferon alfa into human breast milk. J Hum Lact. 2000;16:226–8.
77. Lichtman MLJ. Acute myelogenous leukemia. Williams Hematology, vol. 1047. 6th ed. New York: McGraw-Hill; 2001.
78. Sheehy WT. An evaluation of the effect of pregnancy on chronic granulocytic leukaemia. Am J Obstet Gynecol. 1958;75:788.
79. Ali R, Ozkalemkaş F, Ozkocaman V, Ozçelik T, Ozan U, Kimya Y, Tunali A. Successful pregnancy and delivery in patient with CML and management of CML with leukapheresis during pregnancy; a case report and review of the literature. Jpn J Clin Oncol. 2004;34:215–7.
80. Klaasen R, de Jong P, Wijermans PW. Successful management of chronic myeloid leukemia with leucapheresis during a twin pregnancy. Neth J Med. 2007;65:147–9.
81. Yellu M, Pinkard S, Ghose A, Medlin S. CML in pregnancy: a case report using leukapheresis and literature review. Transfus Apher Sci. 2015;53:289–92.



15 Response-Related Predictors of Survival and of Treatment-Free Remission in CML

Susan Branford, Naranie Shanmuganathan, and Timothy P. Hughes

15.1 Introduction

Patients with chronic-phase CML who receive tyrosine kinase inhibitor (TKI) therapy have a highly variable response, despite the apparent homogeneity of the disease and the highly targeted nature of TKI therapy. In 5–10% of patients progression to blast crisis or accelerated phase will be observed. At the other extreme, 33–68% achieve a deep molecular response (DMR, ≤0.01% *BCR-ABL1* on the international scale) at 5 years [1–3] which increases to 45–81% at 10 years [4, 5]. If DMR is maintained for several years, cessation of therapy can be attempted with the aim to attain treatment-free remission (TFR). Between these extremes, the majority of patients will achieve and maintain a major molecular response (MMR), in which case they have an extremely low risk of a CML-related death, as long as they keep taking their TKI therapy.

Haematological remission is the first evidence of response that clinicians will look for in the first 3 months of TKI therapy. Failure to achieve

S. Branford (✉)
Department of Genetics and Molecular Pathology, SA Pathology, Centre for Cancer Biology, Adelaide, SA, Australia

School of Pharmacy and Medical Science, University of South Australia, Adelaide, SA, Australia

School of Medicine, University of Adelaide, Adelaide, SA, Australia

Precision Medicine Theme, South Australian Health & Medical Research Institute (SAHMRI), Adelaide, SA, Australia
e-mail: Susan.branford@sa.gov.au

N. Shanmuganathan
Department of Genetics and Molecular Pathology, SA Pathology, Centre for Cancer Biology, Adelaide, SA, Australia

School of Pharmacy and Medical Science, University of South Australia, Adelaide, SA, Australia

School of Medicine, University of Adelaide, Adelaide, SA, Australia

Precision Medicine Theme, South Australian Health & Medical Research Institute (SAHMRI), Adelaide, SA, Australia

Department of Haematology, Royal Adelaide Hospital and SA Pathology, Adelaide, SA, Australia

Australasian Leukaemia and Lymphoma Group (ALLG), Melbourne, VIC, Australia

T. P. Hughes
School of Medicine, University of Adelaide, Adelaide, SA, Australia

Precision Medicine Theme, South Australian Health & Medical Research Institute (SAHMRI), Adelaide, SA, Australia

Department of Haematology, Royal Adelaide Hospital and SA Pathology, Adelaide, SA, Australia

Australasian Leukaemia and Lymphoma Group (ALLG), Melbourne, VIC, Australia

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
R. Hehlmann (ed.), *Chronic Myeloid Leukemia*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-71913-5_15

haematological remission by 3 months is rare, but is an unequivocal indication of treatment failure. Beyond that, blood counts are mainly followed to look for haematological toxicity, a relatively common event in the first few months of TKI therapy. Cytogenetic response used to be the mainstay of monitoring in the era of alpha interferon therapy. In the TKI era, cytogenetic analysis of the marrow is mainly relevant as a means to look for additional cytogenetic abnormalities in the leukaemic cells, which may have an adverse impact on outcomes, although the impact may be specific to the abnormality detected [6–8]. Once a patient achieves and maintains a *BCR-ABL1* level below 1%, the value of further cytogenetic studies of the bone marrow is very limited, because Ph+cells will rarely be detected [9]. Furthermore cytogenetic abnormalities in Ph-negative cells in general have minimal impact on patient outcomes, particularly among patients who achieve a *BCR-ABL1* level of <10% IS at 3 months of therapy [10, 11].

The degree of early reduction of the leukaemic cells in the blood and bone marrow and the rapidity of the reduction, as measured by qRT-PCR measurement of the *BCR-ABL1* transcripts in the blood, has proven to be remarkably predictive of subsequent response and outcomes. Within 3 months, the risk of transformation, the probability of a durable response to therapy over many years and the probability of eventual achievement of TFR can all be calculated with a reasonable level of accuracy. This provides the opportunity to give patients reassurance about their long-term prospects in the majority of patients with optimal responses and to intervene in less favourable cases in an attempt to reduce the risk of adverse disease outcomes. This is all dependent on having accurate, sensitive, internationally standardized qRT-PCR monitoring of the blood available, which is conducted and reviewed frequently. The qRT-PCR result at specified time points and the trend of the response over time are critical components of a clinician's oversight of each patient's therapy. These values can also serve as a motivator for the patient to maintain high levels of drug adherence, as any reduction in adherence will likely lead to a rising level of *BCR-ABL1* [12] and/or failure to achieve milestone responses.

15.2 International Standardized Molecular Monitoring for Response Prediction

The IRIS trial (International Randomized Study of Interferon and STI571), which commenced in 2000, was the first clinical trial of imatinib where molecular monitoring using real-time quantitative PCR of *BCR-ABL1* transcripts was included [13]. Comparison of data generated by the three participating laboratories demonstrated consistent differences in the reported values. Clearly, alignment of data to a common scale was required to allow for the appropriate clinical evaluation of the data. At that stage it was not known if molecular monitoring would provide any value for patient management. However, the spectacular response rates of patients in the imatinib arm of the trial and the rapid and profound level of reduction of the leukaemic clone did indeed warrant a more sensitive measure of residual disease than could be offered by cytogenetic analysis. The IRIS study established the clinical relevance of achieving a major molecular response (MMR, ≤0.1% on the international reporting scale) [13]. To this day MMR is a key endpoint in clinical trials of TKIs [14, 15] and is generally considered an optimal response. However, the general acceptance of molecular monitoring for patient management decisions required more than a decade of research, including considerable effort towards method standardization and the introduction of the common international reporting scale (IS). The IS is recognized as the gold standard for molecular monitoring. The development of international method standardization is outlined in Chap. 7.

Internationally standardized quantitative PCR of *BCR-ABL1* transcripts using peripheral blood is the preferred and recommended method for monitoring response to TKI therapy [16, 17]. Molecular monitoring not only provides enhanced sensitivity compared with cytogenetic analysis but also has the major advantage that

peripheral blood is suitable. There is a strong correlation between bone marrow cytogenetic values and peripheral blood *BCR-ABL1* transcript values [9, 18, 19]. Importantly, a *BCR-ABL1* value of ≤1.0% IS is an excellent surrogate for a complete cytogenetic response. However, cytogenetic analysis is still required during therapy in some situations as outlined in clinical recommendations [16, 17]. Molecular monitoring was recommended at 3-month intervals by the European LeukemiaNet (ELN) in 2006 [20]. In 2009, the updated management recommendations included the molecular response level of MMR as a criterion for the evaluation of response [21]. Treatment intervention was not specifically mandated for lack of an MMR. By 2013 there was sufficient supporting evidence from many years of research [22–30] for the ELN to expand the molecular response levels that defined response and to mandate treatment intervention in case of lack of certain time-dependent molecular milestone values [31]. This was enabled by the increasing adoption of the international reporting scale for *BCR-ABL1*. The National Comprehensive Cancer Network (NCCN) also incorporated therapeutic decisions based on molecular values into their clinical practice guidelines for CML in 2013.

Patients from resource-poor countries often do not have access to good quality monitoring due to financial constraints [32]. Studies have shown that this could be detrimental because the frequency of molecular monitoring is associated with patient outcome and less than the recommended frequency is associated with poorer clinical outcomes [33, 34]. This includes increased rates of non-adherence to TKI therapy, which is also associated with poorer outcomes [35–37]. Interestingly, monitoring frequency is not always adhered to in resource-rich countries, which likely impacts long-term patient outcomes [34]. The SIMPLICITY study is an observational study of first-line TKI-treated patients in the USA and six European countries that prospectively enrolled 1242 patients between 2010 and 2015 [38]. It documented monitoring and management patterns in routine clinical practice. Despite both the NCCN and ELN recommending 3-monthly molecular monitoring in the first year of TKI therapy throughout the observation time, the study reported that 68% of patients were not tested for *BCR-ABL1* transcript levels at 3 months of treatment, which decreased to 26% and 9% at 6 and 12 months, respectively. Among patients with a *BCR-ABL1* analysis at 12 months, 23% were not reported on the IS. One would hope that the rate of monitoring using methods standardized to the IS has improved over time considering the importance of the molecular milestone values over the first 12 months of TKI therapy for long-term outcome prediction and therapeutic intervention decisions [16, 17].

15.3 Monitoring Patients with Atypical *BCR-ABL1* Transcripts

It is essential that the *BCR-ABL1* transcript type is characterized at diagnosis to ensure appropriate future monitoring [16]. Qualitative PCR using a simple and widely used multiplex method is capable of detecting typical (e13a2 and e14a2) and atypical transcripts [39]. Approximately 98% of CML patients have the typical *BCR-ABL1* transcripts [40]. The remaining patients have atypical *BCR-ABL1* transcripts where the international reporting scale cannot be easily applied. The atypical fusions most frequently involve different *BCR* exons fused to exon 2 of *ABL1*, and the most common are e1a2, e6a2, e8a2 and e19a2. CML patients are now expected to have long-term survival, and knowledge transference of the *BCR-ABL1* transcript type to new treating clinicians or testing laboratories is critical, irrespective of whether the transcript is typical or atypical. Our laboratory was recently involved in monitoring a patient with the atypical e1a2 transcript. In this case, handover of transcript type to a new laboratory did not occur and a false-negative *BCR-ABL1* value was reported over many months using a method designed for the common transcript types [41]. The patient discontinued TKI therapy without appropriate monitoring for the e1a2 transcript, which was only realized when the patient experienced haematologic relapse.

Patients with atypical *BCR-ABL1* transcripts must be monitored with an alternate method. Qualitative

PCR could be used, although fluorescence in situ hybridization (FISH) may be a better alternative, at least initially, since it provides a quantitative assessment of residual leukaemia. However, FISH methods that measure *BCR-ABL1* have not been standardized to the degree that quantitative PCR methods have been, meaning that TKI failure criteria have not been defined. Furthermore, FISH methods do not have the sensitivity requirements for long-term treatment decisions.

At this stage standardized molecular methods for monitoring CML patients with rare, atypical transcripts are unlikely considering the tremendous international collaborative effort and many years of work that was required to achieve standardization for the common transcripts [42–50]. However, standardization of quantitative PCR methods that measure the e1a2 transcript is underway in Europe for patients with acute lymphoblastic leukaemia (ALL) [51]. Patients with e1a2 CML will likely benefit from this program, but the prognostic implication of achieving specific molecular responses will differ between patients with CML or ALL.

Patient-specific quantitative PCR methods have been developed to monitor patients with atypical transcripts. DNA PCR, which takes advantage of the patient-specific genomic breakpoint for sensitive and specific residual disease measurement, is increasingly utilized for patients with common transcripts [52–56]. DNA PCR recently demonstrated clinical utility for monitoring treatment response for a patient with an atypical e19a2 *BCR-ABL1* fusion [57]. The DNA PCR method was developed as a research tool but has the potential for response monitoring. Whether this strategy could be used to monitor all patients with atypical transcripts outside of the research setting is dependent on available resources in the diagnostic setting.

15.4 Molecular Response-Related Predictors of Outcome

BCR-ABL1 values measured over the first year of TKI therapy are strong predictors of outcome. Outcomes include molecular and cytogenetic response, failure-free survival, progression-free survival and overall survival. Progression was defined as blast crisis or accelerated phase and failure includes events outlined by the ELN [16]. Multiple studies have consistently demonstrated that molecular response in the first few months of commencing TKI therapy is the most critical for long-term outcome prediction [22–29, 58–65]. It has greater prognostic significance than clinical risk scores at diagnosis. The relationship between the degree of initial reduction of *BCR-ABL1* in the first few months of TKI therapy and patient outcome was recognized in 2002 and 2003, which was in the early years after the introduction of imatinib and before the IS was introduced [22–24]. International molecular standardization and larger patient cohorts later confirmed the prognostic significance of ‘early molecular response’ for patients treated with first-line imatinib (Fig. 15.1) and first- and second-generation TKIs [64]. The largest randomized study of first-line imatinib was the German CML Study IV of 1551 patients [1, 64, 65]. At 3 years a survival advantage was demonstrated for patients who achieved an MMR at 12 months compared to those who did not achieve an MMR, 99% (95% CI, 97–100%) versus 95% (95% CI, 93–97), $P = 0.016$. The difference was also observed for progression-free survival: MMR versus no MMR at 12 months, 99% (95% CI, 97–100) versus 95% (95% CI, 93–97); $P = 0.014$ [64]. These results were independent of the treatment approach. At 10 years, the difference in overall survival was still significant for MMR versus no MMR at 12 months: 86.1% (95% CI, 82.1–89.6) versus 80.4% (95% CI, 75.9–84.5). The progression-free survival for MMR versus no MMR at 12 months was 86.6% (95% CI, 82.7–90.0) versus 78.8% (95% CI, 74.3–83.0) [65].

The 7-year follow-up of the IRIS trial found that a *BCR-ABL1* value of >10% IS at 6 months of first-line imatinib and >1% at 12 months was associated with inferior event-free survival and higher rate of progression to accelerated phase or blast crisis compared with other molecular response groups [25]. An MMR at 18 months was associated with a negligible long-term risk of disease progression, and a significantly lower number of patients subsequently lost a complete cytogenetic response compared with patients who

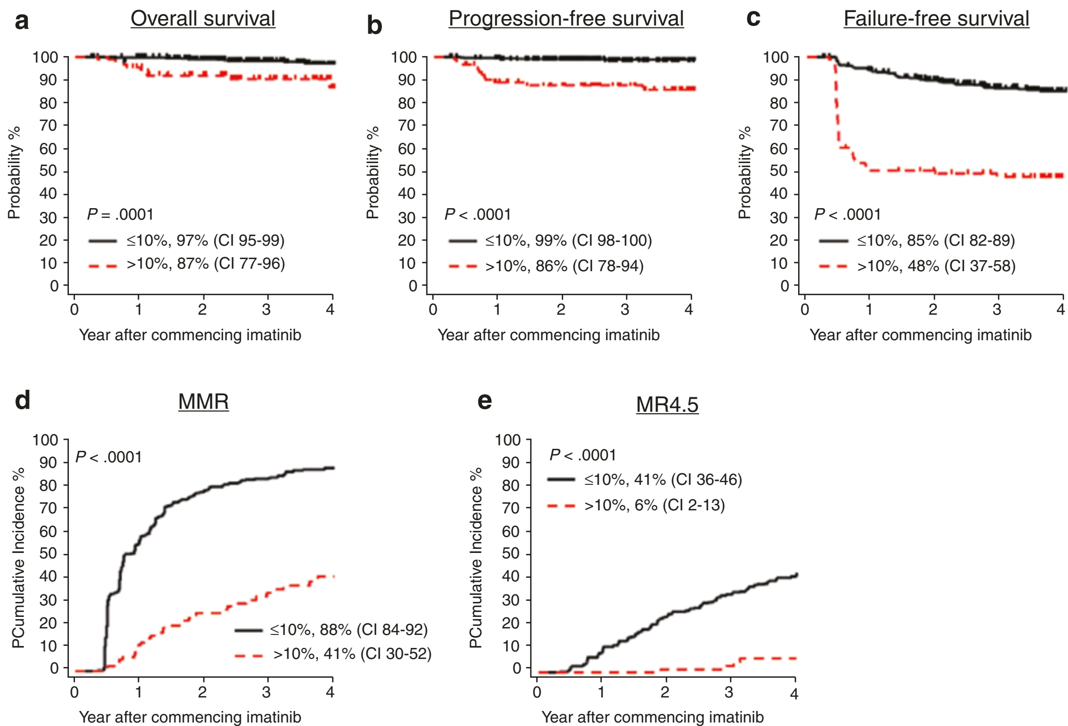


Fig. 15.1 *BCR-ABL1* values >10% IS at 3 months of TKI therapy are consistently associated with poorer outcome. We studied consecutively treated patients with first-line imatinib. Those with *BCR-ABL1* values ≤10% at 3 months (n = 410 patients) had significantly better outcome than patients with *BCR-ABL1* values >10% (n = 97 patients) (**a**–**e**). This research was originally published in Blood. Branford S, Yeung DT, Parker WT, Roberts ND, Purins L, Braley JA, Altamura HK, Yeoman AL, Georgievski J, Jamison BA, Phillis S, Donaldson Z, Leong M, Fletcher L, Seymour JF, Grigg AP, Ross DM, Hughes TP. Prognosis for patients with CML and > 10% *BCR-ABL1* after 3 months of imatinib depends on the rate of *BCR-ABL1* decline. 2014;124:511–8

had *BCR-ABL1* values between 0.1% and 1%. The long-term follow-up of the IRIS trial demonstrated that response persisted and confirmed the importance of achieving an MMR by 12 or 18 months for long-term survival (Table 15.1) [66]. Due to the design of the molecular monitoring time points in the IRIS trial, there were very few samples tested at 3 months. Therefore, no outcome predictions were based on the 3-month value. The predictive value of the early molecular milestone values was confirmed and expanded by many other studies of patients treated with imatinib and second-generation TKIs [26–30, 59, 60, 62, 67, 68]. In particular, the *BCR-ABL1* value at 3 months consistently predicted long-term outcome.

Two seminal papers in 2012 synthesized the concept that molecular values measured over the first 12 months of TKI therapy were powerful outcome predictors [26, 27]. Marin et al. went so far as to claim that the *BCR-ABL1* value at 3 months is the only requirement for predicting outcome, thereby allowing early therapeutic intervention [27]. Two hundred eighty-two consecutive newly diagnosed patients treated with 400 mg imatinib over a 10-year timespan were assessed. Optimal *BCR-ABL1* cutoff values were defined at 3, 6 and 12 months that predicted overall survival, progression-free survival and molecular and cytogenetic outcomes. Patients with a *BCR-ABL1* value at 3 months of >9.84% had significantly inferior responses. When compared with cytogenetic milestone values, *BCR-ABL1* values at 6 and 12 months were superior predictors of overall survival. The prognostic impact of the early *BCR-ABL1* values remained significant for patients who switched to second-generation

Table 15.1 Landmark analysis of outcomes at 10 years by molecular response levels at 12 and 18 months in evaluable patients[a] treated with frontline imatinib therapy in the IRIS trial. From the *New England Journal of Medicine*, Hochhaus A, Larson RA, Guilhot F, Radich JP, Branford S, Hughes TP, Baccarani M, Deininger MW, Cervantes F, Fujihara S, Ortmann C-E, Menssen HD, Kantarjian H, O'Brien SG, Druker BJ, Long-Term Outcomes of Imatinib Treatment for Chronic Myeloid Leukemia, 376, 917–927. Copyright © 2017 Massachusetts Medical Society. Reprinted with permission

	MMR or better	No MMR	*P*
At 12 months			
No. of patients who could be evaluated	153	151	
Deaths, *n* (%)	15 (9.8)	22 (14.6)	
Not related to CML	11 (7.2)	7 (4.6)	
Related to CML	4 (2.6)	15 (9.9)	
Estimated 10-year overall survival—% (95% CI)	91.1 (86.5–95.7)	85.3 (79.5–91.1)	0.15
Estimated 10-year freedom from CML-related death—% (95% CI)	97.8 (95.4–100)	89.4 (84.3–94.5)	0.007
At 18 months			
No. of patients who could be evaluated	164	89	
Deaths, *n* (%)	12 (7.3)	13 (14.6)	
Not related to CML	12 (7.3)	4 (4.5)	
Related to CML	0	9 (10.1)	
Estimated 10-year overall survival—% (95% CI)	93.0 (89.0–97.0)	85.6 (77.9–93.2)	0.04
Estimated 10-year freedom from CML-related death—% (95% CI)	100 (100–100)	90.5 (84.1–96.8)	< 0.001

[a]A total of 305 patients were considered able to be evaluated for molecular response at 12 months; however, one patient discontinued study treatment at 11 months (the patient was considered able to be evaluated for molecular response at 12 months on the basis of an 11-month assessment) and was therefore excluded from the 12-month landmark analysis. Patients who died or who had data censored before each landmark analysis were excluded from that landmark analysis. The deaths reported here are those that occurred in patients with the indicated level of molecular response at 12 months or 18 months who died at some point after 12 months or 18 months, respectively. Two-sided P values were calculated with the use of the log-rank test. CML denotes chronic myeloid leukaemia

TKIs for imatinib failure. Furthermore, the prognostic value of the 3-month *BCR-ABL1* level was independent of imatinib dose intensity. A proportion of patients had dose reduction or temporary cessation due to adverse effects, and these patients had significantly higher *BCR-ABL1* values than imatinib-tolerant patients. The imatinib-intolerant patients had similar or worse outcomes than imatinib-tolerant patients within the same *BCR-ABL1* value category, suggesting that the effect of the early molecular response was not purely biological. The optimal *BCR-ABL1* values for outcome prediction identified in this study were close to those that are now used in international guidelines and recommendations: 10% IS, 1% IS and 0.1% IS [16, 17].

Hanfstein et al. examined a large cohort of 1303 first-line imatinib-treated patients and correlated the 3- and 6-month molecular and cytogenetic response with progression-free and overall survival [26]. High-risk groups were identified according to response levels. Molecular values at 3 months >10% IS and at 6 months >1% IS were highly predictive of inferior outcomes. Similar results were obtained when patients were grouped according to the lack of achievement of a major cytogenetic response (>35% Philadelphia positive) at 3 months and lack of a complete cytogenetic response at 6 months. The study confirmed the strong correlation between a *BCR-ABL1* value of ≤1.0% IS and the achievement of a complete cytogenetic response and ≤10% IS and a major cytogenetic response. Treatment optimization was recommended for patients missing the 3- and 6-month landmark values.

The findings from these and other studies that followed formed the basis of consensus international recommendations and guidelines by experts for monitoring treatment response and for therapeutic intervention decisions. A *BCR-ABL1* value ≤10% at 3 months has become known as an early molecular response (EMR). Expert therapeutic intervention recommendations have been refined over the years, and decisions regarding molecular cutoff values were made with considerable discussion among the experts since there was not always a clear consensus, particularly regarding

therapeutic intervention for patients with *BCR-ABL1* values >10%. The ELN recognized the strong association between *BCR-ABL1* >10% IS at 3 months and inferior outcome. However, in 2013 the ELN was wary of recommending treatment change based on a single *BCR-ABL1* measurement at 3 months [31]. Internationally standardized molecular methods had not been widely adopted at that stage, and the quality of the molecular values could not be guaranteed in all testing laboratories. Therefore, the ELN recommended repeat testing within 1–3 months, and if the *BCR-ABL1* value was still >10%, then a change of therapy was warranted.

The NCCN did initially adopt the option to change therapy at 3 months for *BCR-ABL1* >10%. However, based on subsequent studies, the NCCN later updated the guidelines and suggested additional testing for patients with 3-month *BCR-ABL1* values that were only slightly above 10% or those with a steep decline from the baseline value. The NCCN recognized that these patients may achieve <10% at 6 months, and the outcome was generally favourable. In 2014 Hanfstein et al. had reported that the velocity of *BCR-ABL1* reduction over the first 3 months of TKI was a more reliable predictor of outcome than a single measurement at 3 months [69]. This was a rational analysis since some patients start with a relatively low *BCR-ABL1* transcript value at diagnosis, even <10%. Theoretically, a patient could have no response to TKI and no *BCR-ABL1* reduction from the baseline value at 3 months and still be within the optimal range. The study reported that patients with an approximately threefold reduction of *BCR-ABL1* at the 3-month measurement had a lower probability of progression and higher overall survival. Similarly, we examined more than 500 patients and found that the initial rate of *BCR-ABL1* reduction had prognostic significance [63]. However, we calculated the rate of reduction as the *BCR-ABL1* halving time, which incorporated the number of days over which *BCR-ABL1* measurements occurred. We recognized that the day of sample collection of the 3-month *BCR-ABL1* measurement does not always occur on day 90 after starting TKI. Evaluation of a milestone variable requires assignment of a *BCR-ABL1* value to a specific time point, and the recommendation is to assign values to a particular time point if the day of collection falls within ±1.5 months [70]. The 3-month sample collection time point could theoretically occur between 1.5 and 4.5 months; however every effort should be made to perform the test as close to 3 months as possible. We demonstrated that an early sample collection could alter the 3-month *BCR-ABL1* category and alter the fold reduction of *BCR-ABL1* from baseline. For patients with >10% at 3 months, a long *BCR-ABL1* halving time was an independent predictor of outcome. Thus, examining the kinetics of *BCR-ABL1* decline over time allows earlier therapeutic intervention decisions without further *BCR-ABL1* measurement.

15.4.1 Can the Initial Slope of *BCR-ABL1* Decline be Measured Using *ABL1* Control Gene Methods?

The ELN and NCCN recognize the prognostic value of assessing the kinetics of *BCR-ABL1* decline from the baseline value. However, the ELN only recommends qualitative PCR for the detection and characterization of *BCR-ABL1* as part of the diagnostic workup, whereas the NCCN states that quantitative PCR for *BCR-ABL1* should be performed at diagnosis. The accumulating evidence that the tempo of the initial fall in *BCR-ABL1* is highly informative suggests that measuring the *BCR-ABL1* level at diagnosis is critical for the assessment of the significance of the 3-month *BCR-ABL1* value.

Originally it was thought that methodological differences would preclude the broad adoption of assessment of the initial kinetics for response prediction. Most methods use *ABL1* as the control gene where the primers that amplify *ABL1* are located in an exon that is common to both *ABL1* and *BCR-ABL1*. Therefore, the primers also amplify *BCR-ABL1*, which could theoretically introduce a bias. The methods are based on that of a seminal paper published in 2003 by Gabert et al. on behalf of the Europe Against

Cancer Programme [71]. The authors of that paper did acknowledge that the method had the potential to underestimate the ratio of *BCR-ABL1/ABL1* when a large proportion of cells express *BCR-ABL1*. The theoretical maximum ratio is 100%. However, the data demonstrated that this bias had a minor impact on the relative quantification of *BCR-ABL1* transcripts at diagnosis [71]. The median *BCR-ABL1/ABL1%* ratio at diagnosis for peripheral blood samples was 117%. Values up to 440% were obtained for peripheral blood at diagnosis [71]. These results were unexpected but were obtained with both a plasmid standard curve and without a standard curve using the ΔCt method. The authors pointed out that similar results were reported in papers that have set the standard for monitoring *BCR-ABL1* transcripts using *qRT-PCR* [72, 73]. It was noted that the correlation between *qRT-PCR* and the percentage of Philadelphia-positive metaphases was very good, although there was a wide range in the *BCR-ABL1/ABL1* ratio, especially in patients who showed 100% Philadelphia-positive metaphases [73]. The wide range of ratios at diagnosis was later confirmed using *GUSB* as the control gene, where *BCR-ABL1/GUSB* values ranged from 0.1% to 230% [69], and using *BCR-ABL1/ABL1* where values ranged from 0.01% to 599% [74].

A recent study compared the linearity of *BCR-ABL1/ABL1* ratios and *BCR-ABL1/GUSB* ratios for a subset of Spanish patients treated with frontline nilotinib in the ENEST1st trial [75]. The sub-study evaluated whether the kinetics of *BCR-ABL1* transcript decline in the first 3 months after commencing nilotinib had predictive value for the subsequent achievement of a DMR (≤0.01% IS) at 18 months. The study found that *BCR-ABL1* transcripts declined linearly with both control genes. Furthermore, the use of *ABL1* allowed for an earlier prediction of DMR (2 months) compared with *GUSB* (3 months). The use of *GUSB* to predict an earlier and more accurate response than *ABL1* was not supported by the results. The authors concluded that the dynamic determination of *BCR-ABL1* transcripts using either internal control gene is valid and predictive of subsequent DMR [75]. The tight correlation between ratios measured using *ABL1* or *GUSB* as control genes was in agreement with the results of an earlier study published in 2014 by Huet et al. [76]. That study used the halving time of *BCR-ABL1/ABL1%* from the diagnosis ratio to demonstrate that the halving time could predict MMR at 1 year. The authors also reported that the halving time, log reduction or transcript level at 3 months had a similar ability to predict molecular response, irrespective of the control gene that was used [76]. A number of other studies have now established the reliability of assessing the response kinetics derived from the *BCR-ABL1/ABL1* ratios [62, 74, 77, 78]. Significant differences in overall survival, progression-, event- and failure-free survival, and molecular responses were reported according to the initial *BCR-ABL1/ABL1* halving time [74].

15.5 Predictors of a Deep Molecular Response and Treatment-Free Remission

BCR-ABL1 values measured over the first year of therapy not only predict overall and progression-free survival but also long-term molecular responses. In particular, they predict the subsequent achievement of a DMR. Of note, the term 'complete molecular response' is now rarely used in favour of terms that describe the sensitivity of detection achieved for each sample: MR4 (0.01%), MR4.5 (0.0032%) and MR5 (0.001%) [46] (see Chap. 7). The term 'complete molecular response' incorrectly implied a complete eradication of disease, which can never be determined with certainty. However, detection of residual *BCR-ABL1* is dependent on the quality of RNA and the limit of detection of the quantitative PCR method. Many factors can lead to degradation of *BCR-ABL1* transcripts, including delayed sample stabilization or processing. The molecular response (MR) terms provide a better guide to the depth of response achieved for individual samples and are more readily standardized across laboratories and clinical studies. DMR is the general term to describe *BCR-ABL1* ≤0.01%

IS and is the prerequisite for a trial of TFR. TFR has been a major focus of research for more than a decade [79–88]. Achieving an MMR within 3 to 6 months of commencing imatinib therapy is associated with the highest frequency of achieving the TKI discontinuation criteria [1, 27, 58, 59]. A strategy to improve the rates of early MMR is to use more potent TKIs frontline, although this decision needs to be made in the context of the motivation of the patient for TFR and their comorbidities.

The ELN considers lack of MMR by 12 months of TKI as a warning as long as the *BCR-ABL1* value is ≤1%, meaning the current treatment must be carefully considered. Treatment failure at 12 months and beyond is indicated by *BCR-ABL1* values >1%, in which case treatment should be changed. Warning also indicates that additional molecular analysis is warranted if the kinetics of the response are uncertain. MMR is considered an optimal response as it predicts a CML-specific survival close to 100% because progression to advanced-phase disease is very uncommon [16]. A recent analysis of the German CML Study IV investigated when it is necessary to regard lack of MMR as treatment failure, indicating a switch of therapy is warranted. For progression-free survival, the landmark time point of 2.5 years to achieve MMR showed the largest difference in those with or without MMR [89]. A specific time to achieve DMR for progression-free survival was not identified. The updated ELN recommendations now suggest that a change of treatment may be considered if MMR is not reached by 36–48 months [16].

Recent updates of the NCCN clinical practice guidelines no longer list MMR at 12 months as TKI-sensitive disease [17, 90]. A *BCR-ABL1* value ≤1.0% (equivalent to a complete cytogenetic response) at 12 months is now considered TKI-sensitive disease [17]. The claim is that evaluation of the prognostic significance of MMR has been assessed for different outcome measures at multiple time points, without adjusting for multiple comparisons. This has reduced the validity of the conclusions. Nevertheless, the NCCN recognizes the value of MMR at 12 months and indicates that it is associated with a low probability of progression and is essential for eventually achieving the criteria for TKI cessation for a trial of TFR. However MMR is no longer formally listed in the early treatment response milestones.

In recent years, the *BCR-ABL1* transcript type has been reported as a predictor of some outcome measures for TKI-treated patients. In general the e14a2 transcript type is more favourable and has been associated with higher rates of complete cytogenetic response, MMR or DMR [91–97]. However, Sharma et al. reported superior complete cytogenetic response for patients with the e13a2 transcript [98]. Two studies reported an association between e14a2 transcripts and a more favourable progression-free and failure-free survival [99, 100]. However, this association is not generally reported. Interestingly, one study reported a higher overall survival at 10 years for imatinib-treated patients with the e13a2 transcript [96]. We studied 523 patients consecutively treated in clinical trials of imatinib and found that the transcript type was associated with DMR only [101]. This is consistent with the general concept that the e14a2 transcript is associated with faster responses. For patients treated with first-line nilotinib, it was also the case that the e14a2 transcript was associated with higher rates of DMR [102]. Predictors of a sustained DMR have been investigated [58, 103]. A recent report by Etienne et al. reported that 46% of a cohort of 398 de novo chronic phase patients sustained a DMR, defined as MR4.5 for at least 24 months [103]. Female sex, the e14a2 transcript type and low clinical risk score were associated with sustained DMR.

The duration of DMR before TKI discontinuation was the strongest predictor of TFR in the EURO-SKI study, which is the largest treatment cessation study [87]. Relapse-free survival also improved, but only marginally, with every additional year of first-line imatinib before cessation. That study did not report an association between TFR and the *BCR-ABL1* transcript type; however, smaller cessation studies have reported an association [104, 105]. Claudiani et al. published the first report that the e14a2 transcript was associated with a higher rate of sustained TFR in a retrospective analysis of 64 patients [105]. We recently corroborated

this finding in an Australian cohort [106]. Whether the higher rate of TFR for patients with the e14a2 transcript is associated with longer duration of DMR before cessation in these patients is unknown. However, it is possible because the e14a2 transcript is associated with more rapid and deeper molecular responses. Future analyses incorporating transcript type, duration of DMR and other factors into multivariate models involving large patient cohorts may resolve the issue of whether the transcript type is an independent predictor of TFR. The e14a2 transcript is also predicted to be more immunogenic than the e13a2 transcript [107, 108]. Whether this leads to increased immune-mediated clearance of residual leukaemia that contributes to TFR is unknown.

One question that remains unresolved is the importance of the depth of response at the time of TFR attempt. Some studies have reported that in TFR-eligible patients, deeper responses are associated with a higher probability of attaining TFR. Some of these studies used digital PCR to measure *BCR-ABL1*, which is a more sensitive assay for detecting residual leukaemia [109, 110]. However the importance of these findings will need to be assessed in the context of other variables using multivariate analysis derived from large numbers of TFR study patients.

There may be a role for DNA PCR in the prediction of TFR. Ross et al. [52] developed a highly sensitive method (sensitivity$10^{-6.2}$) to amplify patient-specific *BCR-ABL1* genomic breakpoints and to assess whether more sensitive detection of *BCR-ABL1* could predict TFR. However, this was not the case for the 18 patients studied. All patients had undetectable *BCR-ABL1* mRNA transcripts with a sensitivity of MR4.5 at TKI cessation. DNA PCR detected *BCR-ABL1* in almost all patients, irrespective of whether TFR was maintained or not. Furthermore, detectable DNA *BCR-ABL1* persisted in remission. A recent study also used qRT-PCR for *BCR-ABL1* transcripts and DNA PCR of patient-specific *BCR-ABL1* genomic breakpoints to measure residual disease in patients who ceased TKI [56]. Combining the DNA and RNA values of 42 patients proved valuable for the prediction of DMR maintenance and molecular relapse after TKI cessation/interruption. *BCR-ABL1* was measured using digital PCR during DMR, prior to TKI cessation or treatment interruption. Three response groups were identified and a traffic light stratification model was suggested: (1) double negative for DNA and RNA (green), (2) DNA positive and RNA negative (yellow) and (3) double positive for DNA and RNA (red). Significant differences in the molecular relapse-free survival were observed between the groups after cessation. The highest rate occurred in the patients who were double negative (80–100%) and the lowest rate in the patients who were double positive (20%). The traffic-light stratification system is a highly promising tool to predict the chance of TFR.

15.6 The Impact of Response Dynamics for TFR

The impact of early molecular response dynamics on the success of a TFR attempt has recently been highlighted [106]. We investigated a cohort of 115 patients attempting TFR and found that patients with a rapid initial decline of *BCR-ABL1* transcripts after commencing TKI, measured by the *BCR-ABL1* halving time, had a substantially higher rate of TFR at 12 months after cessation compared with patients who experienced slower initial responses to TKI therapy [106]. The *BCR-ABL1* value measured at 3 months of TKI was not predictive of TFR at 12 months after cessation. An update of the published data is shown in Fig. 15.2, which included additional patients who attempted TFR and had >12 months of follow-up after cessation (n = 123 patients). Division of patients based on halving time quartiles illustrates the importance of early molecular kinetics. In this analysis shorter *BCR-ABL1* halving times correlate with rapid initial response to TKI therapy. For example, patients with a halving time of <8.8 days (quartile 1) had an 85% probability of remaining in TFR at 12 months after cessation, whereas virtually all patients with a halving time of >22.1 days (quartile 4) experienced molecular relapse and required TKI recommencement. Moreover, rapid initial *BCR-ABL1* decline (equating to a short halving time) also correlated with the probability of achieving TFR eligibility [106]. These data support the critical importance

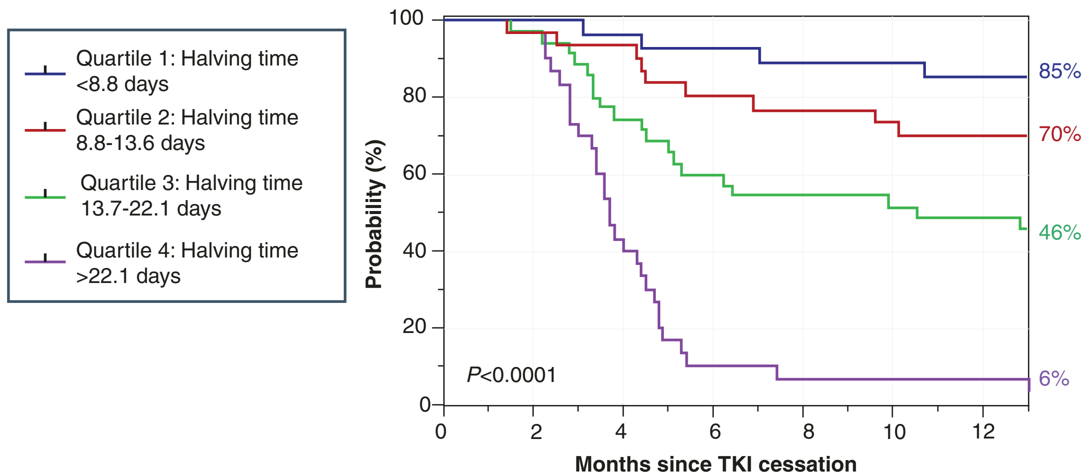


Fig. 15.2 Importance of the initial molecular response kinetics to TKI therapy for the subsequent achievement of TFR. The *BCR-ABL1* halving time after commencing TKI was calculated for 123 patients who attempted TFR. Dividing the halving time into quartile values demonstrated a significant difference in the subsequent achievement of TFR at 12 months after ceasing TKI. Patients with the shortest halving time, which correlates with the most rapid reduction of *BCR-ABL1*, had the highest rate of TFR. Conversely, patients with the slowest decline had very little probability of TFR. The graph shows the probability of TFR according to the halving time quartiles

of the initial kinetics of *BCR-ABL1* decline after commencing TKI for multiple outcome measures, including optimal response and attainment of TFR. A model incorporating independent predictors of sustained TFR, including the initial rate of *BCR-ABL1* decline, could enhance clinical management decisions.

an ongoing risk of relapse for many years after TKI cessation. In a recent review Rousselot et al. found that patients who maintained MR4.5 for the first 2 years had a negligible risk of late relapse, whereas around 18% of patients who did not maintain MR4.5 over the first 2 years relapsed at later time points [111].

15.7 Molecular Monitoring after Therapy Ceases in a TFR Attempt

Once a patient stops therapy in a TFR attempt, frequent molecular monitoring is mandatory. This is because molecular relapse can develop early after stopping and is typically quite rapid. In order to avoid cytogenetic and haematological relapse, it is recommended that TKI therapy is resumed as soon as MMR is lost [84]. To facilitate this, monthly monitoring is recommended by both the ELN and NCCN [16, 17]. The precise details of the monitoring schedule differ, although both agree on monthly monitoring for at least the first 6 months (Table 15.2).

Long-term monitoring is also necessary because while most relapses occur early there is

15.8 Prioritizing qRT-PCR Monitoring where Resources Are Limited

While long-term qRT-PCR monitoring every 3 months is the consensus recommendation from NCCN and ELN, this will not always be possible in resource-limited settings [32, 112]. Where PCR tests are in limited supply, prioritization of testing may be required. In general, the greatest impact of molecular monitoring will be in the first 1–2 years of therapy. If patients are refractory to TKI therapy or develop drug resistance, salvage measures can sometimes be effective [113–115]. The greatest risk of these adverse outcomes is in the first 2 years of therapy. Once a patient has been demonstrated to achieve MMR, the value of 3-monthly monitoring is greatly reduced. Loss

Table 15.2 Summary of ELN and NCCN recommendations regarding treatment-free remission

ELN 2020	NCCN 2020
Recommend consideration of TFR in appropriate patients after careful discussion employing the concept of shared decision making	Emphasizes full discussion with patient regarding risks and adverse events
Mandatory	Mandatory
CML in first CP only	CML in CP, no prior treatment failure or AP/BC
Motivated patient with agreement to more frequent monitoring after stopping	Motivated patient
High-quality PCR using the international scale (IS) with rapid turnaround	High-quality PCR with rapid turnaround (2 weeks) sensitivity at least MR4.5
Minimal (stop allowed)	A 18 years
Typical e13a2 or e14a2 *BCR-ABL1* transcript	Quantifiable *BCR-ABL1* transcript
First-line or second-line if intolerant to first-line TKI	Not specified
TKI >5 years (>4 years if second generation TKI)	TKI >3 years
DMR >2 years	Stable MR4 ≥2 years, documented on at least four tests, performed at least 3 months apart
No prior treatment failure	Prompt resumption of TKI within 4 weeks of loss of MMR
Optimal—STOP recommended for consideration	Optimal
TKI >5 years	TKI >6 years
MR4 >3 years	MR4 >3 years
MR4.5 >2 years	
Monitoring	Monitoring
Monthly for 6 months	Monthly for 12 months
Then 2 monthly until 12 months	Then 2 monthly from 12–24 months
Then 3 monthly ongoing	Then 3 monthly ongoing

of MMR is rare in patients who continue to take their therapy. Recognition of eligibility for TFR, however, will be dependent on ongoing accurate, sensitive monitoring after MMR is achieved. A period of 2–3 years of deep molecular response must be documented before TFR attempts are recommended. This can limit the availability of TFR for many patients who are not monitored regularly. This remains a challenge in many countries.

In the TFR setting frequent monitoring is essential to ensure patients who relapse avoid haematological relapse. If monthly monitoring is not practical, less frequent monitoring than monthly in the first 6 months is possible without endangering the patient [116]. We modelled the safe minimum frequency of monitoring after TKI cessation over the first 12 months. The current recommendation of monthly monitoring after TKI cessation in the first 6–12 months is a historical and cautious measure from the first cessation trials where the rate and timing of relapse was unknown [80, 117]. We found that monitoring every 2 months in the first 6 months and then every 3 months between 6 and 12 months may provide a safe balance between reduced testing frequency and timely detection of molecular relapse [116]. This assumes that monitoring will return to monthly if MR4.5 is lost in the first 12 months. Less frequent monitoring after cessation should make a TFR attempt a practical option for more patients.

15.9 Molecular Monitoring in Advanced-Phase CML

In patients who present with blast phase or progress to blast phase after TKI therapy, the role of molecular monitoring is less clear. There is no evidence that time-dependent achievement of specific levels of molecular response provides reassurance of long-term response, unlike the situation in chronic-phase CML. One study does suggest that deeper responses than MMR are associated with an improved prospect of survival, but nearly all of the patients with long-term survival received an allogeneic transplant, so it is not clear whether achieving deeper molecular responses provides substantial benefit in the non-transplant setting [118]. The focus of therapy in the setting of blast crisis is initially to achieve haematological remission and subsequently a cytogenetic and molecular response. If possible, patients with responsive disease should proceed to an allograft, since this is the only approach with a reasonable prospect of long-term disease control. For patients who do not have the prospect of an allogeneic transplant, the approach is largely palliative.

15.10 Molecular Monitoring Post Allograft

Molecular monitoring post allograft in CML patients is an important indicator of remission status. This is particularly important because disease relapse post allograft remains a significant challenge—especially in patients allografted for advanced-phase disease. Early detection of relapse by molecular monitoring provides an opportunity to re-induce remission much earlier, and perhaps more effectively, than would be the case if haematological monitoring alone was being used. Re-introduction of TKI therapy and/or donor leukocyte infusions can be very effective if used in the earlier stages of relapse post allograft. qRT-PCR screening of the blood 3–6 monthly is generally recommended for patients in chronic phase from the time of the allograft. For patients allografted for advanced phase or second chronic phase CML, bone marrow molecular monitoring is also indicated. The predictive value of low-level detection of *BCR-ABL1* after an allograft is quite limited, but progressive rises in *BCR-ABL1* level often herald cytogenetic and haematological relapse [119].

15.11 Future Directions of Molecular Monitoring

For frontline monitoring of response to therapy, it seems likely that the current monitoring strategy of regular qRT-PCR tests will remain the standard practice. There are no clear advantages of DNA-based PCR in this setting [120]. For assessment prior to TFR, to determine whether it is reasonable to proceed with a TFR attempt, current monitoring provides a broad indication of who should attempt TFR, but even strict adherence to the current ELN requirements of 3 years of MR4 and/or 2 years of MR4.5 will only lead to successful TFR in about 50% of cases. The *BCR-ABL1* halving time after commencing TKI therapy provides strong predictive value, but may not be readily available for many patients currently being considered for TFR [106]. *BCR-ABL1* quantitative measurement prior to commencing TKI is not always performed. Indeed, the ELN states that a quantitative *BCR-ABL1* assessment is not mandatory at diagnosis [16]. However, our data suggest it provides substantial benefit for outcome prediction, including for TFR success [63, 106]. Furthermore, multiple studies have now confirmed that various methods and control genes can reliably assess the rate of decline for outcome prediction [62, 74, 77, 78, 121–124]. Several studies suggest digital PCR or sensitive DNA-based PCR when a patient is being considered for TFR may prove to be more informative [109, 110]. These approaches will remain experimental until further prospective validation is undertaken.

15.12 Conclusions

Since the early analysis of the IRIS study, it has been clear that measurement of the level of *BCR-ABL1* transcripts in the blood represents an excellent surrogate for the overall level of leukaemia in the blood and bone marrow. Furthermore it has been demonstrated that the level of *BCR-ABL1* achieved at specific time points and the tempo of the reduction in the transcript level together provide remarkably reliable insight into the prospects of long-term survival and TFR.

Optimal management of CML patients requires regular accurate and sensitive qRT-PCR monitoring adjusted to the international scale. Any compromise on this practice will likely lead to less favourable outcomes, including a higher risk of CML-related deaths and fewer patients being able to achieve TFR.

References

1. Hehlmann R, Muller MC, Lauseker M, Hanfstein B, Fabarius A, Schreiber A, et al. Deep molecular response is reached by the majority of patients treated with imatinib, predicts survival, and is achieved more quickly by optimized high-dose imatinib: results from the randomized CML-study IV. J Clin Oncol. 2014;32(5):415–23. https://doi.org/10.1200/JCO.2013.49.9020.
2. Hochhaus A, Saglio G, Hughes TP, Larson RA, Kim DWW, Issaragrisil S, et al. Long-term benefits and risks

of frontline nilotinib vs imatinib for chronic myeloid leukemia in chronic phase: 5-year update of the randomized ENESTnd trial. Leukemia. 2016;30(5):1044–54. https://doi.org/10.1038/leu.2016.5.

3. Cortes JE, Saglio G, Kantarjian HM, Baccarani M, Mayer J, Boque C, et al. Final 5-year study results of DASISION: the Dasatinib versus imatinib study in treatment-naive chronic myeloid leukemia patients trial. J Clin Oncol. 2016;34(20):2333–40. https://doi.org/10.1200/JCO.2015.64.8899.
4. Kalmanti L, Saussele S, Lauseker M, Muller MC, Dietz CT, Heinrich L, et al. Safety and efficacy of imatinib in CML over a period of 10 years: data from the randomized CML-study IV. Leukemia. 2015;29(5):1123–32. https://doi.org/10.1038/leu.2015.36.
5. Hughes TP, Saglio G, Larson RA, Kantarjian HM, Kim D-W, Issaragrisil S, et al. Long-term outcomes in patients with chronic myeloid leukemia in chronic phase receiving frontline nilotinib versus imatinib: ENESTnd 10-year analysis. Blood. 2019;134(Supplement_1):2924. https://doi.org/10.1182/blood-2019-128761%JBlood.
6. Wang W, Cortes JE, Tang G, Khoury JD, Wang S, Bueso-Ramos CE, et al. Risk stratification of chromosomal abnormalities in chronic myelogenous leukemia in the era of tyrosine kinase inhibitor therapy. Blood. 2016;127(22):2742–50. https://doi.org/10.1182/blood-2016-01-690230.
7. Alhuraiji A, Kantarjian H, Boddu P, Ravandi F, Borthakur G, DiNardo C, et al. Prognostic significance of additional chromosomal abnormalities at the time of diagnosis in patients with chronic myeloid leukemia treated with frontline tyrosine kinase inhibitors. Am J Hematol. 2018;93(1):84–90. https://doi.org/10.1002/ajh.24943.
8. Wang W, Cortes JE, Lin P, Beaty MW, Ai D, Amin HM, et al. Clinical and prognostic significance of 3q26.2 and other chromosome 3 abnormalities in CML in the era of tyrosine kinase inhibitors. Blood. 2015;126(14):1699–706. https://doi.org/10.1182/blood-2015-05-646489.
9. Ross DM, Branford S, Moore S, Hughes TP. Limited clinical value of regular bone marrow cytogenetic analysis in imatinib-treated chronic phase CML patients monitored by RQ-PCR for BCR-ABL. Leukemia. 2006;20(4):664–70.
10. Chen X, Zheng J, Liang K, He Y, Du W, Li J, et al. Characterisation of clonal Philadelphia-negative cytogenetic abnormalities in a large cohort of chronic myeloid leukaemia. Intern Med J. 2018;48(4):439–44. https://doi.org/10.1111/imj.13527.
11. Issa GC, Kantarjian H, Nogueras Gonzalez G, Borthakur G, Tang G, Wierda W, et al. Clonal chromosomal abnormalities appearing in Philadelphia negative metaphases during CML treatment. Blood. 2017; https://doi.org/10.1182/blood-2017-07-792143.
12. Branford S, Yeung DT, Prime JA, Choi S-Y, Bang J-H, Park JE, et al. BCR-ABL1 doubling times more reliably assess the dynamics of CML relapse compared with the BCR-ABL1 fold rise: implications for monitoring and management. Blood. 2012;119(18):4264–71. https://doi.org/10.1182/blood-2011-11-393041.
13. Hughes TP, Kaeda J, Branford S, Rudzki Z, Hochhaus A, Hensley ML, et al. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. N Engl J Med. 2003;349(15):1423–32.
14. Cortes JE, Gambacorti-Passerini C, Deininger MW, Mauro MJ, Chuah C, Kim D-W, et al. Bosutinib versus imatinib for newly diagnosed chronic myeloid leukemia: results from the randomized BFORE trial. J Clin Oncol. 2018;36(3):231–7. https://doi.org/10.1200/JCO.2017.74.7162.
15. Hughes TP, Mauro MJ, Cortes JE, Minami H, Rea D, DeAngelo DJ, et al. Asciminib in chronic myeloid leukemia after ABL kinase inhibitor failure. N Engl J Med. 2019;381(24):2315–26. https://doi.org/10.1056/NEJMoa1902328.
16. Hochhaus A, Baccarani M, Silver RT, Schiffer C, Apperley JF, Cervantes F, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020;34(4):966–84. https://doi.org/10.1038/s41375-020-0776-2.
17. NCCN Clinical practice guidelines in oncology: chronic myeloid leukemia. Version 3.2020.
18. Branford S, Hughes TP, Rudzki Z. Monitoring chronic myeloid leukaemia therapy by real-time quantitative PCR in blood is a reliable alternative to bone marrow cytogenetics. Br J Haematol. 1999;107(3):587–99.
19. Akard LP, Cortes JE, Albitar M, Goldberg SL, Warsi G, Wetzler M, et al. Correlations between cytogenetic and molecular monitoring among patients with newly diagnosed chronic myeloid leukemia in chronic phase: post hoc analyses of the Rationale and Insight for Gleevec High-Dose Therapy study. Arch Pathol Lab Med. 2014;138(9):1186–92. https://doi.org/10.5858/arpa.2013-0584-OA.
20. Baccarani M, Saglio G, Goldman J, Hochhaus A, Simonsson B, Appelbaum F, et al. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. Blood. 2006;108(6):1809–20. https://doi.org/10.1182/blood-2006-02-005686.
21. Baccarani M, Cortes J, Pane F, Niederwieser D, Saglio G, Apperley J, et al. Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. J Clin Oncol. 2009;27(35):6041–51. https://doi.org/10.1200/JCO.2009.25.0779.
22. Merx K, Muller MC, Kreil S, Lahaye T, Paschka P, Schoch C, et al. Early reduction of BCR-ABL mRNA transcript levels predicts cytogenetic response in chronic phase CML patients treated with imatinib after failure of interferon alpha. Leukemia. 2002;16(9):1579–83.
23. Wang L, Pearson K, Ferguson JE, Clark RE. The early molecular response to imatinib predicts cyto-

genetic and clinical outcome in chronic myeloid leukaemia. Br J Haematol. 2003;120(6):990–9.
24. Branford S, Rudzki Z, Harper A, Grigg A, Taylor K, Durrant S, et al. Imatinib produces significantly superior molecular responses compared to interferon alfa plus cytarabine in patients with newly diagnosed chronic myeloid leukemia in chronic phase. Leukemia. 2003;17(12):2401–9.
25. Hughes TP, Hochhaus A, Branford S, Muller MC, Kaeda JS, Foroni L, et al. Long-term prognostic significance of early molecular response to imatinib in newly diagnosed chronic myeloid leukemia: an analysis from the International Randomized Study of Interferon and STI571 (IRIS). Blood. 2010;116(19):3758–65.
26. Hanfstein B, Muller MC, Hehlmann R, Erben P, Lauseker M, Fabarius A, et al. Early molecular and cytogenetic response is predictive for long-term progression-free and overall survival in chronic myeloid leukemia (CML). Leukemia. 2012;26(9):2096–102.
27. Marin D, Ibrahim AR, Lucas C, Gerrard G, Wang L, Szydlo RM, et al. Assessment of BCR-ABL1 transcript levels at 3 months is the only requirement for predicting outcome for patients with chronic myeloid leukemia treated with tyrosine kinase inhibitors. J Clin Oncol. 2012;30(3):232–8.
28. Branford S, Kim D-W, Soverini S, Haque A, Shou Y, Woodman RC, et al. Initial molecular response at 3 months may predict both response and event-free survival at 24 months in imatinib-resistant or -intolerant patients with Philadelphia chromosome-positive chronic myeloid leukemia in chronic phase treated with nilotinib. J Clin Oncol. 2012;30(35):4323–9. https://doi.org/10.1200/jco.2011.40.5217.
29. Marin D, Hedgley C, Clark RE, Apperley J, Foroni L, Milojkovic D, et al. Predictive value of early molecular response in patients with chronic myeloid leukemia treated with first-line dasatinib. Blood. 2012;120(2):291–4. https://doi.org/10.1182/blood-2012-01-407486.
30. Jain P, Kantarjian H, Nazha A, O'Brien S, Jabbour E, Romo CG, et al. Early responses predict better outcomes in patients with newly diagnosed chronic myeloid leukemia: results with four tyrosine kinase inhibitor modalities. Blood. 2013;121(24):4867–74. https://doi.org/10.1182/blood-2013-03-490128.
31. Baccarani M, Deininger MW, Rosti G, Hochhaus A, Soverini S, Apperley JF, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood. 2013;122(6):872–84. https://doi.org/10.1182/blood-2013-05-501569.
32. Malhotra H, Radich J, Garcia-Gonzalez P. Meeting the needs of CML patients in resource-poor countries. Hematology. 2019;2019(1):433–42. https://doi.org/10.1182/hematology.2019000050.
33. Goldberg SL, Chen L, Guerin A, Macalalad AR, Liu N, Kaminsky M, et al. Association between molecular monitoring and long-term outcomes in chronic myelogenous leukemia patients treated with first line imatinib. Curr Med Res Opin. 2013;29(9):1075–82. https://doi.org/10.1185/03007995.2013.812034.
34. Guerin A, Chen L, Dea K, Wu EQ, Goldberg SL. Economic benefits of adequate molecular monitoring in patients with chronic myelogenous leukemia. J Med Econ. 2014;17(2):89–98. https://doi.org/10.3111/13696998.2013.862251.
35. Darkow T, Henk HJ, Thomas SK, Feng W, Baladi JF, Goldberg GA, et al. Treatment interruptions and non-adherence with imatinib and associated healthcare costs: a retrospective analysis among managed care patients with chronic myelogenous leukaemia. PharmacoEconomics. 2007;25(6):481–96. doi:2564 [pii]
36. Marin D, Bazeos A, Mahon F-X, Eliasson L, Milojkovic D, Bua M, et al. Adherence is the critical factor for achieving molecular responses in patients with Chronic Myeloid Leukemia who achieve complete cytogenetic responses on imatinib. J Clin Oncol. 2010;28(14):2381–8. https://doi.org/10.1200/jco.2009.26.3087.
37. Ibrahim AR, Eliasson L, Apperley JF, Milojkovic D, Bua M, Szydlo R, et al. Poor adherence is the main reason for loss of CCyR and imatinib failure for chronic myeloid leukemia patients on long-term therapy. Blood. 2011;117(14):3733–6. https://doi.org/10.1182/blood-2010-10-309807.
38. Goldberg SL, Cortes JE, Gambacorti-Passerini C, Hehlmann R, Khoury HJ, Michallet M, et al. First-line treatment selection and early monitoring patterns in chronic phase-chronic myeloid leukemia in routine clinical practice: SIMPLICITY. Am J Hematol. 2017;92(11):1214–23. https://doi.org/10.1002/ajh.24887.
39. Cross NC, Melo JV, Feng L, Goldman JM. An optimized multiplex polymerase chain reaction (PCR) for detection of BCR-ABL fusion mRNAs in haematological disorders. Leukemia. 1994;8(1):186–9.
40. Baccarani M, Castagnetti F, Gugliotta G, Rosti G, Soverini S, Albeer A, et al. The proportion of different BCR-ABL1 transcript types in chronic myeloid leukemia. An international overview. Leukemia. 2019; https://doi.org/10.1038/s41375-018-0341-4.
41. Sharplin K, Altamura H, Taylor K, Wellwood J, Taylor D, Branford S. Chronic myeloid leukaemia: the dangers of not knowing your BCR-ABL1 transcript. Leuk Res. 2019;87:106231. https://doi.org/10.1016/j.leukres.2019.106231.
42. Hughes T, Branford S. Molecular monitoring of BCR-ABL as a guide to clinical management in chronic myeloid leukaemia. Blood Rev. 2006;20(1):29–41.
43. Branford S, Fletcher L, Cross NCP, Muller MC, Hochhaus A, Kim D-W, et al. Desirable performance characteristics for BCR-ABL measurement on an international reporting scale to allow consistent interpretation of individual patient response and comparison of response rates between clinical trials. Blood. 2008;112(8):3330–8. https://doi.org/10.1182/blood-2008-04-150680.

44. Muller MC, Cross NCP, Erben P, Schenk T, Hanfstein B, Ernst T, et al. Harmonization of molecular monitoring of CML therapy in Europe. Leukemia. 2009;23(11):1957–63.
45. White HE, Matejtschuk P, Rigsby P, Gabert J, Lin F, Lynn Wang Y, et al. Establishment of the first World Health Organization International Genetic Reference Panel for quantitation of BCR-ABL mRNA. Blood. 2010;116(22):e111–7. https://doi.org/10.1182/blood-2010-06-291641.
46. Cross NCP, White HE, Muller MC, Saglio G, Hochhaus A. Standardized definitions of molecular response in chronic myeloid leukemia. Leukemia. 2012;26(10):2172–5.
47. Cross NC, White HE, Colomer D, Ehrencrona H, Foroni L, Gottardi E, et al. Laboratory recommendations for scoring deep molecular responses following treatment for chronic myeloid leukemia. Leukemia. 2015;29(5):999–1003. https://doi.org/10.1038/leu.2015.29.
48. Cross NC, White HE, Ernst T, Welden L, Dietz C, Saglio G, et al. Development and evaluation of a secondary reference panel for BCR-ABL1 quantification on the International Scale. Leukemia. 2016;30(9):1844–52. https://doi.org/10.1038/leu.2016.90.
49. Cross NCP, White HE, Evans PAS, Hancock J, Copland M, Milojkovic D, et al. Consensus on BCR-ABL1 reporting in chronic myeloid leukaemia in the UK. Br J Haematol. 2018;182(6):777–88. https://doi.org/10.1111/bjh.15542.
50. Langabeer SE. Standardized molecular monitoring for variant BCR-ABL1 transcripts in chronic myeloid leukemia. Arch Pathol Lab Med. 2015;139(8):969. https://doi.org/10.5858/arpa.2014-0522-LE.
51. Pfeifer H, Cazzaniga G, van der Velden VHJ, Cayuela JM, Schafer B, Spinelli O, et al. Standardisation and consensus guidelines for minimal residual disease assessment in Philadelphia-positive acute lymphoblastic leukemia (Ph + ALL) by real-time quantitative reverse transcriptase PCR of e1a2 BCR-ABL1. Leukemia. 2019;33(8):1910–22. https://doi.org/10.1038/s41375-019-0413-0.
52. Ross DM, Branford S, Seymour JF, Schwarer AP, Arthur C, Bartley PA, et al. Patients with chronic myeloid leukemia who maintain a complete molecular response after stopping imatinib treatment have evidence of persistent leukemia by DNA PCR. Leukemia. 2010;24(10):1719–24. https://doi.org/10.1038/leu.2010.185.
53. Mattarucchi E, Spinelli O, Rambaldi A, Pasquali F, Lo Curto F, Campiotti L, et al. Molecular monitoring of residual disease in chronic myeloid leukemia by genomic DNA compared with conventional mRNA analysis. J Mol Diagn: JMD. 2009;11(5):482–7. https://doi.org/10.2353/jmoldx.2009.080150.
54. Bartley PA, Latham S, Budgen B, Ross DM, Hughes E, Branford S, et al. A DNA real-time quantitative PCR method suitable for routine monitoring of low levels of minimal residual disease in chronic myeloid leukemia. J Mol Diagn: JMD. 2015;17(2):185–92. https://doi.org/10.1016/j.jmoldx.2014.10.002.
55. Alikian M, Ellery P, Forbes M, Gerrard G, Kasperaviciute D, Sosinsky A, et al. Next-generation sequencing-assisted DNA-based digital PCR for a personalized approach to the detection and quantification of residual disease in chronic myeloid leukemia patients. J Mol Diagn: JMD. 2016;18(2):176–89. https://doi.org/10.1016/j.jmoldx.2015.09.005.
56. Machova Polakova K, Zizkova H, Zuna J, Motlova E, Hovorkova L, Gottschalk A, et al. Analysis of chronic myeloid leukaemia during deep molecular response by genomic PCR: a traffic light stratification model with impact on treatment-free remission. Leukemia. 2020; https://doi.org/10.1038/s41375-020-0882-1.
57. Pagani IS, Dang P, Saunders VA, Braley J, Thieleke A, Branford S, et al. Clinical utility of genomic DNA Q-PCR for the monitoring of a patient with atypical e19a2 BCR-ABL1 transcripts in chronic myeloid leukemia. Leuk Lymphoma. 2020:1–3. https://doi.org/10.1080/10428194.2020.1772476.
58. Branford S, Yeung DT, Ross DM, Prime JA, Field CR, Altamura HK, et al. Early molecular response and female sex strongly predict stable undetectable BCR-ABL1, the criteria for imatinib discontinuation in patients with CML. Blood. 2013;121(19):3818–24. https://doi.org/10.1182/blood-2012-10-462291.
59. Hughes TP, Saglio G, Kantarjian HM, Guilhot F, Niederwieser D, Rosti G, et al. Early molecular response predicts outcomes in patients with chronic myeloid leukemia in chronic phase treated with frontline nilotinib or imatinib. Blood. 2014;123(9):1353–60. https://doi.org/10.1182/blood-2013-06-510396.
60. Lapusan S, Yong A, Savani BN, Mohty M. Achieving early molecular response in chronic myeloid leukemia in chronic phase to reduce the risk of progression: clinical relevance of the 3- and 6-month time points. Eur J Haematol. 2015;95(2):103–12. https://doi.org/10.1111/ejh.12453.
61. Boquimpani C, Schaffel R, Biasoli I, Bendit I, Spector N. Molecular responses at 3 and 6 months after switching to a second-generation tyrosine kinase inhibitor are complementary and predictive of long-term outcomes in patients with chronic myeloid leukemia who fail imatinib. Leuk Lymphoma. 2015;56(6):1787–92. https://doi.org/10.3109/10428194.2014.974047.
62. Fava C, Rege-Cambrin G, Dogliotti I, Gottardi E, Berchialla P, Di Gioacchino B, et al. Early BCR-ABL1 reduction is predictive of better event-free survival in patients with newly diagnosed chronic myeloid leukemia treated with any tyrosine kinase inhibitor. Clin Lymphoma Myeloma Leuk. 2016;16(Suppl):S96–S100. https://doi.org/10.1016/j.clml.2016.03.008.
63. Branford S, Yeung DT, Parker WT, Roberts ND, Purins L, Braley JA, et al. Prognosis for patients with CML and >10% BCR-ABL1 after 3 months of imatinib depends on the rate of BCR-ABL1 decline.

Blood. 2014;124(4):511–8. https://doi.org/10.1182/blood-2014-03-566323.
64. Hehlmann R, Lauseker M, Jung-Munkwitz S, Leitner A, Muller MC, Pletsch N, et al. Tolerability-adapted imatinib 800 mg/d versus 400 mg/d versus 400 mg/d plus interferon-alpha in newly diagnosed chronic myeloid leukemia. J Clin Oncol. 2011;29(12):1634–42. https://doi.org/10.1200/JCO.2010.32.0598.
65. Hehlmann R, Lauseker M, Saussele S, Pfirrmann M, Krause S, Kolb HJ, et al. Assessment of imatinib as first-line treatment of chronic myeloid leukemia: 10-year survival results of the randomized CML study IV and impact of non-CML determinants. Leukemia. 2017;31(11):2398–406. https://doi.org/10.1038/leu.2017.253.
66. Hochhaus A, Larson RA, Guilhot F, Radich JP, Branford S, Hughes TP, et al. Long-term outcomes of imatinib treatment for chronic myeloid leukemia. N Engl J Med. 2017;376(10):917–27. https://doi.org/10.1056/NEJMoa1609324.
67. Neelakantan P, Gerrard G, Lucas C, Milojkovic D, May P, Wang L, et al. Combining BCR-ABL1 transcript levels at 3 and 6 months in chronic myeloid leukemia: implications for early intervention strategies. Blood. 2013;121(14):2739–42.
68. Jabbour E, Kantarjian HM, Saglio G, Steegmann JL, Shah NP, Boque C, et al. Early response with dasatinib or imatinib in chronic myeloid leukemia: 3-year follow-up from a randomized phase 3 trial (DASISION). Blood. 2014;123(4):494–500. https://doi.org/10.1182/blood-2013-06-511592.
69. Hanfstein B, Shlyakhto V, Lauseker M, Hehlmann R, Saussele S, Dietz C, et al. Velocity of early BCR-ABL transcript elimination as an optimized predictor of outcome in chronic myeloid leukemia (CML) patients in chronic phase on treatment with imatinib. Leukemia. 2014;28(10):1988–92.
70. Pfirrmann M, Hochhaus A, Lauseker M, Sausele S, Hehlmann R, Hasford J. Recommendations to meet statistical challenges arising from endpoints beyond overall survival in clinical trials on chronic myeloid leukemia. Leukemia. 2011;25(9):1433–8.
71. Gabert J, Beillard E, van der Velden VH, Bi W, Grimwade D, Pallisgaard N, et al. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia—a Europe against Cancer program. Leukemia. 2003;17(12):2318–57.
72. Emig M, Saussele S, Wittor H, Weisser A, Reiter A, Willer A, et al. Accurate and rapid analysis of residual disease in patients with CML using specific fluorescent hybridization probes for real time quantitative RT-PCR. Leukemia. 1999;13(11):1825–32.
73. Schoch C, Schnittger S, Bursch S, Gerstner D, Hochhaus A, Berger U, et al. Comparison of chromosome banding analysis, interphase- and hypermetaphase-FISH, qualitative and quantitative PCR for diagnosis and for follow-up in chronic myeloid leukemia: a study on 350 cases. Leukemia. 2002;16(1):53–9.
74. Zhang J, Wang Y, Wang J, Hu J, Chen S, Jin J, et al. Early BCR-ABL1 decline in imatinib-treated patients with chronic myeloid leukemia: results from a multicenter study of the Chinese CML alliance. Blood Cancer J. 2018;8(7):61. https://doi.org/10.1038/s41408-018-0093-4.
75. Stuckey R, Casado LF, Colomer D, Gomez-Casares MT, Casas L, Garcia-Gutierrez V, et al. Early prediction of subsequent molecular response to nilotinib in patients with chronic myeloid leukemia: comparison of the quantification of BCR-ABL1 ratios using ABL1 or GUSB control genes. J Mol Diagn: JMD. 2020;22(10):1217–24. https://doi.org/10.1016/j.jmoldx.2020.06.016.
76. Huet S, Cony-Makhoul P, Heiblig M, Tigaud I, Gazzo S, Belhabri A, et al. Major molecular response achievement in CML patients can be predicted by BCR-ABL1/ABL1 or BCR-ABL1/GUS ratio at an earlier time point of follow-up than currently recommended. PLoS One. 2014;9(9):e106250. https://doi.org/10.1371/journal.pone.0106250.
77. Pennisi MS, Stella S, Vitale SR, Puma A, Di Gregorio S, Romano C, et al. BCR-ABL1 Doubling-Times and Halving-Times May Predict CML Response to Tyrosine Kinase Inhibitors. Front Oncol. 2019;9:764. https://doi.org/10.3389/fonc.2019.00764.
78. Karpurmath SV, Seshachalam A, Selvaraj K, Rajamani P, Satish K, Reddy N, et al. Halving time of BCR-ABL1 in Chronic Myeloid Leukemia, Is it a better bet than day 90 value—Multicenter study from south India. Clin Lymphoma Myeloma Leuk. 2019; https://doi.org/10.1016/j.clml.2019.09.606.
79. Mahon FX, Rea D, Guilhot J, Guilhot F, Huguet F, Nicolini F, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre stop imatinib (STIM) trial. Lancet Oncol. 2010;11(11):1029–35. https://doi.org/10.1016/S1470-2045(10)70233-3.
80. Ross DM, Branford S, Seymour JF, Schwarer AP, Arthur C, Yeung DT, et al. Safety and efficacy of imatinib cessation for CML patients with stable undetectable minimal residual disease: results from the TWISTER study. Blood. 2013;122(4):515–22. https://doi.org/10.1182/blood-2013-02-483750.
81. Rea D, Nicolini FE, Tulliez M, Guilhot F, Guilhot J, Guerci-Bresler A, et al. Discontinuation of dasatinib or nilotinib in chronic myeloid leukemia: interim analysis of the STOP 2G-TKI study. Blood. 2017;129(7):846–54. https://doi.org/10.1182/blood-2016-09-742205.
82. Mahon F, Boquimpani C, Kim D, et al. Treatment-free remission after second-line nilotinib treatment in patients with chronic myeloid leukemia in chronic phase: results from a single-group, phase 2, open-label study. Ann Intern Med. 2018;168(7):461–70. https://doi.org/10.7326/M17-1094.
83. Clark RE, Polydoros F, Apperley JF, Milojkovic D, Rothwell K, Pocock C, et al. De-escalation of tyrosine kinase inhibitor therapy before complete

treatment discontinuation in patients with chronic myeloid leukaemia (DESTINY): a non-randomised, phase 2 trial. Lancet Haematol. 2019;6(7):e375–e83. https://doi.org/10.1016/S2352-3026(19)30094-8.

84. Rousselot P, Charbonnier A, Cony-Makhoul P, Agape P, Nicolini FE, Varet B, et al. Loss of major molecular response as a trigger for restarting tyrosine kinase inhibitor therapy in patients with chronic-phase chronic myelogenous leukemia who have stopped imatinib after durable undetectable disease. J Clin Oncol. 2014;32(5):424–30. https://doi.org/10.1200/JCO.2012.48.5797.
85. Lee SE, Choi SY, Song HY, Kim SH, Choi MY, Park JS, et al. Imatinib withdrawal syndrome and longer duration of imatinib have a close association with a lower molecular relapse after treatment discontinuation: the KID study. Haematologica. 2016;101(6):717–23. https://doi.org/10.3324/haematol.2015.139899.
86. Hochhaus A, Masszi T, Giles FJ, Radich JP, Ross DM, Gomez Casares MT, et al. Treatment-free remission following frontline nilotinib in patients with chronic myeloid leukemia in chronic phase: results from the ENESTfreedom study. Leukemia. 2017;31(7):1525–31. https://doi.org/10.1038/leu.2017.63.
87. Saussele S, Richter J, Guilhot J, Gruber FX, Hjorth-Hansen H, Almeida A, et al. Discontinuation of tyrosine kinase inhibitor therapy in chronic myeloid leukaemia (EURO-SKI): a prespecified interim analysis of a prospective, multicentre, non-randomised, trial. Lancet Oncol. 2018;19(6):747–57. https://doi.org/10.1016/S1470-2045(18)30192-X.
88. Shah NP, Garcia-Gutierrez V, Jimenez-Velasco A, Larson S, Saussele S, Rea D, et al. Dasatinib discontinuation in patients with chronic-phase chronic myeloid leukemia and stable deep molecular response: the DASFREE study. Leuk Lymphoma. 2020;61(3):650–9. https://doi.org/10.1080/10428194.2019.1675879.
89. Saussele S, Hehlmann R, Fabarius A, Jeromin S, Proetel U, Rinaldetti S, et al. Defining therapy goals for major molecular remission in chronic myeloid leukemia: results of the randomized CML study IV. Leukemia. 2018;32(5):1222–8. https://doi.org/10.1038/s41375-018-0055-7.
90. Radich JP, Deininger M, Abboud CN, Altman JK, Berman E, Bhatia R, et al. Chronic myeloid leukemia, version 1.2019, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Cancer Netw. 2018;16(9):1108–35. https://doi.org/10.6004/jnccn.2018.0071.
91. Lucas CM, Harris RJ, Giannoudis A, Davies A, Knight K, Watmough SJ, et al. Chronic myeloid leukemia patients with the e13a2 BCR-ABL fusion transcript have inferior responses to imatinib compared to patients with the e14a2 transcript. Haematologica. 2009;94(10):1362–7. https://doi.org/10.3324/haematol.2009.009134.
92. Castagnetti F, Gugliotta G, Palandri F, Breccia M, Stagno F, Levato L, et al. The BCR-ABL1 Transcript Type Does Not Influence the Response and the Outcome of Chronic Myeloid Leukemia Patients Treated Frontline with Nilotinib. ASH Annual Meeting Abstracts. 2012;120(21):1680.
93. Hanfstein B, Lauseker M, Hehlmann R, Saussele S, Erben P, Dietz C, et al. Distinct characteristics of e13a2 versus e14a2 BCR-ABL1 driven chronic myeloid leukemia under first-line therapy with imatinib. Haematologica. 2014;99(9):1441–7. https://doi.org/10.3324/haematol.2013.096537.
94. Dmytrenko IV, Fedorenko VG, Shlyakhtychenko TY, Sholoyko VV, Lyubarets TF, Malinkina TV, et al. Assessment of response to imatinib therapy in patients with chronic myeloid leukemia with e13a2 and e14a2 transcripts of BCR/ABL1 gene. Probl Radiat Med Radiobiol. 2015;20:328–40.
95. Lin H-XX, Sjaarda J, Dyck J, Stringer R, Hillis C, Harvey M, et al. Gender and BCR-ABL transcript type are correlated with molecular response to imatinib treatment in patients with chronic myeloid leukemia. Eur J Haematol. 2016;96(4):360–6. https://doi.org/10.1111/ejh.12597.
96. Pagnano KBB, Miranda EC, Delamain MT, Duarte GO, de Paula EV, Lorand-Metze I, et al. Influence of BCR-ABL transcript type on outcome in patients with chronic-phase chronic myeloid leukemia treated with imatinib. Clin Lymphoma Myeloma Leuk. 2017;17(11):728–33. https://doi.org/10.1016/j.clml.2017.06.009.
97. Greenfield G, McMullan R, Robson N, McGimpsey J, Catherwood M, McMullin MF. Response to Imatinib therapy is inferior for e13a2 BCR-ABL1 transcript type in comparison to e14a2 transcript type in chronic myeloid leukaemia. BMC Hematol. 2019;19(1):7. https://doi.org/10.1186/s12878-019-0139-2.
98. Sharma P, Kumar L, Mohanty S, Kochupillai V. Response to Imatinib mesylate in chronic myeloid leukemia patients with variant BCR-ABL fusion transcripts. Ann Hematol. 2010;89(3):241–7. https://doi.org/10.1007/s00277-009-0822-7.
99. Jain P, Kantarjian H, Patel KP, Gonzalez GN, Luthra R, Shamanna RK, et al. Impact of BCR-ABL transcript type on outcome in patients with chronic-phase CML treated with tyrosine kinase inhibitors. Blood. 2016;127(10):1269–75. https://doi.org/10.1182/blood-2015-10-674242.
100. Castagnetti F, Gugliotta G, Breccia M, Iurlo A, Levato L, Albano F, et al. The BCR-ABL1 transcript type influences response and outcome in Philadelphia chromosome-positive chronic myeloid leukemia patients treated frontline with imatinib. Am J Hematol. 2017;92(8):797–805. https://doi.org/10.1002/ajh.24774.
101. Marum JE, Branford S. Current developments in molecular monitoring in chronic myeloid leukemia. Ther Adv Hematol. 2016;7(5):237–51. https://doi.org/10.1177/2040620716657994.
102. Birrell GW, JRRJJTMFL. Exon skipping in the ATM gene in normal individuals: the effect of blood

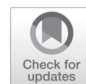
sample storage on RT-PCR analysis. Hum Mutat. 2001;17(1):75–6.

103. Etienne G, Dulucq S, Bauduer F, Adiko D, Lifermann F, Dagada C, et al. Incidences of Deep Molecular Responses and Treatment-Free Remission in de Novo CP-CML Patients. Cancers (Basel). 2020;12:9. https://doi.org/10.3390/cancers12092521.
104. D'Adda M, Farina M, Schieppati F, Borlenghi E, Bottelli C, Cerqui E, et al. The e13a2 BCR-ABL transcript negatively affects sustained deep molecular response and the achievement of treatment-free remission in patients with chronic myeloid leukemia who receive tyrosine kinase inhibitors. Cancer. 2019;125(10):1674–82. https://doi.org/10.1002/cncr.31977.
105. Claudiani S, Apperley JF, Gale RP, Clark R, Szydlo R, Deplano S, et al. e14a2 BCR-ABL1 transcript is associated with a higher rate of treatment-free remission in individuals with chronic myeloid leukemia after stopping tyrosine kinase inhibitor therapy. Haematologica. 2017;102(8):e297–e9. https://doi.org/10.3324/haematol.2017.168740.
106. Shanmuganathan N, Pagani IS, Ross DM, Park S, Yong AS, Braley JA, et al. Early BCR-ABL1 kinetics are predictive of subsequent achievement of treatment-free remission in chronic myeloid leukemia. Blood. 2021;137:1196–1207. https://doi.org/10.1182/blood.2020005514.
107. Greiner J, Schmitt M. Leukemia-associated antigens as target structures for a specific immunotherapy in chronic myeloid leukemia. Eur J Haematol. 2008;80(6):461–8. https://doi.org/10.1111/j.1600-0609.2008.01053.x.
108. Rojas JM, Knight K, Wang L, Clark RE. Clinical evaluation of BCR-ABL peptide immunisation in chronic myeloid leukaemia: results of the EPIC study. Leukemia. 2007;21(11):2287–95. https://doi.org/10.1038/sj.leu.2404858.
109. Nicolini FE, Dulucq S, Boureau L, Cony-Makhoul P, Charbonnier A, Escoffre-Barbe M, et al. Evaluation of residual disease and TKI duration are critical predictive factors for molecular recurrence after stopping Imatinib first-line in chronic phase CML patients. Clin Cancer Res. 2019;25(22):6606–13. https://doi.org/10.1158/1078-0432.CCR-18-3373.
110. Mori S, Vagge E, le Coutre P, Abruzzese E, Martino B, Pungolino E, et al. Age and dPCR can predict relapse in CML patients who discontinued imatinib: the ISAV study. Am J Hematol. 2015;90(10):910–4. https://doi.org/10.1002/ajh.24120.
111. Rousselot P, Loiseau C, Delord M, Cayuela JM, Spentchian M. Late molecular recurrences in patients with chronic myeloid leukemia experiencing treatment-free remission. Blood Adv. 2020;4(13):3034–40. https://doi.org/10.1182/bloodadvances.2020001772%JBloodAdvances.
112. Pagnano KBB. BCR-ABL1 level monitoring in chronic myeloid leukemia by real time polymerase chain reaction in Brazil - not so real. Rev Bras Hematol Hemoter. 2017;39(3):197–8. https://doi.org/10.1016/j.bjhh.2017.05.005.
113. Yeung DT, Osborn MP, White DL, Branford S, Braley J, Herschtal A, et al. TIDEL-II: first-line use of imatinib in CML with early switch to nilotinib for failure to achieve time-dependent molecular targets. Blood. 2015;125(6):915–23. https://doi.org/10.1182/blood-2014-07-590315.
114. Cortes J, Rousselot P, Kim D-W, Ritchie E, Hamerschlak N, Coutre S, et al. Dasatinib induces complete hematologic and cytogenetic responses in patients with imatinib-resistant or -intolerant chronic myeloid leukemia in blast crisis. Blood. 2007;109(8):3207–13. https://doi.org/10.1182/blood-2006-09-046888.
115. Giles FJ, le Coutre PD, Pinilla-Ibarz J, Larson RA, Gattermann N, Ottmann OG, et al. Nilotinib in imatinib-resistant or imatinib-intolerant patients with chronic myeloid leukemia in chronic phase: 48-month follow-up results of a phase II study. Leukemia. 2013;27(1):107–12. https://doi.org/10.1038/leu.2012.181.
116. Shanmuganathan N, Braley JA, Yong ASM, Hiwase DK, Yeung DT, Ross DM, et al. Modeling the safe minimum frequency of molecular monitoring for CML patients attempting treatment-free remission. Blood. 2019;134(1):85–9. https://doi.org/10.1182/blood.2019000120.
117. Rousselot P, Huguet F, Rea D, Legros L, Cayuela JM, Maarek O, et al. Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. Blood. 2007;109(1):58–60.
118. Chen Z, Medeiros LJ, Kantajian HM, Zheng L, Gong Z, Patel KP, et al. Differential depth of treatment response required for optimal outcome in patients with blast phase versus chronic phase of chronic myeloid leukemia. Blood Cancer J. 2017;7(2):e521. https://doi.org/10.1038/bcj.2017.4.
119. Arpinati M, Tolomelli G, Bochicchio MT, Castagnetti F, Amabile M, Bandini G, et al. Molecular monitoring of BCR-ABL transcripts after allogeneic stem cell transplantation for chronic myeloid leukemia. Biol Blood Marrow Transplant. 2013;19(5):735–40. https://doi.org/10.1016/j.bbmt.2013.01.007.
120. Pagani IS, Dang P, Kommers IO, Goyne JM, Nicola M, Saunders VA, et al. BCR-ABL1 genomic DNA PCR response kinetics during first-line imatinib treatment of chronic myeloid leukemia. Haematologica. 2018;103(12):2026–32. https://doi.org/10.3324/haematol.2018.189787.
121. Wącław J, Zawada M, Czekalska S, Ochrem B, Sacha T. Comparison of ABL1 and Gusb Reference Genes in the qRT-PCR Analysis of Halving Time and Early Molecular Response to TKI Therapy in Patients with Chronic Myeloid Leukemia. Blood. 2016;128(22):Abstract 5424. https://doi.org/10.1182/blood.V128.22.5424.5424.
122. Huet S, Cony-Makhoul P, Heiblig M, Tigaud I, Gazzo S, Belhabri A, et al. Major molecular response achievement in CML Patients can be predicted by BCR-ABL1/ABL1 or BCR-ABL1/GUS ratio at an

earlier time point of follow-up than currently recommended. PloS One. 2014;9(9):e106250-e. https://doi.org/10.1371/journal.pone.0106250.

123. Pritchard J, Lustgarten S, Hodgson J, Baccarani M, Cortes J, Deininger M et al., editors. Analysis of the relationship between dose and BCR-ABL halving time in CP-CML patients treated with ponatinib or imatinib. Poster presented at: 20th European Hematology Association Congress; 2015 Jun 11–14; Vienna, Austria.

124. Stuckey R, Casado L-F, Colomer D, Gómez-Casares MT, Casas L, García-Gutierrez V, et al. Early prediction of subsequent molecular response to nilotinib in patients with chronic myeloid leukemia: comparison of the quantification of BCR-ABL1 ratios using ABL1 or GUSB control genes. J Mol Diagn. 2020; https://doi.org/10.1016/j.jmoldx.2020.06.016.



16 Discontinuation or Cessation of Tyrosine Kinase Inhibitor Treatment in Chronic Myeloid Leukemia Patients with Deep Molecular Response

Susanne Saußele and Francois-Xavier Mahon

16.1 Introduction

Chronic myeloid leukemia (CML) is more than ever the model of targeted therapy for human malignancies. The success of the first tyrosine kinase inhibitor (TKI) imatinib has profoundly changed the outcome for CML patients. Since TKI-treated CML patients have a near-normal life expectancy [1], important issues must be considered in the future: (a) long-term toxicities directly influencing the quality of life and ethical aspects of the treatment and (b) the economic impact of treating patients for their lifetime.

One of the best ways to consider these points is to ask the relevant question about stopping TKIs in good responding patients. Such a strategy has been proposed now as a result of several studies including more than 3000 patients in deep molecular remission (DMR, BCR-ABL1 (IS) < 0.01%) who have stopped a TKI. The main prognostic factors are the duration of DMR and the TKI treatment duration. However, many questions about the depth of molecular remission, other predictive factors including immunological factors, and safety are still open and unresolved. Based on recent data published to date, the recommendations of the National Comprehensive Cancer Network (NCCN) and the European LeukemiaNet (ELN) propose criteria when discontinuing TKI treatment safely in responding patients with CML is most appropriate [2, 3].

16.2 TFR Studies

The initiative was started with a pilot study in 12 patients with CML when it was proposed to discontinue imatinib (Rousselot et al. 2007). After a median follow-up of 18 months, 50% of patients remained off therapy without confirmed reappearance of peripheral blood BCR-ABL1 transcripts [4]. This pilot study provided a proof of concept that imatinib discontinuation could be achieved in selected CML patients. It was followed by a multicenter study entitled “Stop Imatinib” (STIM) trial [5]. Prospectively, 100 patients with chronic-phase CML receiving imatinib therapy in DMR were included. Fifty-one percent of the patients had been previously treated with IFN, and the other half were treated with imatinib only. Molecular relapse, which was arbitrarily defined as two positive RQ-PCR results over a period of 1 month showing a significant rise (1 log) in BCR-ABL1 transcripts, was a trigger for imatinib treatment

S. Saußele (✉)
III. Med. Klinik, Medizinische Fakultät Mannheim der Universität Heidelberg, Mannheim, Germany
e-mail: susannne.saussele@medma.uni-heidelberg.de

F.-X. Mahon
Bergonié Cancer Institute, University of Bordeaux, INSERM U916, Bordeaux, France

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2021
R. Hehlmann (ed.), *Chronic Myeloid Leukemia*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-71913-5_16

again. Molecular recurrence-free survival rate at 65 months was 39%. For those patients who achieved the first 6 months without relapse (landmark analysis), the probability of relapse was 10% at 24 months [6]. Most patients who experienced molecular relapse did so within 6 months of imatinib cessation and remained responsive to re-treatment with imatinib as observed in the pilot study. Comparable results were reported in the Australasian Leukaemia and Lymphoma Group (ALLG) CML8 study (TWISTER) [7]. With a median follow-up of 8.6 years (range 5.7–11.2 years), 18 patients remained in continuous TFR (45.0%) [8]. Most relapses occurred within 6 months of stopping imatinib, and no relapses occurred beyond 27 months.

One of the important issues regards the definition DMR which was not uniform when the first trials started. New definitions were introduced in 2012 (see later). Other attempts at imatinib discontinuation, which did not meet the criterion of DMR, exhibited rapid molecular relapses [9–12].

Multiple TKI discontinuation studies have been published or are still ongoing confirming these results. In addition, registries on TFR outside clinical trials demonstrated the feasibility of TFR in routine care [13, 14].

Most trials confirmed that the duration of response, especially the duration of DMR, was important (). The validation of this criterion was reinforced using mathematical models confirming a biphasic dynamic of BCR-ABL1 transcript decline with a two-slope model of TKI the α slope corresponded to the rapid initial decrease in BCR-ABL1 transcript levels (cycling cells) after the start of treatment, and the β slope corresponded to the longer-term BCR-ABL1 dynamics (less proliferative cells) [15]. Another model based on the biphasic decline of BCR-ABL1 transcript levels suggested that 31% of the patients would remain in DMR after treatment cessation after a fixed period of 2 years in MR5, whereas 69% are expected to relapse [16]. Most recently, another mathematical model demonstrated an antileukemic immunologic effect in 21 patients with CML for whom BCR-ABL1/ABL1 time courses had been quantified before and after TKI cessation. Immunologic control was conceptually necessary to explain TFR as observed in about half of the patients [17].

Because the identification of patients who would benefit most from discontinuing TKIs remains a key issue, the question of the duration of molecular response before discontinuation is crucial.

It is also one of the objectives of the European Stop Kinase Inhibitor (EURO-SKI) trial from the European LeukemiaNet (ELN) that was running in 11 countries. The criteria for discontinuation were less strict than those in the STIM studies: the duration of TKI treatment prior to enrolment had to be at least 3 years and the PCR level below 0.01% within the previous year, i.e., a sustained DMR of 4 log had to be confirmed. Results of a planned interim analysis with the final analysis still pending [18] showed the following:

After a median follow-up of 27 months, molecular relapse-free survival of 755 evaluable patients was 61% (95% CI 57–64) at 6 months and 50% (46–54) at 24 months. No plateau was reached. Of these 755 patients, 371 (49%) lost MMR after TKI discontinuation, four (1%) died while in MMR for reasons unrelated to CML (myocardial infarction, lung cancer, renal cancer, and heart failure), and 13 (2%) restarted TKI therapy while in MMR. An additional six (1%) patients died in CP-CML after loss of MMR and re-initiation of TKI therapy for reasons unrelated to CML, and two (<1%) patients lost MMR despite restarting TKI therapy. In the prognostic analysis in 405 patients who received imatinib as first-line treatment, longer treatment-free duration and longer DMR duration were associated with increasing probability of MMR maintenance at 6 months with DMR duration being the most impotant factor.

These results were similar to another French trial of 218 patients [19].

The depth of response is an important factor in the decision to discontinue TKI treatment. The definition of molecular response and the standardization of BCR-ABL1 transcript measurement remain a concern. For this reason, the CML Working Group of the ELN has proposed revised definitions of MR taking into account the sensitivity of molecular tests, i.e., MR4 indicates ≥4-log reduction (BCR-ABL1 (IS) ≤ 0·01%),

MR4.5 indicates ≥4.5-log reduction (BCR-ABL1 (IS) ≤ 0·0032%), and MR5 indicates ≥5-log reduction (BCR-ABL1 (IS) ≤0.001%) [20, 21]. Different European laboratories working in a European molecular network validated this standardization and performed the molecular analyses of the EURO-SKI trial. Terms like complete molecular remission (CMR) or undetectable levels of minimal residual disease (UMRD) should not be used anymore. They indicate a negative RQ-PCR result and must be associated with a defined PCR assay sensitivity; however, it should be noted that leukemic cells may still be present even if RQ-PCR results are negative [22]. Current RQ-PCR methods can reliably detect up to a 5-log reduction in BCR-ABL1, but newer techniques, such as DNA-based PCR, RNA-based digital PCR, and replicated PCR, have demonstrated increased sensitivities and may enable the assessment of even deeper levels of molecular response [23].

The Imatinib Suspension and Validation (ISAV) trial is the first study using digital PCR in parallel with qRT-PCR [24]. The method seems to be more sensitive in this study as the prediction of relapse was more accurate. One hundred twelve patients with at least 2-year imatinib treatment and at least 18 months undetectable transcripts in qRT-PCR were followed for a median of 21.6 months. Cumulative incidence of relapses was 52% after 36 months. Relapse was defined as loss of MMR (two consecutive positive PCRs with one result at least above 0.1%).

However, it should be noted that after using an ultrasensitive PCR technique, a low level of BCR-ABL1 transcripts has been found in the blood of normal individuals, suggesting that a complete absence of transcripts may not be required to eradicate the disease [25, 26]. Most patients have detectable BCR-ABL1 DNA by highly sensitive methods (27). In the TWISTER study, nine patients in long-term TFR were monitored by highly sensitive individualized BCR-ABL1 DNA PCR. This technique provided more precise quantification and demonstrated a BCR-ABL1 DNA decrease from a median of MR5.0 in the first year of TFR to MR6.1 in the sixth year of TFR [8]. In the EURO-SKI trial, DNA and mRNA BCR-ABL1 measurements by qPCR were compared in 2189 samples (129 patients) and by digital PCR in 1279 sample (62 patients). A high correlation was found at levels of disease above MR4, but there was a poor correlation for samples during DMR. A combination of both methods resulted in a better prediction of molecular recurrence-free survival (MRFS). At 18 months after treatment cessation, patients with negative results for DNA- and RNA-based PCR had an MRFS of 80% and 100%, respectively, compared with those who were DNA positive/RNA negative (MRFS = 57% and 67%) or DNA positive/RNA positive (MRFS = 20% for both cohorts) [27].

Such a strategy should be prospectively validated and can improve TFR results.

In a lineage analysis (granulocytes, monocytes, B cells, T cells, and NK cells) of residual CML cells of 20 patients who were in TFR for >1 year, MRD was identified predominantly in the lymphoid compartment and not in granulocytes. B cells were more often BCR-ABL1 positive than T cells and at higher levels. These data suggest that MRD in the blood of TFR patients need not imply the persistence of multipotent CML cells [28].

We still do not know the threshold of residual disease which will allow us to safely stop TKI with the lowest rate of molecular recurrence. What is the definition of molecular relapse triggering re-treatment? It is also a very important question. It is absolutely necessary to use exactly the same criteria to compare studies to exclude misinterpreting the results. In the STIM studies, molecular relapse was defined by positivity of BCR-ABL1 transcript in qRT-PCR confirmed by a second analysis point indicating the increase of 1 log in relation to the first analysis point, at two successive assessments, or loss of MMR at one point. This definition leads to propose the term molecular recurrence instead of molecular relapse [29]. By comparison to the STIM study which was the first clinical trial proposing to stop TKI, the criteria triggering re-treatment after molecular relapse have now evolved. Since many studies pertaining to TKI cessation have been launched, we need to underline clearly the criteria for treatment re-challenge in future trials.

The French multicenter observational study (A-STIM [According to Stop Imatinib]) validated loss of MMR as a trigger for restarting TKI therapy in CP-CML patients who have stopped imatinib after achieving durable molecular response. In a first publication in 2014, 80 patients with CP-CML had stopped imatinib after sustained DMR of 2 years with the same definition as compared to STIM study [30]. Molecular relapse was less stringently defined as loss of MMR at any time for triggering re-treatment. The median follow-up after discontinuation was 31 months (range, 8–92 months). TFR was estimated 61% at 36 months, but it was estimated around 37%, i.e., similar to STIM or TWISTER results when STIM criteria were used.

Meanwhile, longer follow-up of the A-STIM study revealed very late loss of MMR. In total, 218 pts. were followed and the TFR rate was estimated to be 45.6% after 7 years. For 9/65 (14%) patients experiencing loss of MMR, molecular recurrence occurred after 2 years in TFR. The probability of remaining in TFR was 65.4% for patients having experienced fluctuations in minimal residual disease (MRD), at least two consecutive measurements BCR-ABL1 (IS) >0.0032% or loss of MR4, whereas it was 100% for those with stable DMR.

In addition, some studies reported cases where sudden BC after stopping occurred. A long-term molecular follow-up therefore remains mandatory for CML patients in TFR [31].

Another concept was followed in the DESTINY (De-Escalation and Stopping Treatment with Imatinib, Nilotinib, or sprYcel) study. TKI treatment was de-escalated to half the standard dose for 12 months before cessation. Analysis was performed according to MR level before study entry. Recurrence-free survival was 72% for DMR patients, 36% for the MMR group [32].

To address the feasibility of discontinuing nilotinib or dasatinib, academic- and pharma-sponsored studies were implemented.

Stopping after first-line therapy with either dasatinib or nilotinib was investigated in Dasfree and ENESTfreedom trial, respectively.

In the single-arm, phase 2 ENESTfreedom trial, patients ⩾2 years of frontline nilotinib therapy were enrolled. Patients with sustained DMR during the 1-year nilotinib consolidation phase were eligible for the TFR phase. In total, 215 patients entered the consolidation phase, of whom 190 entered the TFR phase. The median duration of nilotinib before stopping treatment was 43.5 months, the shortest of all TFR studies so far. At 48 weeks after stopping nilotinib, 98 patients (51.6%) remained in MMR or better [33].

In the Dasfree trial, a single-arm phase 2 trial, 84 patients were enrolled after first- or second-line therapy with dasatinib. At 2 years, TFR was 46%. Multivariate analyses revealed statistically significant associations between 2-year TFR and duration of prior dasatinib, line of therapy, and age (>65 years) [34].

Other studies confirmed these data such as the Japanese DADI trial (dasatinib discontinuation). Of 58 patients who discontinued dasatinib, 32 (55%) had TFR at 6 months with a median follow-up of 3·months. However, the definition of response and retreatment is not clearly described in the paper [35]. In a French trial, a first interim analysis reported outcomes of 60 patients with a minimum follow-up of 12 months. Twenty-six patients (43.3%) lost MMR. TFR rates at 12 and 48 months were 63.3% and 53.6%, respectively. In a univariate analysis, prior suboptimal response or TKI resistance was the only baseline factor associated with significantly worse outcome [36].

Other studies like ENESTPath and ENESTop focused on patients who switch to nilotinib in order to reach sustained DMR before entering a TFR phase. Whereas ENEStpath is still ongoing, in ENESTop, 163 patients who had switched from imatinib to nilotinib (for reasons including resistance, intolerance, and physician preference) entered the consolidation phase. One hundred twenty-six were eligible to stop the TKI. At 48 weeks and 96 weeks, 58% and 53% of patients maintained TFR, respectively [37].

16.3 Which Clinical and Biological Factors Might Predict TFR?

Besides the duration and depth of response, which other factors may be used to suggest the possibility of interrupting TKI treatment? In the

STIM study several potential factors for prediction of molecular recurrence were retrospectively assessed [5]. The probability of remaining in stable DMR after discontinuation was favorable in the low Sokal risk group when compared to the intermediate or high Sokal risk groups. Using multivariate analysis and logistic regression at 8 months, Sokal risk and imatinib therapy duration were confirmed as two independent prognostic factors for prediction of molecular relapse after imatinib cessation.

In EURO-SKI no prognostic score was found to be significantly associated with TFR. Despite this, longer treatment duration and longer DMR duration were associated with increasing probability of MMR maintenance at 6 months with DMR duration being the stronger factor. The final analysis is pending.

Other criteria such as age, sex, or depth of MR were significant in some smaller studies but not confirmed in others.

Using the criteria of the STIM and TWISTER studies, it should be possible to predict which patients are ideal for discontinuation of TKIs. Recently Branford and colleagues found in a study of 415 patients treated with imatinib for 8 years that the cumulative rate of stable $MR^{4.5}$ (for at least 2 years) was 43%. In these patients, the time to achieve MMR was correlated with the time to achieve stable $MR^{4.5}$.[38] In addition the only two independent factors, i.e., female sex and a low level of BCR-ABL1 value at 3 months, were statistically strongly linked to the prediction of sustained $MR^{4.5}$. Factors associated with sustained $MR^{4.5}$ and undetectable transcripts induced by TKI (imatinib, dasatinib, and nilotinib) were also analyzed in a multivariable analysis ($N = 495$) by Falchi et al. and showed that older age, higher baseline hemoglobin, higher baseline platelets, TKI modality, and response at 3 months were significant [39]. A long-term analysis has been performed in the German CML study IV. From more than 1500 patients the cumulative incidence of confirmed MR4.5 was 54% after 9 years [40]. The study demonstrated a link between MR4.5 achievement and better survival.

Most recently, the time for the BCR-ABL1 value to halve from the time of diagnosis ha been shown to be the strongest independent predictor of sustained TFR. Early molecular response dynamics were assessed from 115 patients attempting subsequent TFR after ≥12 months follow-up. The probability of sustained TFR at 12 months was 55%. TFR rate was 80% in patients with a BCR-ABL1 halving time of <9.35 days compared with only 4% if the halving time was >21.85 days ($P < 0.001$). The e14a2 BCR-ABL1 transcript type and duration of TKI exposure before attempting TFR were also independent predictors of sustained TFR [41].

In addition, immunological effects seem to play an important role in maintaining TFR. Several studies have reported that low NK cell numbers may predict early disease relapse after TKI discontinuation [42–45]. These studies suggest that NK cell-based immune surveillance may contribute to CML control after TKI cessation. In one of the studies, NK cell numbers were significantly different in early relapses (≤5 months after TKI stop) versus late relapses (>5 months after TKI stop) [44]. Thus, different mechanisms may be involved in return of the disease at different time points. It further remains to be determined if pharmacological use of agent(s) that stimulate NK cell function can increase the number of CML patients achieving deep molecular response and long-term TFR after TKI cessation. Whether NK cell number and function may also be used to predict disease relapse after TKI discontinuation needs to be investigated.

In EURO-SKI it was prospectively demonstrated in 122 patients that the expression of the T-cell inhibitory receptor (CTLA-4) ligand CD86 (B7.2) on plasmacytoid dendritic cells (pDC) affects relapse risk after TKI cessation. TFR rate was 30.1% for patients with >95 CD86 + pDC per 105 lymphocytes, but 70.0% for patients with <95 CD86 + pDC. Moreover, only patients with lower pDC derived a significant benefit from longer TKI exposure [46]. Other factors were retrospectively investigated like KIRs and pharmacogenetic factors influencing TKI uptake [47, 48].

16.4 Can we Cure CML?

The answer to this question depends on the definition of cure. If the definition of cure is "Absence of long-term leukemia relapse after treatment discontinuation," we have proven that it requires at least sustained DMR in TKI-treated patients. But we may never be able to prove that cure requires the eradication of residual leukemic cells. For instance, in the TWISTER study using PCR on DNA which is a non-routine technique increasing the sensitivity as compared to classical RQ-PCR to analyze patients who were considered in undetectable MR, leukemic cells were exhibited in all cases. In addition, as mentioned before using an ultrasensitive PCR technique, a low level of BCR-ABL1 transcripts has been found in the blood of normal individuals, suggesting that a complete absence of transcripts may not be required to eradicate the disease. However, newer research suggests that only lymphocytes may remain positive.

When patients still in MMR after TKI discontinuation were analyzed, clearly BCR-ABL1 fluctuations (defined by more than two consecutive positive values) were observed like, e.g., in A-STIM [26]. It means for those patients that leukemic cells persist, but the burden of the residual disease increases only in few patients even without treatment. These results are in agreement with the observation in patients who stopped interferon alpha in remission with clear evidence of residual disease without clinical relapse [49]. To speculate we could take the example from microbiology and infectious diseases where persistence of bacteria does not necessarily imply relapse. That is why John Goldman proposed some years ago the concept of "operational cure" [50]. This type of definition allows for the fact that, using an ultrasensitive PCR technique, low level of BCR-ABL1 transcripts can be found in the blood of normal individuals [22, 23].

In spite of these considerations if we want to decrease the rate of molecular recurrence after stopping TKI, we need to understand why quiescent leukemic stem cells (LSCs) are insensitive to TKIs, which is illustrated by the large number of publications focused on targeting the LSCs [51, 52]. Compared to normal stem cells, LSCs exhibit aberrant or nonregulated self-renewal, survival, and dormancy. Several strategies have been proposed including inhibiting survival/renewal pathways, sensitizing LSC (cycling or differentiating), immune targeting, or modifying the bone marrow niche; JAK/STAT, JAK2 kinase, the protein phosphatase 2A (PP2A), arachidonate 5-lipoxygenase gene (ALOX5), histone deacetylases (HDACs), sirtuin 1 (SIRT1), and BCL6 are among the most relevant targets for such a strategy [53–57]. Two of the most important pathways for self-renewal of CML-LSCs are the Wnt-catenin and the hedgehog (Hh) pathways [58, 59].

16.5 Side Effects after TKI Discontinuation

While imatinib and other TKIs can induce side effects in the musculoskeletal system, it has been assumed that such adverse events are reversible upon cessation of therapy. However, in all stopping trials, a substantial number of patients reported musculoskeletal pain starting or worsening 1–6 weeks after stopping TKI therapy. This was specifically investigated in a sub-cohort of the EURO-SKI trial where it occurred in 15 out of 50 patients [60]. The pain was localized to various parts of the body, including the shoulder and hip regions and/or extremities, sometimes resembling polymyalgia rheumatica. Symptoms were mild in most individuals, leading only to use of nonprescription drugs (paracetamol or NSAID), but some were more severely afflicted with manifestations interfering with everyday activities and requiring steroid therapy. Over time these symptoms seem to resolve. The rate of molecular recurrence in patients with musculoskeletal pain did not differ from those without these symptoms. These findings were confirmed in other studies [61].

This phenomenon is not restricted to imatinib pretreatment. Physicians should be aware of the possibility of adverse events appearing after stopping long-term TKI therapy. Further

investigations into underlying mechanisms are also warranted [61].

In conclusion, the subset of patients with DMR leading to cessation of treatment is heterogeneous. Around 40–60% of CML patients with stable DMR on TKI for at least 2 years are likely to remain in a prolonged TFR after treatment is stopped. Meanwhile international recommendations have included TFR as a treatment option to be considered in appropriate patients [2, 3].

Guidelines for TFR

NCCN[1]	ELN[2]
1. Age >18 2. Chronic phase disease, no history of AP, BC 3. TKI therapy ror >3 years 4. Quantifiable BCR-ABL1 transcripts 5. Stable MR (MR4) >2 years 6. Access to reliable PCR test 7. Monthly monitoring >1 year 8. TKI resumption within 4 weeks after MMR Loss 9. Consultation with CML Speciality center	1. Institutional criteria met and patient consent 2. Typical e13a2- or e14a2-BCR–ABL1 transcripts 3. Chronic phase disease 4. No prior treatment failure 5. First-line or second line (only intolerance) therapy Minimal criteria 1. Duration of TKI therapy >5 years (>4 years for 2nd gen. TKI) 2. Duration of DMR (MR^4 or better) >2 years Optimal criteria: DMR >2 y for MR4.5, >3 y for MR4

TFR: Treatment-free remission
1. Radich Jp. et al. /Nat/Compr Canc Net 2018 2. Hochhaus A .et al Leukemics 2020.

Little is known about the possibility of stopping a second time. So far this seems to be successful in about 25% of patients [62]. Studies such as NAUT and DasStop2 which include IFN treatment are ongoing. A TFR approach as second stop should only be performed within clinical trials.

A long-term follow-up of different cessation studies will be necessary to affirm operational cure.

References

1. Bower H, Bjorkholm M, Dickman PW, et al. Life expectancy of patients with chronic myeloid Leukemia approaches the life expectancy of the general population. J Clin Oncol. 2016;34(24):2851–7.
2. Deininger M, Shah NP, Altman JK, et al. Chronic myeloid leukemia. NCCN clinical practice guidelines in oncology. www.nccn.org/: National Comprehensive Cancer Network; 2020.
3. Hochhaus A, Baccarani M, Silver RT, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020;34:966.
4. Rousselot P, Huguet F, Rea D, et al. Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. Blood. 2007;109(1):58–60.
5. Mahon FX, Rea D, Guilhot J, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre stop imatinib (STIM) trial. Lancet Oncol. 2010;11(11):1029–35.
6. Etienne G, Guilhot J, Rea D, et al. Long-term follow-up of the French stop imatinib (STIM1) study in patients with chronic myeloid leukemia. J Clin Oncol. 2017;35(3):298–305.
7. Ross DM, Branford S, Seymour JF, et al. Patients with chronic myeloid leukemia who maintain a complete molecular response after stopping imatinib treatment have evidence of persistent leukemia by DNA PCR. Leukemia. 2010;24(10):1719–24.
8. Ross DM, Pagani IS, Shanmuganathan N, et al. Long-term treatment-free remission of chronic myeloid leukemia with falling levels of residual leukemic cells. Leukemia. 2018;32(12):2572–9.
9. Cortes J, O'Brien S, Kantarjian H. To the editor: discontinuation of imatinib therapy after achieving a molecular response. Blood. 2004;104(7):2204–5.
10. Mauro MJ, Druker BJ, Maziarz RT. Divergent clinical outcome in two CML patients who discontinued imatinib therapy after achieving a molecular remission. Leuk Res. 2004;28:S71–S3.
11. Merante S, Orlandi E, Bernasconi P, et al. Outcome of four patients with chronic myeloid leukemia after

imatinib mesylate discontinuation. Haematologica. 2005;90(7):979–81.
12. Michor F, Hughes TP, Iwasa Y, et al. Dynamics of chronic myeloid leukaemia. Nature. 2005;435(7046):1267–70.
13. Hernández-Boluda JC, Pereira A, Pastor-Galán I, et al. Feasibility of treatment discontinuation in chronic myeloid leukemia in clinical practice: results from a nationwide series of 236 patients. Blood Cancer J. 2018;8(10):91.
14. Fava C, Rege-Cambrin G, Dogliotti I, et al. Observational study of chronic myeloid leukemia Italian patients who discontinued tyrosine kinase inhibitors in clinical practice. Haematologica. 2019;104(8):1589–96.
15. Stein AM, Bottino D, Modur V, et al. BCR-ABL transcript dynamics support the hypothesis that leukemic stem cells are reduced during imatinib treatment. Clin Cancer Res. 2011;17(21):6812–21.
16. Horn M, Glauche I, Muller MC, et al. Model-based decision rules reduce the risk of molecular relapse after cessation of tyrosine kinase inhibitor therapy in chronic myeloid leukemia. Blood. 2013;121(2):378–84.
17. Hähnel T, Baldow C, Guilhot J, et al. Model-based inference and classification of immunologic control mechanisms from TKI cessation and dose reduction in patients with CML. Cancer Res. 2020;80(11):2394–406.
18. Saussele S, Richter J, Guilhot J, et al. Discontinuation of tyrosine kinase inhibitor therapy in chronic myeloid leukaemia (EURO-SKI): a prespecified interim analysis of a prospective, multicentre, non-randomised, trial. Lancet Oncol. 2018;19(6):747–57.
19. Nicolini FE, Dulucq S, Boureau L, et al. Evaluation of residual disease and TKI duration are critical predictive factors for molecular recurrence after stopping imatinib first-line in chronic phase CML patients. Clin Cancer Res. 2019;25(22):6606–13.
20. Cross NCP, White HE, Müller MC, et al. Standardized definitions of molecular response in chronic myeloid leukemia. Leukemia. 2012;26(10):2172–5.
21. Cross NC, White HE, Colomer D, et al. Laboratory recommendations for scoring deep molecular responses following treatment for chronic myeloid leukemia. Leukemia. 2015;29(5):999–1003.
22. Branford S, Seymour JF, Grigg A, et al. BCR-ABL messenger RNA levels continue to decline in patients with chronic phase chronic myeloid leukemia treated with imatinib for more than 5 years and approximately half of all first-line treated patients have stable undetectable BCR-ABL using strict sensitivity criteria. Clin Cancer Res. 2007;13(23):7080–5.
23. Melo JV, Ross DM. Minimal residual disease and discontinuation of therapy in chronic myeloid Leukemia: can we aim at a cure? ASH Education Program Book. 2011;2011(1):136–42.
24. Mori S, Vagge E, le Coutre P, et al. Age and dPCR can predict relapse in CML patients who discontinued imatinib: the ISAV study. Am J Hematol. 2015;90(10):910–4.
25. Biernaux C, Loos M, Sels A, et al. Detection of major bcr-abl gene expression at a very low level in blood cells of some healthy individuals. Blood. 1995;88(8):3118–22.
26. Bose S, Deininger M, Gora-Tybor J, et al. The presence of typical and atypical BCR-ABL fusion genes in leukocytes of normal individuals: biologic significance and implications for the assessment of minimal residual disease. Blood. 1998;92(9):3362–7.
27. Machova Polakova K, Zizkova H, Zuna J, et al. Analysis of chronic myeloid leukaemia during deep molecular response by genomic PCR: a traffic light stratification model with impact on treatment-free remission. Leukemia. 2020;34(8):2113–24.
28. Pagani IS, Dang P, Saunders VA, et al. Lineage of measurable residual disease in patients with chronic myeloid leukemia in treatment-free remission. Leukemia. 2020;34(4):1052–61.
29. Deininger M. Hematology: curing CML with imatinib--a dream come true? Nat Rev Clin Oncol. 2011;8(3):127–8.
30. Rousselot P, Charbonnier A, Cony-Makhoul P, et al. Loss of major molecular response as a trigger for restarting tyrosine kinase inhibitor therapy in patients with chronic-phase chronic myelogenous Leukemia who have stopped Imatinib after durable undetectable disease. J Clin Oncol. 2014;32(5):424–30.
31. Rousselot P, Loiseau C, Delord M, et al. Late molecular recurrences in patients with chronic myeloid leukemia experiencing treatment-free remission. Blood Adv. 2020;4(13):3034–40.
32. Clark RE, Polydoros F, Apperley JF, et al. De-escalation of tyrosine kinase inhibitor therapy before complete treatment discontinuation in patients with chronic myeloid leukaemia (DESTINY): a non-randomised, phase 2 trial. Lancet Haematol. 2019;6(7):e375–e83.
33. Radich JP, Hochhaus A, Masszi T, Hellmann A, Stentoft J, Casares MTG, García-Gutiérrez JV, Conneally E, le Coutre PD, Gattermann N, Martino B, Saussele S, Giles FJ, Ross DM, Aimone P, Li S, Titorenko K, Saglio G. Treatment-free remission following frontline nilotinib in patients with chronic phase chronic myeloid leukemia: 5-year update of the ENESTfreedom trial. Leukemia. 2021. https://doi.org/10.1038/s41375-021-01205-5. Online ahead of print.
34. Shah NP, Garcia-Gutierrez V, Jimenez-Velasco A, et al. Dasatinib discontinuation in patients with chronic-phase chronic myeloid leukemia and stable deep molecular response: the DASFREE study. Leuk Lymphoma. 2020:650–9.
35. Kimura S, Imagawa J, Murai K, et al. Treatment-free remission after first-line dasatinib discontinuation in patients with chronic myeloid leukaemia (first-line DADI trial): a single-arm, multicentre, phase 2 trial. Lancet Haematol. 2020;7(3):e218–e25.
36. Rea D, Nicolini FE, Tulliez M, et al. Discontinuation of dasatinib or nilotinib in chronic myeloid leukemia:

interim analysis of the STOP 2G-TKI study. Blood. 2017;129(7):846–54.

37. Mahon FX, Boquimpani C, Kim DW, et al. Treatment-free remission after second-line Nilotinib treatment in patients with chronic myeloid Leukemia in chronic phase: results from a single-group, phase 2. Open-Label Study Ann Intern Med. 2018;168(7):461–70.
38. Branford S, Yeung DT, Ross DM, et al. Early molecular response and female sex strongly predict stable undetectable BCR-ABL1, the criteria for imatinib discontinuation in patients with CML. Blood. 2013;121(19):3818–24.
39. Falchi L, Kantarjian HM, Wang X, et al. Significance of deeper molecular responses in patients with chronic myeloid leukemia in early chronic phase treated with tyrosine kinase inhibitors. Am J Hematol. 2013;88(12):1024–9.
40. Hehlmann R, Müller MC, Lauseker M, et al. Deep molecular response is reached by the majority of patients treated with imatinib, predicts survival, and is achieved more quickly by optimized high-dose imatinib: results from the randomized CML-study IV. J Clin Oncol. 2014;32(5):415–23.
41. Shanmuganathan N, Pagani IS, Ross DM, et al. Early BCR-ABL1 kinetics are predictive of subsequent achievement of treatment-free remission in chronic myeloid leukemia. Blood. 2021;137(9):1196–207.
42. Rea D, Henry G, Khaznadar Z, et al. Natural killer-cell counts are associated with molecular relapse-free survival after imatinib discontinuation in chronic myeloid leukemia: the IMMUNOSTIM study. Haematologica. 2017;102(8):1368–77.
43. Dumas PY, Bérard E, Bréal C, et al. Killer immunoglobulin-like receptor genotypes and chronic myeloid leukemia outcomes after imatinib cessation for treatment-free remission. Cancer Med. 2019;8(11):4976–85.
44. Ilander M, Olsson-Stromberg U, Schlums H, et al. Increased proportion of mature NK cells is associated with successful imatinib discontinuation in chronic myeloid leukemia. Leukemia. 2016;31(5):1108–16.
45. Irani YD, Hughes A, Clarson J, et al. Successful treatment-free remission in chronic myeloid leukaemia and its association with reduced immune suppressors and increased natural killer cells. Br J Haematol. 2020;191(3):433–41.
46. Schütz C, Inselmann S, Sausslele S, et al. Expression of the CTLA-4 ligand CD86 on plasmacytoid dendritic cells (pDC) predicts risk of disease recurrence after treatment discontinuation in CML. Leukemia. 2017;31(4):829–36.
47. Caocci G, Martino B, Greco M, et al. Killer immunoglobulin-like receptors can predict TKI treatment-free remission in chronic myeloid leukemia patients. Exp Hematol. 2015;43(12):1015–8.e1
48. Rinaldetti S, Pfirrmann M, Manz K, et al. Effect of ABCG2, OCT1, and ABCB1 (MDR1) gene expression on treatment-free remission in a EURO-SKI subtrial. Clin Lymphoma Myeloma Leuk. 2018;18(4):266–71.
49. Mahon FX, Delbrel X, Cony-Makhoul P, et al. Follow-up of complete cytogenetic remission in patients with chronic myeloid leukemia after cessation of interferon alfa. J Clin Oncol. 2002;20(1):214–20.
50. Goldman J, Gordon M. Why do chronic myelogenous leukemia stem cells survive allogeneic stem cell transplantation or imatinib: does it really matter? Leuk Lymphoma. 2006;47(1):1–7.
51. Gallipoli P, Abraham SA, Holyoake TL. Hurdles toward a cure for CML: the CML stem cell. Hematol Oncol Clin North Am. 2011;25(5):951–66. v
52. Rea D, Rousselot P, Guilhot J, et al. Curing chronic myeloid leukemia. Curr Hematol Malig Rep. 2012;7(2):103–8.
53. Hantschel O, Warsch W, Eckelhart E, et al. BCR-ABL uncouples canonical JAK2-STAT5 signaling in chronic myeloid leukemia. Nat Chem Biol. 2012;8(3):285–93.
54. Yaoyu C, Cong P, Con S, et al. Novel therapeutic agents against cancer stem cells of chronic myeloid leukemia. Anti Cancer Agents Med Chem. 2010;10(2):111–5.
55. Zhang B, Strauss AC, Chu S, et al. Effective targeting of quiescent chronic myelogenous leukemia stem cells by histone deacetylase inhibitors in combination with imatinib mesylate. Cancer Cell. 2010;17(5):427–42.
56. Li L, Wang L, Li L, et al. Activation of p53 by SIRT1 inhibition enhances elimination of CML leukemia stem cells in combination with imatinib. Cancer Cell. 2012;21(2):266–81.
57. Hurtz C, Hatzi K, Cerchietti L, et al. BCL6-mediated repression of p53 is critical for leukemia stem cell survival in chronic myeloid leukemia. J Exp Med. 2011;208(11):2163–74.
58. Dierks C, Beigi R, Guo G-R, et al. Expansion of Bcr-Abl-positive leukemic stem cells is dependent on hedgehog pathway activation. Cancer Cell. 2008;14(3):238–49.
59. Kleppe M, Levine RL. Targeting β-catenin in CML: leukemia stem cells beware! Cell Stem Cell. 2012;10(4):351–3.
60. Richter J, Soderlund S, Lubking A, et al. Musculoskeletal pain in patients with chronic myeloid leukemia after discontinuation of imatinib: a tyrosine kinase inhibitor withdrawal syndrome? J Clin Oncol. 2014;32(25):2821–3.
61. Berger MG, Pereira B, Rousselot P, et al. Longer treatment duration and history of osteoarticular symptoms predispose to tyrosine kinase inhibitor withdrawal syndrome. Br J Haematol. 2019;187(3):337–46.
62. Legros L, Nicolini FE, Etienne G, et al. Second tyrosine kinase inhibitor discontinuation attempt in patients with chronic myeloid leukemia. Cancer. 2017;123(22):4403–10.