Cytogenetics of Chronic Myeloid Leukemia (CML)

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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].

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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].

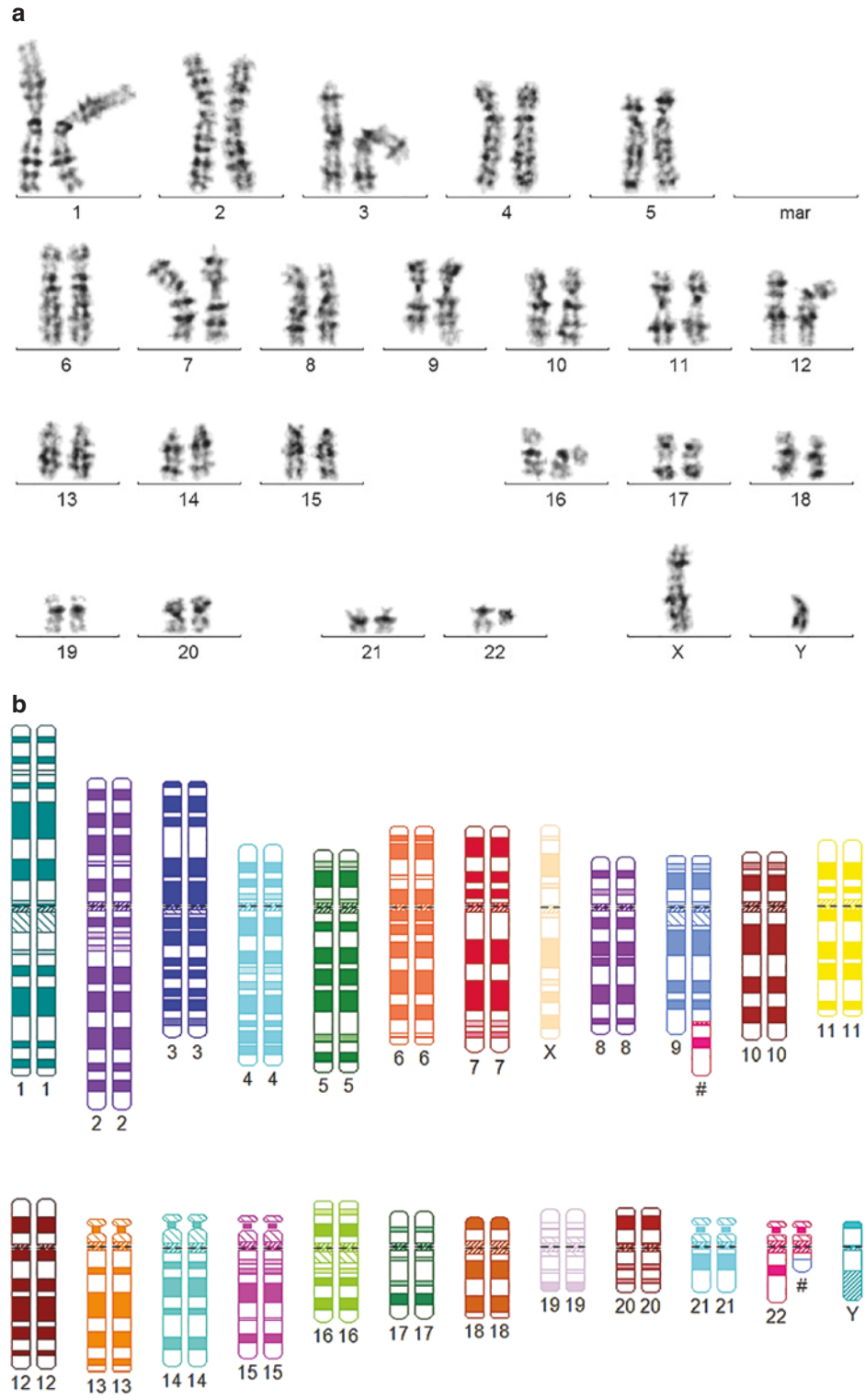

Fig. 1.1 (**a**) Karyogram and (**b**) scheme of the translocation t(9;22)(q34;q11) generated with CyDAS [17]

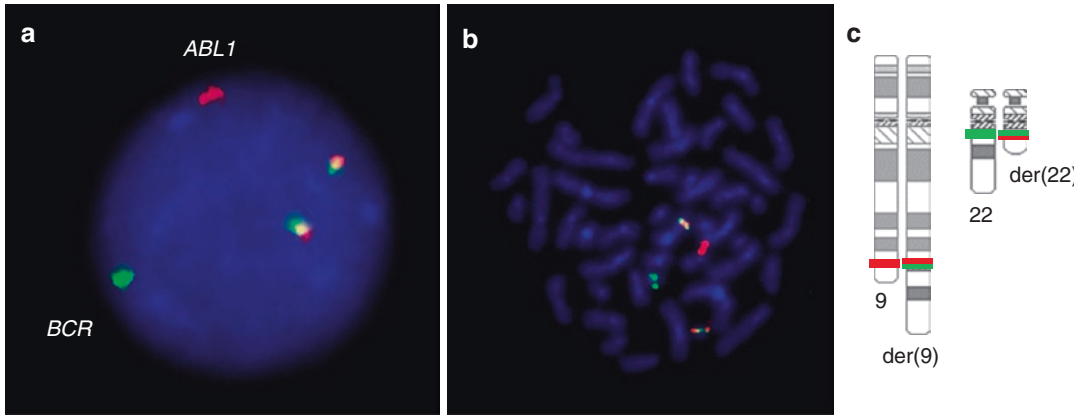

Fig. 1.2 (**a**) Interphase FISH using dual-color dual-fusion probes, with *BCR* labelled in green and *ABL1* in red to detect the *BCR-ABL1* rearrangement that leads to two yellow fusion signals. (**b**) Metaphase FISH with the *BCR-ABL1* probes. (**c**) Scheme of the *BCR* and *ABL1* probes binding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

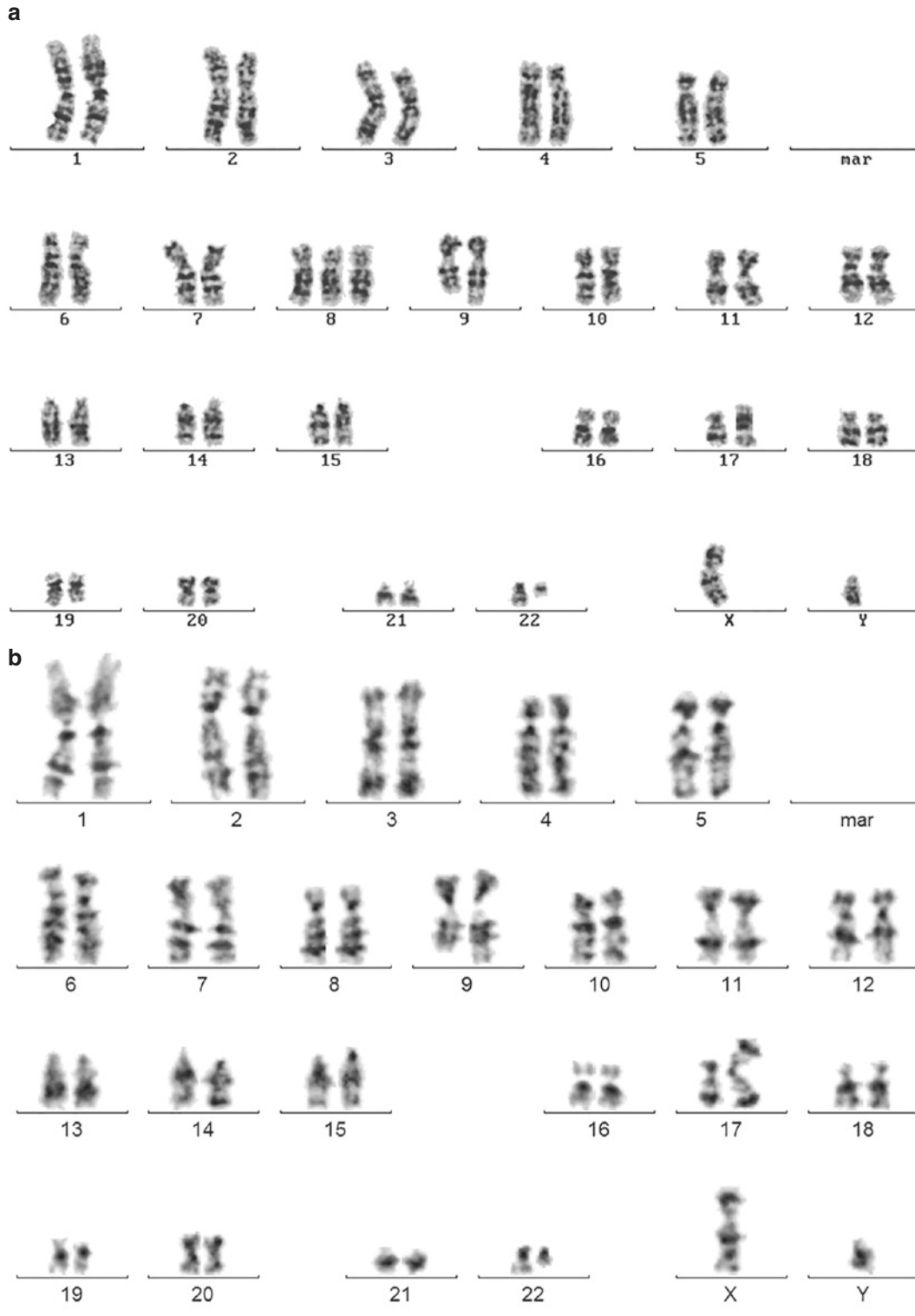

Fig. 1.3 (**a**) Karyogram of the translocation t(9;22)(q34;q11) with trisomy 8 and (**b**) i(17)(q10) as additional chromosomal aberrations (ACAs), respectively

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 ≤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 (≤0.01% *BCR-ABL1/ABL1*), MR4.5 (≤0.0032% *BCR-ABL1/ABL1*), and MR5 (≤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://mitelmandatabase.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.