12 Clinical Relevance of *RUNX1* and *CBFB* Alterations in Acute Myeloid Leukemia and Other Hematological Disorders

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Abstract

The translocation t(8;21), leading to a fusion between the *RUNX1* gene and the *RUNX1T1* locus, was the first chromosomal translocation identified in cancer. Since the first description of this balanced rearrangement in a patient with acute myeloid leukemia (AML) in 1973, *RUNX1* translocations and point mutations have been found in various myeloid and lymphoid neoplasms. In this chapter, we summarize the currently available data on the clinical relevance of core binding factor gene alterations in hematological disorders. In the first section, we discuss the prognostic implications of the core binding factor translocations *RUNX1-RUNX1T1* and *CBFB-MYH11* in AML patients. We provide an overview of the cooperating genetic events in patients with CBF-rearranged AML and their clinical implications, and review current treatment approaches for CBF AML and the utility of minimal residual disease monitoring. In the next sections, we summarize the available data on rare *RUNX1* rearrangements in various hematologic neoplasms and the role of *RUNX1* translocations in therapy-related myeloid neoplasia. The final three sections of the chapter cover the spectrum and clinical significance of *RUNX1* point mutations in AML and myelodysplastic syndromes, in familial platelet disorder with associated myeloid malignancy, and in acute lymphoblastic leukemia.

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Keywords

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12.1 Introduction

The balanced translocation t(8;21)(q22;q22), initially described by Janet D. Rowley in 1973 in a patient with acute myeloid leukemia (AML), was the first reciprocal chromosomal translocation identified in cancer cells (Rowley 1973). In 1991, Miyoshi and co-workers cloned and sequenced a novel gene, located on the breakpoint on chromosome 21, in AML patients with this translocation (Miyoshi et al. 1991). The gene was initially called *AML1*, and is now named Runt-related transcription factor 1 (*RUNX1*) due to its homology with the Drosophila gene Runt. Soon afterwards, it was established that on the molecular level, t(8;21)(q22;q22) leads to the formation of a chimeric fusion transcript that today is named *RUNX1-RUNX1T1* (Erickson et al. 1992; Miyoshi et al. 1993). The RUNX1 protein is part of a heterodimeric transcription factor called the "core binding factor", or CBF. Today, three different DNA-binding CBFα subunits (*RUNX1, RUNX2* and *RUNX3)* and one common non-DNA-binding CBFβ subunit (encoded by the *CBFB* gene) have been identified in humans (Speck and Gilliland 2002). In 1993, Liu and co-workers identified *CBFB* and the myosin heavy chain gene *MYH11* as the fusion partners in another recurrent balanced chromosomal rearrangement in AML, namely inv(16)(p13q22) and its variant, t(16;16)(p13;q22) (Liu et al. 1993). These seminal discoveries pointed towards an important role of CBF genes not only in normal hematopoiesis, but also in leukemia, and opened the road for further studies revealing that the *RUNX1* gene is frequently altered in myeloid malignancies and other hematological disorders through various mechanisms including chromosomal translocations, point mutations and deletions. In this chapter, we will review the clinical and prognostic significance of *RUNX1* alterations and the *CBFB-MYH11* fusion in AML and other hematological disorders.

12.2 Balanced Translocations Involving the Core Binding Factor Subunits in AML: t(8;21)(q22;q22); *RUNX1-RUNX1T1* and inv(16)(p13q22)/t(16;16)(p13;q22); *CBFB-MYH11*

12.2.1 Background

Between 1978 and 1984, the International Workshops on Chromosomes in Leukemia established that t(8;21)(q22;q22) is a recurrent event in AML, and closely linked to M2 morphology according to the French-American-British (FAB) classification (i.e., acute myelogenous leukemia with maturation) (Rowley and de la Chapelle 1978; Rowley 1980; Bloomfield et al. 1984). In subsequent studies, the incidence of the *RUNX1-RUNX1T1* rearrangement in adult AML was 4–8% (Slovak et al. 2000; Byrd et al. 2002; Mrózek 2004), and it was 7% in a very large cohort of 5876 patients aged 16–59 years (Grimwade et al. 2010). *RUNX1-RUNX1T1* is extremely rare in infants but occurs in 11–14% of children and adolescents, and represents the single most common balanced translocation in pediatric AML (Leverger et al. 1988; Raimondi et al. 1999; Mrózek et al. 2004; von Neuhoff et al. 2010; Harrison et al. 2010). The incidence of *RUNX1-RUNX1T1* decreases with age, and the translocation is less frequent in patients aged ≥60 years (Bloomfield et al. 1984; Byrd et al. 2002; Grimwade et al. 2010).

The pericentric inversion inv(16)(p13q22) was first described as a recurrent abnormality in AML in 1983. The original publications described an association with myelomonocytic leukemia with abnormal eosinophils (FAB M4eo), and reported that affected patients had favorable response to treatment (Le Beau et al. 1983; Bloomfield et al. 1984). The incidence of *CBFB-*

MYH11 in adults was 5% in the large British Medical Research Council (MRC) cohort, and 2–9% in other series, and it is found in 6–9% of pediatric AML (Raimondi et al. 1999; Mrózek et al. 2004; Grimwade et al. 2010; von Neuhoff et al. 2010; Harrison et al. 2010). Similar to *RUNX1-RUNX1T1*, the *CBFB-MYH11* fusion is less frequent in older adults (i.e., ≥60 years) (Byrd et al. 2002).

12.2.2 Prognosis of AML Patients with t(8;21)(q22;q22); *RUNX1-RUNX1T1* and inv(16)(p13q22) /t(16;16)(p13;q22); *CBFB-MYH11*

The reports from the International Workshops on Chromosomes in Leukemia established that karyotype is an important prognostic factor in AML, and revealed that both CBF rearrangements, t(8;21)(q22;q22) and inv(16)(p13q22), associate with relatively favorable outcomes. This finding was confirmed by long-term follow-up of the initial cohorts, although treatment was not uniform in these early series (Rowley 1980; Larson et al. 1983; Bloomfield et al. 1984; Swansbury et al. 1994). Later studies in patients who received more standardized, cytarabine-based induction and consolidation chemotherapy on cooperative group trials consistently showed that patients with CBF rearrangements had higher complete remission (CR) rates compared to patients with cytogenetically normal AML, and longer disease-free survival (DFS) and overall survival (OS) (Keating et al. 1987; Fenaux et al. 1989; Slovak et al. 2000). In a study of 1213 patients enrolled on 5 consecutive Cancer and Leukemia Group B (CALGB) treatment protocols, Byrd and colleagues demonstrated that the *RUNX1-RUNX1T1* and *CBFB-MYH11* fusions associated with high CR rates (91% and 85%, respectively), a low rate of primary refractory disease, and favorable DFS and OS (Byrd et al. 2002). In a more recent analysis of 5876 younger adults (<60 years) treated on trials of the MRC, *RUNX1-RUNX1T1* positive patients had a CR rate of 97% and a 10-year OS of 61%, while those with *CBFB-MYH11* had a CR rate of 92% and a 10-year OS of 55% (Fig. 12.1) (Grimwade et al. 2010). When patients with acute promyelocytic leukemia (APL) are excluded, t(8;21)(q22;q22) and inv(16)(p13q22) / t(16;16)(p13;q22) represent the most favorable cytogenetic subset in this very large cohort. In older adults (≥60 years) with CBF leukemias who received at least one cycle of induction chemotherapy, the CR rate was 88%, but 5-year OS was only 31% (Prébet et al. 2009). Pediatric AML patients with CBF rearrangements have excellent outcomes with reported OS rates of ~90% at 5 years and ~80% at 10 years (von Neuhoff et al. 2010; Harrison et al. 2010). Based on these data, it is generally accepted today that the CBF rearrangements, t(8;21)(q22;q22), inv(16)(p13q22) and t(16;16)(p13;q22), define a favorable cytogenetic subset of adult and pediatric AML patients, and this is reflected by current risk stratification systems and international guidelines (Grimwade et al. 2010; Döhner et al. 2010; Creutzig et al. 2012).

12.2.3 Role of Cooperating Cytogenetic Changes and Gene Mutations in AML with CBF Gene Rearrangement

Additional chromosomal alterations are found in most AML patients with the translocation t(8;21). Loss of a sex chromosome was identified as the most common secondary alteration in adults and children, followed by deletions in the long arm of chromosome 9 (band 9q22) (Larson et al. 1983; Bloomfield et al. 1984; Raimondi et al. 1999; Kuchenbauer et al. 2006). In a series of 111 adult patients, additional chromosomal abnormalities were found in 70%, including loss of a sex chromosome in 47%, 9q deletion in 15% and trisomy 8 in 6% (Krauth et al. 2014). Loss of a sex chromosome associated with favorable, and trisomy 8 with unfavorable event-free survival (EFS) in this series, although treatment was heterogeneous and the number of patients with +8 was small. Other reports also suggested that specific additional cytogenetic abnormalities, including

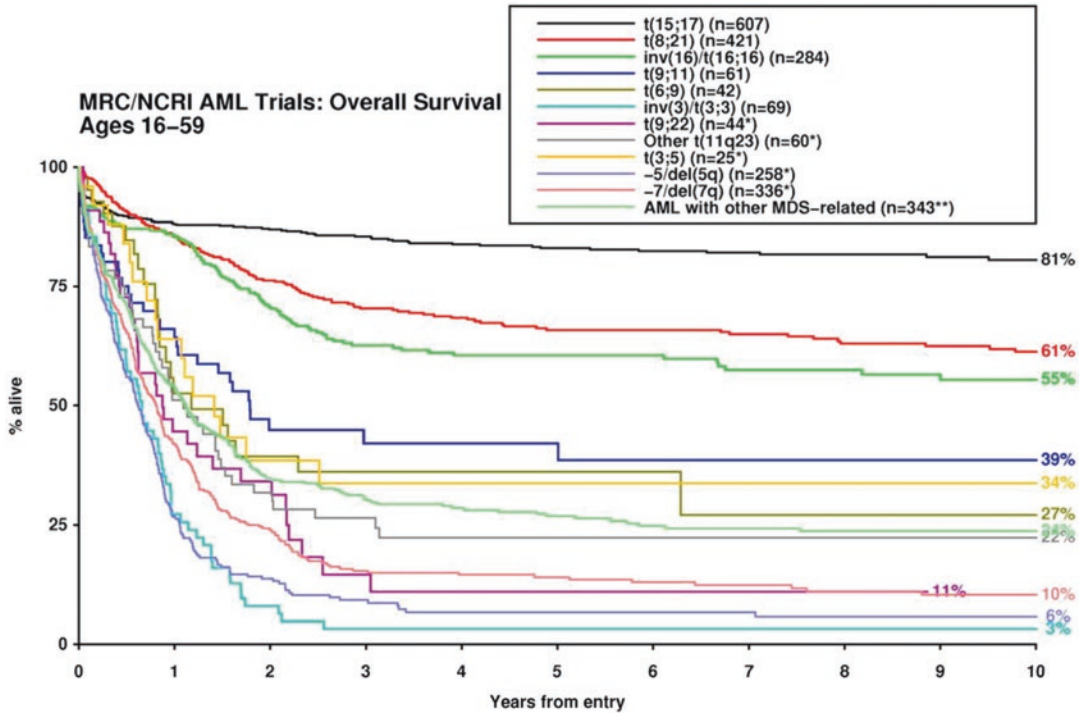

Fig. 12.1 Prognosis of AML patients with t(8;21)(q22;q22); *RUNX1-RUNX1T1* and inv(16)(p13q22)/t(16;16)(p13;q22); *CBFB-MYH11*. Overall survival of younger patients (age, 16–59) treated on British Medical Research Council trials, stratified according to cytogenetic findings. Survival for patients with t(8;21) is shown in red and survival for patients with inv(16) is shown in green (Figure reproduced from Grimwade et al. (2010) with permission)

del(9q) and loss of the Y chromosome in male patients, or *RUNX1-RUNX1T1* rearrangement in the context of a complex karyotype, adversely affect the outcomes of *RUNX1-RUNX1T1*-positive patients (Schoch et al. 1996; Schlenk et al. 2004; Appelbaum et al. 2006). In contrast, several large studies in children and adults demonstrated that the presence of secondary cytogenetic alterations including loss of a sex chromosome, del(9q), or trisomy 8 have no adverse impact on outcomes (Fenaux et al. 1989; Byrd et al. 2002; Grimwade et al. 2010; Harrison et al. 2010). Importantly, patients with *RUNX1-RUNX1T1* and *CBF-MYH11* have favorable outcomes even when these abnormalities occur within a complex karyotype (Byrd et al. 2002; Grimwade et al. 2010).

In AML patients with inv(16) or t(16;16), the most frequently identified additional cytogenetic alterations are trisomy 22, trisomy 11, and deletions on the short arm of chromosome 7, which are found in 14–19%, 10–16%, and 5–6% of patients, respectively (Schlenk et al. 2004; Marcucci et al. 2005; Grimwade et al. 2010; Paschka et al. 2013). At least three independent studies reported that patients with *CBFB-MYH11* and an additional chromosome 22 have a particularly low risk of relapse and favorable survival (Grimwade et al. 2010; Schlenk et al. 2004; Marcucci et al. 2005).

More recent analyses also include information on molecular gene mutations. Data from the German AML Study Group (AMLSG) show that 56% of *RUNX1-RUNX1T1* rearranged AML and 84% of *CBF-MYH11* rearranged AML harbor mutations in *KIT*, *FLT3*, *NRAS* or *KRAS* (Paschka and Döhner 2013). The frequency of *KIT* and *FLT3* gene mutations was similar in both subsets of CBF leukemias. *KIT* mutations were detected in 30% of *RUNX1-RUNX1T1* positive cases and *37% CBF-MYH11* positive cases, and *FLT3* mutations were present in 13% and 17%,

respectively. *RAS* mutations, however, were more common in AML with *CBF-MYH11* (53% vs. 21% in *RUNX1-RUNX1T1* rearranged AML). In another analysis of 11 different genes in 139 *RUNX1-RUNX1T1*-positive AML patients, at least one gene mutation was found in 50%, with *KIT*, *NRAS* and *ASXL1* being most commonly affected (Krauth et al. 2014).

In 2014, Micol and colleagues discovered novel mutations in the additional sex combs-like 2 (*ASXL2*) gene in almost a quarter of patients with *RUNX1-RUNX1T1* fusion. Notably, mutations in this gene were absent in patients with *CBFB-MYH11* rearrangement or mutated *RUNX1* (Micol et al. 2014). More comprehensive genetic analyses have revealed that mutations affecting epigenetic modifiers, including *ASXL1, ASXL2, EZH2* and *KDM6A*, the cohesin complex, and the zink finger transcription factor *ZBTB7A* are common in *RUNX1-RUNX1T1* rearranged AML, but rare or absent in patients with *CBFB-MYH11* (Hartmann et al. 2016; Duployez et al. 2016; Lavallée et al. 2016; Sood et al. 2016). The prognostic relevance of these mutations in CBF AML remains to be determined. SNP-array studies revealed that submicroscopic copy number alterations are rare in CBF rearranged AML (Kühn et al. 2012). Overall, these data indicate that, while activation of receptor tyrosine kinase signaling pathways is a common mechanism in CBF AML, other cooperating pathways may be specific to patients with either *RUNX1-RUNX1T1* or *CBFB-MYH1*.

12.2.3.1 Clinical Relevance of *KIT* Gene Mutations in CBF AML

Mutations in the *KIT* receptor tyrosine kinase in AML were first identified by Beghini and colleagues (Beghini et al. 1998), and were subsequently confirmed to be recurrent events in CBF leukemias, while they are rare in other cytogenetic subsets (Gari et al. 1999; Beghini et al. 2000; Schnittger et al. 2006). In some patients, *KIT* mutations become undetectable in CR while the *RUNX1-RUNX1T1* fusion remains detectable using similarly sensitive methods, suggesting that *KIT* mutations constitute a secondary hit that provides a growth and/or survival advantage to the leukemic cells (Wang et al. 2005). In several cohorts of CBF-rearranged adolescents and adults, *KIT* mutations were found in 20–47% of *RUNX1-RUNX1T1* and 30–45% of *CBFB-MYH11* positive patients and tended to associate with higher white blood counts (Care et al. 2003; Beghini et al. 2004; Wang et al. 2005; Cairoli et al. 2006; Paschka et al. 2006; Allen et al. 2013). In *RUNX1-RUNX1T1* rearranged AML, most of the mutations are activating missense mutations in the tyrosine kinase domain (exon 17), while mutations in the extracellular domain (exon 8) and the transmembrane and juxtamembrane domains (exons 10 and 11) occur more rarely and have not been analyzed in all studies (Allen et al. 2013). In contrast, exon 8 mutations are more common in patients with *CBFB-MYH11* (Paschka et al. 2013; Allen et al. 2013).

In several moderately-sized retrospective series of *RUNX1-RUNX1T1*-positive AML, *KIT*-mutated patients had a higher incidence of relapse (70–100%) compared to *KIT* wild-type patients (~35%), while the results in patients with *CBFB-MYH11* were discordant (Schnittger et al. 2006; Cairoli et al. 2006; Paschka et al. 2006; Nanri et al. 2005b; Boissel et al. 2006). In the largest cohort reported so far by the MRC study group, *KIT* mutations were found in 23% of 199 patients with *RUNX1-RUNX1T1* rearrangement. Only "high-level" *KIT* mutations with a mutant-to-wild type ratio of ≥25% associated with higher relapse risk (41% compared to 25% for *KIT* wild-type patients), while *KIT* mutations present at lower levels had no impact on relapse. Of note, *FLT3*-internal tandem duplications, but not *KIT* mutations, associated with shorter OS in this cohort (Allen et al. 2013). In contrast, 35% of 155 *CBFB-MYH11* rearranged patients in the same study had mutated *KIT*, and mutation status did not affect RFS or OS. In a large series of *CBFB-MYH11* patients from the German AMLSG, *KIT* mutations negatively affected RFS, but not OS (Paschka et al. 2013).

Collectively, these results establish *KIT* mutations as a predictor of higher relapse risk in adult *RUNX1-RUNX1T1* rearranged AML. *KIT* mutations were linked to shorter OS in some studies (Schnittger et al. 2006; Cairoli et al. 2006;

Boissel et al. 2006) but not in others (Paschka et al. 2006; Allen et al. 2013; Nanri et al. 2005b), suggesting that *KIT*-mutated patients may respond favorably to salvage therapy. The prognostic relevance of KIT mutations in AML with *CBFB-MYH11* is less well established, and there are conflicting reports on the prognostic relevance of *KIT* mutations in pediatric CBF AML patients (Paschka and Döhner 2013; Pollard et al. 2010).

12.2.4 Treatment of AML with CBF Leukemias

12.2.4.1 Chemotherapy and the Role of High-Dose Cytarabine

As outlined above, approximately 90% of adult *RUNX1-RUNX1T1*-positive AML patients achieve CR with cytarabine- and anthracycline-based ('7+3'-like) induction chemotherapy. A study from CALGB demonstrated that consolidation therapy with 4 cycles of high-dose cytarabine (HDAC, 3 g/m^2 twice daily on days 1,3 and 5), compared to cytarabine doses of 100 or 400 mg/m^2/day for 5 days, resulted in prolonged DFS particularly in patients with CBF leukemias (Bloomfield et al. 1998). The same group later showed that patients with *RUNX1-RUNX1T1* rearrangement who received three or four cycles of HDAC had superior 5-year DFS (71%) and OS (76%) compared to patients who received only one HDAC course (5 year DFS, 37%; 5-year OS, 44%). In an extended series of 96 *RUNX1-RUNX1T1*-positive patients, those receiving multiple HDAC courses had a 10 year survival of 56%, compared to 43% for those receiving only a single course (Marcucci et al. 2005). In patients with *CBFB-MYH11*, exposure to 3–4 HDAC cycles resulted in improved 5-year DFS compared to those receiving only one cycle (57% vs. 30%), with no improvement in OS (Byrd et al. 2004). A favorable impact of 3 cycles of HDAC consolidation, compared to 4 cycles of multiagent chemotherapy with lower-dose cytarabine, on DFS in CBF leukemias was also confirmed by a Japanese trial (Miyawaki et al. 2011). Daunorubicin dose escalation from 45 to 90 mg/m^2 during induction resulted in a trend towards improved EFS and OS in older patients (≥60 years) with CBF leukemia (Löwenberg et al. 2009). In a large British randomized trial of mostly younger patients and in a retrospective analysis of two French trials, daunorubicin dose escalation from 60 to 90 mg/m^2 during induction was not associated with improved survival in CBF leukemias (Prébet et al. 2014; Burnett et al. 2015). Therefore, '7+3'-like induction chemotherapy (preferentially with a daunorubicin dose of 60 mg/m^2) followed by 3–4 cycles of HDAC consolidation currently can be considered the standard treatment for adult AML patients with CBF rearrangement, although one study suggested that lower cumulative doses of cytarabine may be sufficient (Löwenberg et al. 2011).

12.2.4.2 Gemtuzumab Ozogamicin

Gemtuzumab ozogamicin (GO) is an anti-CD33 antibody coupled to the cytotoxic drug calicheamicin. In a subgroup analysis of the British MRC AML15 trial for patients <60 years, addition of a single dose of GO to induction chemotherapy led to significantly improved OS in patients with CBF leukemias (Burnett et al. 2011). A beneficial effect of GO in CBF leukemia patients was confirmed in a large meta-analysis of five randomized trials, which found a 5-year OS of 78% for patients receiving GO compared to 55% for those not receiving GO (Hills et al. 2014). The optimal dose and schedule of GO administration in CBF AML are unknown. GO was withdrawn from the US market due to concerns about early mortality in one trial (Petersdorf et al. 2013), and the drug is currently approved in Japan, but not in the US or Europe.

12.2.4.3 Allogeneic Stem Cell Transplantation for CBF AML

In a retrospective comparison of 118 AML patients with *RUNX1-RUNX1T1* translocation in first CR who underwent allogeneic stem cell transplantation (alloSCT) from a HLA-identical sibling to 132 patients receiving consolidation chemotherapy on German multicenter trials, patients receiving alloSCT had a reduced relapse

risk, however, this was offset by increased treatment-related mortality. In summary, relapse-free survival (RFS) tended to be better for those patients receiving chemotherapy (Schlenk et al. 2008). For patients with *CBFB-MYH11* rearrangement, a German meta-analysis of 170 patients in first CR also found no RFS benefit of allogeneic SCT over consolidation chemotherapy (Schlenk et al. 2004). Results of a donor-versus-no donor analysis and a large-meta analysis of 24 trials confirmed that AML patients with CBF leukemias do not profit from alloSCT in first CR (Cornelissen et al. 2007; Koreth et al. 2009). While *KIT* mutations predict a higher relapse risk in *RUNX1-RUNX1T1*-positive AML, it is unknown whether alloSCT ameliorates this increased risk. Consequently, there is currently no consensus whether *KIT*-mutated patients should undergo alloSCT in first CR (Allen et al. 2013).

12.2.4.4 Tyrosine Kinase Inhibitors

Functional analyses of *KIT* mutations showed that they lead to constitutive activation of the receptor (Cammenga et al. 2005). Furthermore, *KIT* is overexpressed in *RUNX1-RUNX1T1*-rearranged AML patients irrespective of its mutation status (Bullinger et al. 2004; Valk et al. 2004). Wild-type and mutant *KIT* isoforms can be inhibited by various tyrosine kinase inhibitors (TKIs), providing a rationale for therapeutic use of TKIs in t(8;21) AML (Growney et al. 2005; Nanri et al. 2005a; Schittenhelm et al. 2006; Chevalier et al. 2010; Paschka and Döhner 2013). Mutated *KIT* isoforms exhibit variable sensitivity to different inhibitors. Clinical responses were observed in single patients or small series of patients with advanced disease receiving TKI, including imatinib and dasatinib (Nanri et al. 2005a; Chevalier et al. 2010). However, in a study of 26 high-risk patients with minimal residual disease (MRD) persistence or recurrence, 12 months of dasatinib maintenance did not avert hematological relapse in patients with molecular recurrence, or improve DFS in those with suboptimal MRD response (Boissel et al. 2015). Several prospective studies of dasatinib in CBF leukemias are ongoing.

12.2.4.5 Treatment of Relapsed Disease

Data from the 6th International Workshop on Chromosomes in Leukemia suggested that relapsed *RUNX1-RUNX1T1*-positive AML is relatively sensitive to repeated chemotherapy, and second CRs can be achieved in a considerable fraction of patients (Garson et al. 1989). In a retrospective analysis of 59 patients in first relapse, the rate of second CR after salvage chemotherapy was 88%, and 5-year survival after relapse was 51%. Addition of GO to salvage chemotherapy appeared to be beneficial, with a 5 year OS of 65% compared to 44% for those receiving chemotherapy without GO (Hospital et al. 2014). An analysis by the MRC group showed that relapsed *RUNX1-RUNX1T1* or *CBFB-MYH11*-positive AML patients who received salvage chemotherapy without alloSCT had a 5-year OS of 41% and 47%, respectively, compared to 29 % and 39 % for those receiving an alloSCT, yet this was not a randomized comparison and survival estimates may be biased. Nevertheless, these data indicate that CBF AML frequently remains chemoresponsive at the time of relapse, in contrast to relapsed non-CBF AML which generally is considered incurable without alloSCT (Burnett et al. 2013).

12.2.5 Minimal Residual Disease Monitoring in CBF AML

The *RUNX1-RUNX1T1* and *CBFB-MYH11* fusion transcripts can be detected with high sensitivity by RT-PCR, and this technique may be used to detect persisting leukemic cells in patients in clinical remission. However, it has been shown that some patients who have been in morphological CR for up to 8 years still have detectable *RUNX1-RUNX1T1* transcripts in the bone marrow (BM) and/or blood, although some studies reported that transcript levels tended to decrease and become undetectable over time (Nucifora et al. 1993; Nucifora and Rowley 1994; Kusec et al. 1994; Satake et al. 1995). Clonogenic progenitor assays revealed that the *RUNX1-RUNX1T1* fusion persisted in multipotent

hematopoetic progenitor cells that were able to differentiate into mature trilineage myeloid cells and mature B cells *in vitro* and *in vivo* (Miyamoto et al. 1996, 2000). These studies indicate that *RUNX1-RUNX1T1*-positive pre-leukemic stem cells capable of self-renewal and differentiation can persist in the BM during CR, although their frequency gradually decreases over time. Miyamoto studied *RUNX1-RUNX1T1*-positive patients who had been in CR for 1–12.5 years using a nested RT-PCR assay with a sensitivity of $1:10^{-7}$. They found *RUNX1-RUNX1T1* transcripts in the BM of all 18 patients treated with chemotherapy only, but in none of the 4 patients who had undergone alloSCT (Miyamoto et al. 1996). Another study, however, reported that *RUNX1-RUNX1T1* transcripts were also detectable in 9 of 10 patients in CR after alloSCT (Jurlander et al. 1996). Taken together, these studies establish that *RUNX1-RUNX1T1*-positive cells can persist at low levels in t(8;21) AML patients who achieve long-term remissions. They also demonstrate that the *RUNX1-RUNX1T1* fusion alone is not sufficient to initiate AML, and secondary genetic lesions are needed.

Low-level persistence of *RUNX1-RUNX1T1* transcripts in patients who may be cured limits the utility of qualitative (end-point) RT-PCR assays for the detection of clinically meaningful residual disease. Nevertheless, a French multicenter study of 51 patients suggested that many patients in long-term remission ultimately become PCR-negative. Using a less sensitive one-step qualitative PCR technique, this study showed that patients who achieved PCR negativity during follow-up had a relapse rate of 15%, while all patients with persistently positive PCR results relapsed. This study also suggested the possibility of early MRD-based response assessment, since patients who became PCR-negative after induction and before consolidation chemotherapy had a relapse rate of 11%, compared to 72% for the remaining patients (Morschhauser et al. 2000).

The development of quantitative PCR (qPCR) techniques allowed serial monitoring of *RUNX1-RUNX1T1* and *CBFB-MYH11* transcript levels over time, and establishing critical threshold levels that are predictive of imminent hematological relapse (Tobal and Yin 1996; Marcucci et al. 1998; Krauter et al. 1999; Tobal et al. 2000; Krauter et al. 2003; Buonamici et al. 2002; Leroy et al. 2005). If quantitative MRD monitoring is to be used for clinical decision-making, careful standardization of methods and cut-offs is necessary to ensure comparable results from different laboratories. RNA-based assays for quantitative detection of *RUNX1-RUNX1T1, CBFB-MYH11* and other fusion transcripts have been established and validated by multinational consortia including the "Europe against Cancer" (EAC) initiative (van Dongen et al. 1999; Gabert et al. 2003). Of note, since the genomic breakpoints in the *RUNX1* locus are distributed over a region of ~25 kilobases, MRD monitoring on genomic DNA requires development of patient-specific assays. While this approach is feasible and offers the conceptual advantage of quantifying the proportion of leukemic cells more directly, it suffers from variable sensitivity and greatly increased complexity, and is thus not widely used (Duployez et al. 2014).

The clinical relevance of MRD measurements by qPCR was demonstrated in several large, uniformly treated patient cohorts analyzed according to the EAC recommendations. The British MRC group studied 278 CBF-AML patients aged 15–70 years, and found that a >3 log reduction of *RUNX1-RUNX1T1* transcript levels in BM after the first induction cycle was associated with a cumulative incidence of relapse of only 4% at 5 years, while patients with a lesser reduction had relapse rates exceeding 30%, although this did not translate into significant survival differences. Similarly, detection of <10 *CBFB-MYH11* copies per 10^5 copies of *ABL* in peripheral blood after induction 1 associated with a relatively low 5-year incidence of relapse (21%) and favorable survival after CR. After completion of therapy, BM MRD levels of over 500 *RUNX1-RUNX1T1* copies per 10^5 *ABL* copies were also highly predictive of relapse (relapse rate, 100% versus 7% for those with persistently lower levels) and inferior OS (5-year survival, 57% vs. 94%). For patients with *CBFB-MYH11*, detection of >10 copies in the peripheral blood associated with a

97% risk of relapse and 57% 5-year-survival, compared with a 7% relapse risk and 91% survival in those with MRD levels <10 copies. The median time from qPCR positivity to hematologic relapse was about 5 months, leading the authors to recommend MRD monitoring from BM every 3 months during the first 18 months of follow-up (Yin et al. 2012).

The French AML Intergroup reported data on 198 CBF-AML patients aged 18–60 years. A ≥3 log reduction of BM fusion transcripts after the first consolidation course associated with a lower relapse risk (hazard ratio, 0.31), while the risk of death was not significantly lower (hazard ratio, 0.51). Importantly, the prognostic significance of early MRD reduction with regard to relapse outweighed the impact of *KIT* and *FLT3* gene mutations in a multivariate analysis (Jourdan et al. 2013). Among *RUNX1-RUNX1T1* rearranged patients in this cohort, persistent MRD positivity or molecular relapse in blood after the end of therapy predicted hematological relapse in 21 of 28 patients, while persistent *RUNX1-RUNX1T1* MRD positivity in the BM at 2 years was found in 9% of patients who maintained long-term remissions (Willekens et al. 2016).

The German AMLSG group studied a cohort of 53 *CBFB-MYH11* rearranged patients and identified criteria for risk stratification. Patients who achieved qPCR negativity in at least one BM sample during consolidation therapy had favorable DFS, while qPCR negativity in at least two BM or PB samples during consolidation therapy and early follow-up predicted for superior DFS and OS. Conversion from PCR negativity to PCR positivity after consolidation therapy occurred in 10 patients, and 6 of them relapsed (Corbacioglu et al. 2010). In summary, these studies establish that MRD measurements by qPCR, and particularly early response kinetics during therapy, are strong prognostic markers in CBF AML. However, it remains unclear whether treatment modification in response to unfavorable MRD results is beneficial.

A Chinese study addressed this question and studied the role of MRD-directed treatment in 116 *RUNX1-RUNX1T1*-rearranged AML patients. Patients who did not sustain a 3-log reduction of *RUNX1-RUNX1T1* transcript levels after 2 cycles of intermediate-dose cytarabine-based consolidation chemotherapy were considered high risk and were recommended to undergo alloSCT, while those with better responses were scheduled for 6 cycles of consolidation chemotherapy. The trial was not randomized, and about 40 % of patients crossed over between the two arms for various reasons. In this cohort, alloSCT improved DFS and OS of high-risk, but not of low-risk patients, but this result requires confirmation from controlled trials (Zhu et al. 2013). A follow-up study from the same group investigated the prognostic relevance of MRD detection in the posttransplantation setting. Patients who achieved a >3 log reduction of BM *RUNX1-RUNX1*T1 transcript levels during the first 3 months after alloSCT had significantly lower relapse rates and longer DFS, compared to those with higher transcript levels. A multivariate analysis suggested that MRD levels outweigh *KIT* mutation status as a stronger predictor of post-transplant relapse risk, although this analysis is limited by the relatively small patient cohort (Wang et al. 2014).

12.3 Other Balanced Translocations Involving *RUNX1* in Myeloid Malignancies

Besides the t(8;21)(q22;q22), several other recurrent chromosomal translocations involving the *RUNX1* locus have been described in myeloid neoplasms and are discussed in the following sections.

12.3.1 AML with t(3;21)(q26;q22); *RUNX1-MECOM*

The balanced translocation t(3;21)(q26;q22) was initially identified in patients with chronic myeloid leukemia (CML) in blast crisis, and subsequently found in 3.6% of patients with therapy-related AML or myelodysplastic syndromes (t-AML/t-MDS) (Rubin et al. 1987; Rubin et al. 1990). In a cohort of 6515 adult AML patients,

the translocation occurred in only 0.14%. More recently, this translocation was also observed in CML evolving into myeloid blast crisis after TKI treatment (Paquette et al. 2011). According to the 2016 WHO classification of haematopoietic neoplasms, detection of t(3;21)(q26;q22) is sufficient to establish a diagnosis of "AML with myelodysplasia-related changes" in patients with ≥20% blasts.

The t(3;21)(q26;q22) was shown to lead to the formation of *RUNX1-EVI1* and *RUNX1-MDS1* fusion transcripts (Nucifora et al. 1994; Mitani et al. 1994). The *MDS1* and *EVI1* genes are located closely to each other in chromosome band 3q26, and splicing of the second exon of *MDS1* to the second exon of *EVI1* can lead to the formation of a chimeric *MDS1/EVI1* transcript. Due to this close relation, *MDS1* and *EVI1* now are designated the '*MDS1* and *EVI1* complex locus' (*MECOM*), and the fusion gene in t(3;21)(q26;q22) has thus been named *RUNX1-MECOM*. High *EVI1* expression is found in most patients with t(3;21)(q26;q22). *MDS1/EVI1* levels were also high in some patients with t(3;21), but absent in others, indicating that *RUNX1-MDS1/EVI1* as well as *RUNX1-EVI1* fusions may occur depending on the location of the breakpoint in band 3q26 relative to the *MECOM* locus (Lugthart et al. 2010). With regard to outcomes, t-AML with t(3;21) associated with shorter OS compared to t-AML with t(8;21) in one series (Slovak et al. 2002).

12.3.2 AML with t(16;21)(q24;q22); *RUNX1-CBFA2T3*

The t(16;21)(q24;q22) is a rare, but recurrent chromosomal alteration found in therapy-related myeloid neoplasms. Gamou and colleagues reported that in this translocation, *RUNX1* is fused to *CBFA2T3* (previously called *MTG16*), a member of the conserved ETO family of transcriptional corepressors that shares a high degree of homology with *RUNX1T1*, the *RUNX1* translocation partner in t(8;21) (Gamou et al. 1998; Davis et al. 2003). Only 24 patients with t(16;21)(q24;q22) are currently reported in the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer, including 12 who also had trisomy 8, suggesting a possible association between the two alterations (Mitelman et al. 2016). The clinical significance of this translocation in AML is unknown.

12.3.3 Rare Recurrent Translocations in AML Involving *RUNX1*

A number of additional, very rare but recurrent translocations involving *RUNX1* have been described in AML. In t(1;21)(p36;q22), *RUNX1* is fused to the *PRDM16* gene, a member of the positive regulatory (PR) domain gene family with similarity to *MECOM* (Sakai et al. 2005). In t(1;21)(p22;q22), *RUNX1* is fused to the *CLCA2* calcium channel gene (Giguère and Hébert 2010). In the t(11;21)(p14;q22), the fusion partner is *KIAA1549L*, a poorly characterized gene with unknown function (Abe et al. 2012). Finally, the t(20;21)(q13.2;q22.12) results in a *ZFP64-RUNX1* fusion involving the zinc finger protein *ZFP6* (Richkind et al. 2000). The clinical significance of these alterations is unknown due to their rarity.

12.4 Association of *RUNX1* Translocations with Therapy-Related Neoplasia

Petersen-Biergard and colleagues first reported an association between chromosomal rearrangements involving chromosome band 21q22 and t-MDS or t-AML (Pedersen-Bjergaard and Philip 1991). In 2002, an international workshop identified balanced 21q22 translocations in 15.5% of patients with t-MDS or therapy-related acute leukemias (Slovak et al. 2002). The most common primary diseases were breast cancer, Hodgkin disease and non-Hodgkin lymphoma, and most patients had received topoisomerase II inhibitors and alkylating agents with or without radiotherapy. The median latency of the secondary hematologic disorder was 39 months, significantly longer than for therapy-induced neoplasms with

rearrangements involving *KMT2A* (chromosome band 11q23) or *CBFB* (16q22). A t(8;21) was present in 56% of these patients, and 22 additional translocations with documented involvement of the *RUNX1* locus were found, including t(3;21) in 20% and t(16;21) in 5% of patients. In a small series of 13 patients with t-AML and t(8;21), a CR rate of >90% was observed; however 10 of the 13 patients died after a median of 19 months (Gustafson et al. 2009). Likewise, Krauth and colleagues reported that among patients with t(8;21), those with t-AML had shorter OS compared to *de novo* patients (Krauth et al. 2014). The limited data available from retrospective case series suggests that the prognosis of t-AML patients with *RUNX1* rearrangements other than t(8;21) is relatively poor, with a median survival of less than 1 year (Slovak et al. 2002).

12.5 *RUNX1* Point Mutations in Myeloid Malignancies

12.5.1 *RUNX1* Mutations in AML

When the *RUNX1* gene was initially identified in 1991, Miyoshi et al. described a transcript encoding a 250-amino acid (AA) protein that was later named isoform AML1a, and today is known as transcript variant 3 (Miyoshi et al. 1991). Subsequently, the same group identified two additional transcript variants encoding proteins of 453 and 480 AA, which were designated AML1b (transcript variant 2) and AML1c (transcript variant 1), respectively (Miyoshi et al. 1995). The N-terminus of AML1c differs from that of AML1a and AML1b due to the use of an alternative promoter. All 3 proteins share a highly conserved, 128-AA Runt domain, a protein motif responsible for both DNA binding and heterodimerization. AML1b and AML1c contain a large C-terminal transactivation domain. Currently, the NCBI Gene database lists 13 exons, and 10 alternatively spliced RefSeq transcript isoforms, while the Ensembl database lists 9 protein-coding isoforms.

In 1999, Osato and colleagues were the first to identify somatically acquired *RUNX1* point mutations in 8 of 160 patients with myeloid leukemia (7 AML and 1 CML in blast crisis) (Osato et al. 1999). These mutations, located in the Runt domain, either disturb DNA binding and/or lead to weakened nuclear expression of *RUNX1*. The Runt domain is located in exons 3–5 and ranges from position 50–178 in the 453 AA transcript (position 77–205 in the 480 AA transcript). Early *RUNX1* mutation screening studies therefore often focused on exons 3–5, and did not include exons 1 and 2 or the C-terminal exons encoding the transactivation domain. Aggregate data from multiple cohorts available through the Catalogue of Somatic Mutations in Cancer (Forbes et al. 2015; Schnittger et al. 2011; Tang et al. 2009), and data from our own patients (Metzeler et al. 2016) indicate that *RUNX1* missense mutations cluster in the Runt homology domain (spanning exons 3–5) and are predicted to interfere with DNA binding, while truncating (nonsense and frame shift) mutations are distributed along the entire coding sequence (Fig. 12.2).

The reported incidence of *RUNX1* mutations in AML varies widely between studies (Tang et al. 2009), ranging from 3% in a series of pediatric AML patients (Taketani et al. 2003) to 33% in a cohort of adults with non-complex karyotypes (Schnittger et al. 2011). This large variability may be due to different baseline characteristics of the patient populations under study (e.g., age range, ethnicity, selection of cytogenetic subgroups, and *de novo* vs. secondary AML), and differences in the methods and target regions for mutation analyses. In recent, relatively large adult AML cohorts, the incidence of *RUNX1* mutations generally was in the range of 5–15% (Osato et al. 2001; Tang et al. 2009; Gaidzik et al. 2011; Patel et al. 2012; The Cancer Genome Atlas Research Network 2013; Kihara et al. 2014). The Cancer Genome Atlas Research Network (TCGA) consortium identified *RUNX1* mutations in 10% of 200 AML adult patients studied by whole-genome or whole-exome sequencing (The Cancer Genome Atlas Research Network 2013). Notably, analyses of clonal hierarchies in this cohort suggested that *RUNX1* mutations always were part of the "founding clone" that initiated the disease (Miller et al. 2013).

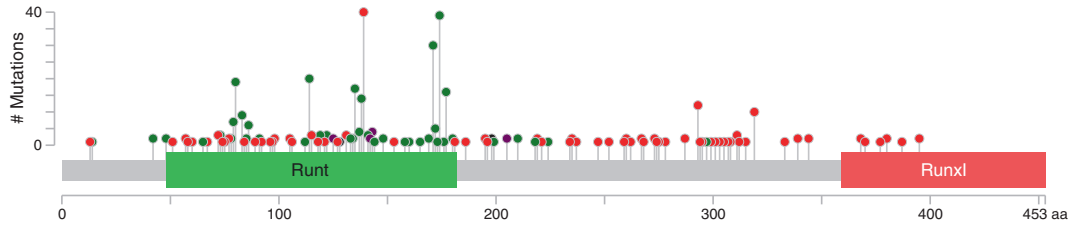

Fig. 12.2 Spectrum of somatic *RUNX1* point mutations in AML patients. Distribution of somatic *RUNX1* point mutations along the coding sequence of transcript variant 2 (NCBI accession number, NM_001001890.2). Truncating mutations (i.e., nonsense and frame shift changes) are shown in *red*, and missense variants are shown in *green* (Data are from the Catalogue of Somatic Mutations in Cancer (COSMIC) (Forbes et al. 2015) and from Metzeler et al. (2016))

12.5.2 Clinical Characteristics of AML Patients with Mutated *RUNX1*

Early studies indicated an association of somatic *RUNX1* mutations with FAB M0 morphology, and with secondary or treatment-related myeloid neoplasia (Asou 2003; Osato 2004). For example, Preudhomme and co-workers identified *RUNX1* mutations in ~10% of AML patients, and in 22% of patients with minimally differentiated (i.e., M0) AML. Twenty-one of 34 AML M0 patients in this series had biallelic *RUNX1* mutations, where a point mutation on one allele was accompanied by another point mutation or deletion of the second allele, while patients with non-M0 AML had monoallelic mutations (Preudhomme et al. 2000; Roumier et al. 2003). The association of *RUNX1* mutations with minimally differentiated AML was confirmed in larger series showing that 24–65% of patients with AML M0 carry *RUNX1* mutations (Dicker et al. 2007; Tang et al. 2009; Schnittger et al. 2011; Kao et al. 2014). Of note, in one large cohort that only included patients younger than 60 years, only 15% of M0 patients had mutated *RUNX1*, but the mutation was still enriched in this subgroup (Gaidzik et al. 2011). Tang and co-workers initially reported an association of *RUNX1* mutation with older age, an association that has been confirmed by multiple subsequent studies (Tang et al. 2009; Schnittger et al. 2011; Mendler et al. 2012; Greif et al. 2012). An association with male sex was also noted in some, but not all of these studies.

12.5.3 Cooperating Genetic Lesions in *RUNX1*-Mutated AML

RUNX1 mutations are found in patients with intermediate-risk (including cytogenetically normal AML) or unfavorable karyotypes, but are absent in those with favorable karyotypes (i.e., CBF leukemias including *RUNX1-RUNX1T1* rearranged AML, and APL) and in patients with balanced translocations involving 11q23 (*KMT2A; MLL*) (Tang et al. 2009; Gaidzik et al. 2011; The Cancer Genome Atlas Research Network 2013). *RUNX1* mutations are particularly common in patients with isolated trisomy 13, a rare cytogenetic subgroup with a *RUNX1* mutation frequency of 75–90% (Dicker et al. 2007; Schnittger et al. 2011; Herold et al. 2014). In several series, an association of *RUNX1* mutations with trisomy 8 was noted (Tang et al. 2009; Gaidzik et al. 2011; Alpermann et al. 2015). Accordingly, in two studies of adult AML patients with sole trisomy 8, *RUNX1* mutations were the most or second most common molecular alterations, occurring in 28% and 32% of patients, respectively (Becker et al. 2014; Alpermann et al. 2015). Finally, two studies reported an association between somatically acquired trisomy 21 and *RUNX1* mutations in AML (Preudhomme et al. 2000; Taketani et al. 2003). In one of these studies, the mutated *RUNX1* allele was present on two of the three copies of chromosome 21 in all 4 patients analyzed (Preudhomme et al. 2000). This indicates that trisomy 21 occurred as a secondary change

after the *RUNX1* mutation, possibly acting by increasing the mutant-to-wild type allelic ratio.

With regard to coexisting molecular genetic alterations, *RUNX1* mutations were shown to be almost mutually exclusive with *NPM1* and *CEBPA* mutations in multiple AML cohorts (Tang et al. 2009; Schnittger et al. 2011; Gaidzik et al. 2011; Mendler et al. 2012; Greif et al. 2012; The Cancer Genome Atlas Research Network 2013). On the other hand, a positive association was observed between mutated *RUNX1* and presence of *KMT2A* (*MLL*) partial tandem duplications (*KMT2A*-PTD) (Tang et al. 2009; Schnittger et al. 2011; Gaidzik et al. 2011; Greif et al. 2012). *RUNX1* has subsequently been shown to interact directly with *KMT2A*. This interaction mediates histone H3K4 tri-methylation in the promoter region of the *SPI1* (PU.1) transcription factor that is involved in hematopoietic stem cell maintenance (Koh et al. 2013). Several groups also found a close association of mutations in *ASXL1* and *RUNX1*, with 22–44% of *RUNX1*-mutated patients carrying *ASXL1* mutations (Mendler et al. 2012; Schnittger et al. 2013; Paschka et al. 2015). Moreover, an association of *RUNX1* mutations with mutated *IDH2* was identified in two large studies (Gaidzik et al. 2011; The Cancer Genome Atlas Research Network 2013). While these associations suggest functional synergism between the *RUNX1* and frequently co-mutated genes, this has not yet been proven experimentally.

12.5.4 *RUNX1* Mutations and Prognosis in AML

Several relatively large patient cohorts provide information on the prognostic relevance of *RUNX1* gene mutations. Tang and colleagues identified *RUNX1* mutations in 13% of 470 patients with non-M3 AML (Tang et al. 2009). In the 330 patients who received standard induction and consolidation chemotherapy, multivariate analyses showed that *RUNX1* mutations associated with lower CR rates and shorter OS, but not with differences in DFS. In another cohort of 449 patients with normal or non-complex abnormal karyotypes who received non-uniform treatment, mutated *RUNX1* associated with inferior EFS and OS (Schnittger et al. 2011).

These two reports both showed an unfavorable impact of mutated *RUNX1* in subgroup analyses of patients with cytogenetically normal AML (CN-AML), and several studies focused on this cytogenetic subset. Mendler and colleagues identified *RUNX1* mutations in 12.5% of *de novo* CN-AML patients, and found an association with lower CR rates and inferior RFS, EFS and OS (Mendler et al. 2012). Similar results were obtained in multivariate analyses, and in subgroup analyses of younger (<60 years) and older (≥60 years) patients. Updated survival data are shown in Fig. 12.3. Of note, patients did not undergo alloSCT in first CR in this series. In another, smaller study focusing on CN-AML, an association between mutated *RUNX1* and inferior OS was observed in the entire cohort and in the subgroups of patients aged ≥60 years, and those within the European LeukemiaNet (ELN) Intermediate-I genetic group (Greif et al. 2012).

In the largest cohort published so far, Gaidzik and colleagues studied 945 patients aged 18–60 years treated on trials of the AMLSG. Only 5.6% of the patients carried *RUNX1* mutations, potentially due to the exclusion of older patients. *RUNX1* mutations associated with lower CR rate, and shorter RFS and OS. In multivariate analyses, *RUNX1* mutations were a significant predictor of shorter EFS and shorter RFS censored at the time of alloSCT, but not for CR rate, RFS without censoring, or OS (Gaidzik et al. 2011). In another relatively large cohort of 664 patients aged 18–86 years, *RUNX1* mutations associated with unfavorable OS only in patients younger than 60 years, and particularly in those with intermediate-risk cytogenetics (Metzeler et al. 2016).

At least two groups have addressed the impact of postremission therapy on outcomes of *RUNX1*-mutated patients. In the study by Tang and colleagues, *RUNX1* mutations were not associated with OS in the subset of patients who underwent alloSCT, suggesting that allografting might ameliorate the unfavorable prognostic impact of the mutation (Tang et al. 2009). In agreement with

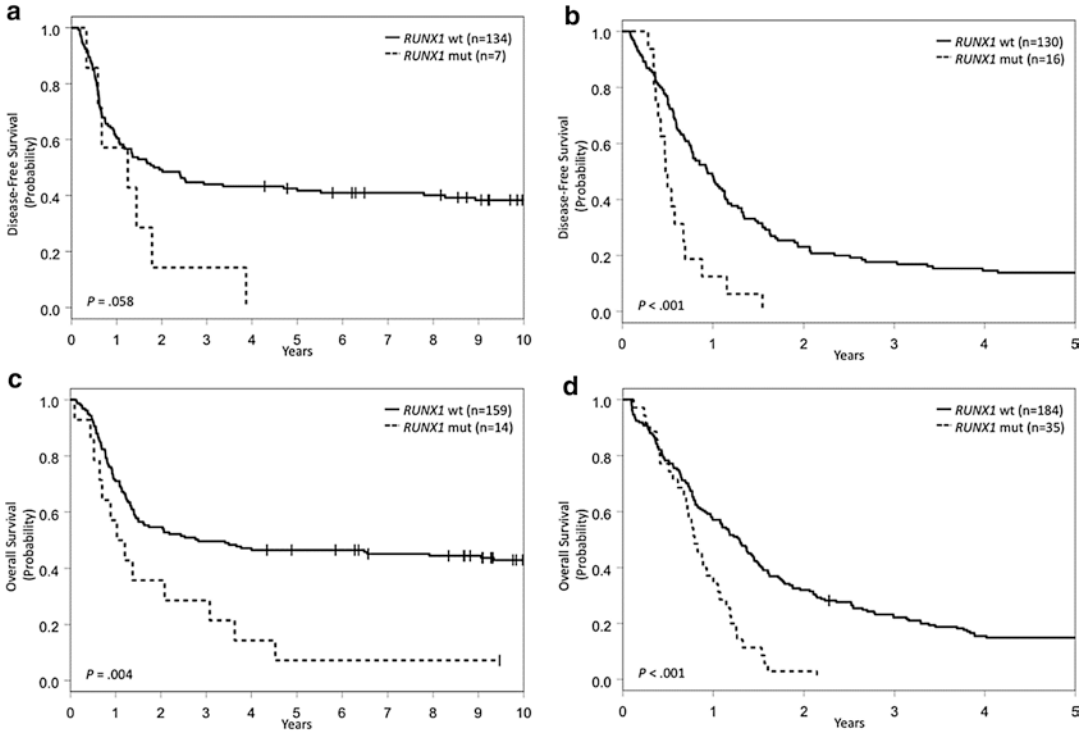

Fig. 12.3 *RUNX1* mutations and prognosis of patients with *de novo* cytogenetically normal AML. *Top*: Disease-free survival of patients with *de novo* cytogenetically normal AML (**a**) aged <60 years and (**b**) aged ≥60 years, according to *RUNX1* mutation status. *Bottom*: Overall survival of patients with *de novo* cytogenetically normal AML (**c**) aged <60 years and (**d**) aged ≥60 years, according to *RUNX1* mutation status (Bloomfield et al. unpublished data)

these results, Gaidzik et al. found that the RFS of *RUNX1* mutated patients who underwent alloSCT was comparable to *RUNX1*-wild type patients, while all patients who did not receive a transplant uniformly relapsed, most within 1 year (Gaidzik et al. 2011).

In summary, while univariate analyses consistently showed an unfavorable prognosis of *RUNX1*-mutated adult AML patients, the results of multivariate analyses adjusting for potential confounders are less clear. These discrepancies may be due to different baseline characteristics, for example regarding the age range of included patients, differences in treatment regimens including the use of alloSCT, and the effects of other genetic alterations that are considered in the multivariate models. With regard to the last point, several groups have recently tried to combine the prognostic information conveyed by various genetic alterations into integrative risk stratification algorithms. In two of these models, *RUNX1* mutations emerged as a factor associated with relatively unfavorable OS (Kihara et al. 2014; Grossmann et al. 2012), while in a third study, *RUNX1* mutations were found in only 5% of patients and were not included in the proposed risk stratification system (Patel et al. 2012).

Besides their prognostic relevance at baseline, *RUNX1* mutations could also serve as novel markers for MRD detection, yet the heterogeneity of the mutations make monitoring via conventional PCR assays difficult. This issue may be solved through the use of next-generation sequencing (NGS) techniques, as demonstrated in a cohort of 103 intensively treated, *RUNX1*-mutated patients with available follow-up samples (Kohlmann et al. 2014). Although the sensitivity of the NGS assay was relatively limited, residual disease was detected in 46 of the 103 patients at time points ranging from 60 to

198 days after initial diagnosis. Detectable *RUNX1* mutation associated with shorter EFS and OS. Notably, *RUNX1* mutations detected at the time of initial diagnosis were stable in relapsed disease in 51 of 57 evaluable patients (89%), while in 6 patients (11%), mutations were lost.

12.5.5 Reasons for the Different Outcomes of AML with Mutations and Balanced Translocations Involving *RUNX1*

Two major modes of *RUNX1* gene alteration, point mutations and balanced translocations leading to chimeric fusion genes, are found in AML. As outlined above, the clinical consequences of these two types of alterations are remarkably different. *RUNX1* point mutations generally associate with inferior outcomes and FAB M0 morphology, while the *RUNX1-RUNX1T1* gene fusion associates with favorable outcomes and a more differentiated (FAB M2) phenotype. The causes for these discrepant effects are not well understood. Among the potential factors that have been implicated are differences in the spectrum of co-mutated partner genes, differences in the residual *RUNX1* activity of the mutant allele, variable dominant-negative effects on the intact second allele, and effects of the translocation fusion partner in the case of balanced translocations (Osato et al. 2001).

12.5.6 *RUNX1* Point Mutations in Myelodysplastic Syndromes and Other Myeloid Malignancies

RUNX1 mutations in patients with MDS were first described in 2000 (Imai et al. 2000). Harada and colleagues subsequently found *RUNX1* mutations in 24% of MDS patients with elevated BM blasts (refractory anemia with excess blasts, RAEB) or post-MDS AML, while mutations were rarely observed in low-risk MDS without increased blast count (Harada et al. 2004). Of note, the frequency of *RUNX1* mutations was particularly high (50%) in a cohort of patients who developed MDS or AML after chemotherapy, radiotherapy or radiation exposure due to the atomic bombs used against Japan (Harada et al. 2003; Harada et al. 2004). A link between exposure to ionizing radiation and *RUNX1*-mutated myelodysplasia was confirmed by a study of radiation-exposed residents near a former Soviet nuclear test site (Zharlyganova et al. 2008). In three more recently published, larger series of MDS patients, *RUNX1* mutations were found in 8–11% of individuals (Bejar et al. 2011; Papaemmanuil et al. 2013; Haferlach et al. 2014). In two of these studies, analyses of recurrently mutated genes by targeted NGS revealed that mutated *RUNX1* frequently co-occur with mutations in *SRSF2*, *ASXL1*, *EZH2*, and *STAG2* (Papaemmanuil et al. 2013; Haferlach et al. 2014).

In MDS, *RUNX1* mutations are relatively rare in patients with low-risk disease and are found more frequently in patients with increased BM blasts (RAEB) and those transforming to post-MDS secondary AML (s-AML) (Papaemmanuil et al. 2013; Haferlach et al. 2014). In the light of this association, it is not surprising that mutated *RUNX1* associated with inferior leukemia-free survival in one cohort (Dicker et al. 2010). Moreover, *RUNX1* mutations associated with shorter OS even after adjustment for the International Prognostic Scoring System (IPSS) risk group and age in another series (Bejar et al. 2011). Finally, *RUNX1* mutations were included in a recently proposed 14-gene score that identifies MDS patients with shorter OS (Haferlach et al. 2014). In a study of 38 paired samples from MDS patients who later progressed to s-AML, 9 (24%) had *RUNX1* mutations already at the MDS stage, and only one *RUNX1*-wild type patient gained a mutation at the time of progression. Although the median time between MDS diagnosis and progression to s-AML was only 9 months, these data suggest that *RUNX1* mutations are a predisposing factor for s-AML transformation that is already present during the MDS phase, and not a marker that is acquired at the time of progression (Flach et al. 2011).

Mutations in *RUNX1* were also detected in 9–15% of chronic myelomonocytic leukemia (CMML), but do not seem to be prognostically relevant in this entity (Itzykson et al. 2013; Kohlmann et al. 2010). Finally, in a study of 70 patients with advanced, *KIT*-mutated systemic mastocytosis, *RUNX1* mutations were found in 23 % and associated with shorter OS (Jawhar et al. 2016).

12.6 Familial Platelet Disorder with Associated Myeloid Malignancy

Familial platelet disorder with associated myeloid malignancy (FPDMM; also known as familial platelet disorder with propensity to acute myelogenous leukemia, FPD/AML; Online Mendelian Inheritance in Man [OMIM] identifier, #601399) is an extremely rare, heritable condition caused by heterozygous germline *RUNX1* mutations. This syndrome was initially described in 1985 by Dowton and colleagues as an autosomal dominant disorder of platelet production and function in a large family with bleeding diathesis, and 6 members of the same family developed hematologic neoplasms (Dowton et al. 1985). Through linkage analysis, a critical region on chromosome 21 was identified in several affected families, and mutation analysis of regional candidate genes revealed mutations in *RUNX1* in six of the seven families (Ho et al. 1996; Song et al. 1999).

In the meantime, at least 20 affected kindreds have been described in the literature, and 19 of them were found to carry diverse types of *RUNX1* mutations including missense, frameshift and nonsense mutations as well as large deletions affecting the *RUNX1* locus (Preudhomme et al. 2009). Truncating changes lead to loss of the C-terminal transactivation domain resulting in haploinsufficiency of *RUNX1*. Missense mutations frequently affect conserved residues in the Runt domain that are involved in DNA binding, and may exert a dominant-negative effect on the remaining, intact allele through heterodimerization (Michaud et al. 2002). Patients with FPDMM typically present with mild thrombocytopenia, an "aspirin-like" platelet aggregation defect with abnormal response to epinephrine and arachidonic acid, a dense granule storage pool deficiency, and prolonged bleeding time. The lifetime incidence of leukemia among affected individuals is reported to be 20–50% (Osato 2004). In a series of asymptomatic individuals with germline *RUNX1* mutations aged <50 years, clonal hematopoiesis was detected in 67%, a proportion that is much higher than expected during normal aging (Churpek et al. 2015). Progression to AML is often accompanied by somatically acquired "second hits" (mutations or deletions) involving the second *RUNX1* allele, as well as gains of additional mutations in genes recurrently mutated in sporadic AML (Antony-Debré et al. 2016). Recently, somatic mutations in the *CDC25C* gene, which is not known to be mutated in sporadic AML, were reported in 7 of 13 FPDMM patients from Japan, including 4 of 7 patients who had developed AML. This finding was not reproduced in a European cohort (Yoshimi et al. 2014; Antony-Debré et al. 2016).

12.7 *RUNX1* Gene Alterations in Acute Lymphoblastic Leukemia

Although *RUNX1* translocations were first detected in AML, they are also found in acute leukemias of lymphoid lineage, particularly in childhood B-cell precursor acute lymphoblastic leukemia (BCP-ALL). The t(12;21)(p13;q22), leads to rearrangement of *RUNX1* with the *ETV6* gene. The resulting *ETV6-RUNX1* fusion transcript (previously designated *TEL-AML1*) is controlled by the *ETV6* promoter and contains the N-terminal "helix-loop-helix" (HLH) domains of *ETV6* fused to a large C-terminal part of the *RUNX1* coding sequence, including the Runt and transactivation domains. This is in contrast to *RUNX1* fusion genes found in AML, which are under the control of the *RUNX1* promoter and lack the *RUNX1* C-terminus including the transactivation domain (Golub et al. 1995; Romana et al. 1995a).

The t(12;21)(p13;q22) is commonly cryptic and missed by metaphase cytogenetics, and thus was initially considered to be a rare event.

However, using fluorescence-*in situ* hybridization, the *ETV6-RUNX1* rearrangement can be detected in approximately 15–35% of pediatric BCP-ALL, particularly in patients aged 1–9 years, while it is rare (<3%) in adult BCP-ALL (Romana et al. 1995b; Fears et al. 1996; Zelent et al. 2004). Thus, t(12;21)(p13;q22) is the most common structural chromosomal alteration in pediatric cancer. The translocation is accompanied by a deletion of the second *ETV6* allele on the other chromosome in >50% of patients, suggesting that loss of *ETV6* function plays a role in this disease (Raynaud et al. 1996; Schwab et al. 2013). Overall, *ETV6-RUNX1*-rearranged childhood BCP-ALL patients seem to harbor a relatively high number of copy number alterations including deletions of *CDKN2A*/B, *PAX5* and *BTG1*, each occurring in 15–20% of patients (Kim et al. 1996; Mullighan et al. 2007; Schwab et al. 2013). Furthermore, mutations in the histone H3K36 methyltransferase *NSD2* are found in 20% of *ETV6-RUNX1* rearranged childhood ALL (Jaffe et al. 2013). Several studies conclusively demonstrated that children with the *ETV6-RUNX1* rearrangement have excellent treatment outcomes (Shurtleff et al. 1995; Borkhardt et al. 1997; Moorman et al. 2010; Bhojwani et al. 2012), although in some series a high frequency of late relapses (≥5 years after diagnosis) was noted (Forestier et al. 2008). In one large study of 1725 children and adolescents with BCP-ALL, those with *ETV6-RUNX1* had ~50% reduced risk of relapse or death compared to other genetic subsets, with no late relapses. These associations persisted in multivariate analyses adjusting for other known risk factors (Moorman et al. 2010).

Studies of monozygotic twins and neonatal blood spots (Guthrie cards) revealed that the *RUNX1-ETV6* rearrangement is frequently acquired before birth, and BCP-ALL can develop in affected children with a reported latency of up to 14 years (Ford et al. 1998; Wiemels et al. 1999a, b). Notably, using highly sensitive assays, *RUNX1-ETV6* fusion transcripts can be found in up to 1% of cord blood samples from healthy newborns (Mori et al. 2002). In these children, the rearrangement is present in 1 of 10^3–10^4 mononuclear cells, indicating that the offspring of the single cell that initially acquired the translocation gained a proliferative advantage and underwent clonal expansion. On the other hand, the proportion of newborns with detectable *RUNX1-ETV6* transcripts in cord blood samples exceeds the incidence of *RUNX1-ETV6*-positive childhood BCP-ALL by a factor of 100, indicating that affected newborns have a low absolute risk of developing ALL, and that the acquisition of secondary genetic lesions is necessary for the development of overt leukemia. Although more rare, *RUNX1-RUNX1T1* fusion transcripts have also been detected in healthy newborns (Mori et al. 2002). Quantitative PCR assays have been developed for the detection of MRD in children with t(12;21) (Pallisgaard et al. 1999; Seeger et al. 2001; Drunat et al. 2001).

Besides the t(12;21), *RUNX1* point mutations have been found in sporadic childhood ALL (Song et al. 1999) and affect about 15% of children with early T-cell precursor (ETP)-ALL (Zhang et al. 2012). *RUNX1* mutations also occur in T-ALL developing in patients with FPDMM (Owen et al. 2008; Preudhomme et al. 2009; Prébet et al. 2013).

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