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REVIEW ARTICLE

Mutations of the *TET2* and *CBL* genes: novel molecular markers in myeloid malignancies

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Abstract Despite recent progress in molecular research in myeloid malignancies, in subsets of patients with myelodysplastic syndrome (MDS) so far no underlying mutation was identified. In the myeloproliferative neoplasms (MPNs), the *JAK2*V617F alone cannot explain the phenotypic heterogeneity. In acute myeloid leukemia (AML), clinical variability exists within distinct subgroups. Thus, the search for novel molecular markers continues. Recently, mutations of the tet oncogene family member 2 (*TET2*) and Casitas B-cell lymphoma (*CBL*) genes became the focus of interest. With diverse genetic methods, *TET2* on chromosome 4q24 was identified as candidate tumor suppressor gene. Sequencing studies revealed heterogeneous mutations in 10–25% of patients with acute myeloid leukemia (AML), MDS, and MPNs, while the frequency might be higher in chronic myelomonocytic leukemia (CMML). The prognostic impact is being explored. The *CBL* gene is involved in the degradation of tyrosine kinases. In rare cases of human AML (<2%), *CBL* mutants were identified, with a higher frequency in core binding factor leukemias. Presence of these mutations was suggested to be involved in aberrant *FLT3* expression. In the MPNs, a 2–8% frequency of *CBL* mutations was reported. These novel mutations deepened insights in the mechanisms of leukemogenesis, might contribute to the identification of new therapeutic targets, and improve diagnostics in the myeloid malignancies.

Keywords *TET2* mutation · *CBL* mutation · Acute myeloid leukemia (AML) · Myelodysplastic syndrome (MDS) · Myeloproliferative neoplasms (MPNs)

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Introduction

In recent years, an increasing pattern of recurrent molecular alterations has evolved in acute myeloid leukemia (AML). Examples are mutations of the nucleophosmin (*NPM1*) gene being described by Falini et al. [1] or of the CCAAT/enhancer-binding protein alpha (*CEPBA*) gene which has a coding function for a critical myeloid transcription factor [2]. These prognostically favorable genetic alterations and their association with normal karyotype AML were recognized as provisional entities by the new World Health Organization (WHO) classification in 2008 [3]. Considering other recurrent mutations, e.g. the *FLT3*-ITD/TKD (internal tandem duplications/tyrosine kinase domain mutations of the *FLT3* gene), *MLL*-PTD (partial tandem duplications of the mixed lineage leukemia gene), or *RUNX1* (runt-related transcription factor 1), molecular alterations can by now be identified in >80% of patients with normal karyotype AML [4]. In 55% of patients, cytogenetic analysis reveals aberrant karyotypes with a strong prognostic power [5–7]. Thus, a biological and clinical relevant subcategorization based on the cytogenetic and molecular genetic features is possible in most AML cases. However, as the clinical outcomes are highly variable even within distinct genetic subgroups, additional mechanisms are assumed to play a role in pathogenesis.

Overexpression of receptor tyrosine kinases due to mutations [8] or autocrine activation at the mRNA and protein levels is a frequent phenomenon are in AML. *FLT3* has been demonstrated to be overexpressed in patients with evidence of a *FLT3* mutation as well as in patients with *FLT3* wild type [9]. The potential functions of *FLT3* in AML in wild type cases remain to be clarified.

In myelodysplastic syndromes (MDS), molecular alterations were identified in smaller subsets of patients, such as *RUNX1*/*AML1* mutations in 15–20% of therapy-associated MDS (t-MDS) cases [10] or mutations of the *FLT3* gene in single advanced MDS cases [11]. Mutations of the *RAS* (family of retrovirus-associated DNA sequences) oncogenes were described in 5% of t-MDS cases [12] and in 12% of *RUNX1*-mutated advanced MDS cases [13]. Cytogenetic alterations exist in >50% of all MDS cases [7, 14]. Similar to AML, the karyotypes have strong prognostic power, but variances of the clinical profiles are seen as well within distinct cytogenetic subgroups. Further, in those ~50% of MDS patients with absence of cytogenetic abnormalities, the pathogenetic events causing the malignant conditions need further intensive investigation.

With respect to the myeloproliferative neoplasms (MPNs), the *JAK2*V617F activating mutation has been identified in >95% of patients with polycythemia vera (PV), but ~45% of patients with primary myelofibrosis (PMF) or essential thrombocytosis (ET) show no evidence of this molecular marker [15, 16]. Other activating mutations, e.g., of the *MPL* gene or exon 12 of *JAK2* are limited to small percentages of patients with *JAK2*V617F unmutated MPNs [17, 18].

Thus, research focuses on the identification of novel leukemogenic markers in patients with AML, MDS, or MPNs. Recently, two novel molecular mutations—involving the *TET2* [19–21] and Casitas B-cell lymphoma (*CBL*) [22, 23] genes—became focus of interest in these myeloid malignancies.

Identification of mutations in the *TET2* gene

The tet oncogene family member 2 (*TET2*) gene is localized on chromosome 4q24. It spreads over 11 exons and contains 150 kb. The 4q24 breakpoint has been demonstrated to be involved in other AML associated translocations, e.g. the t (3;4)(q26;q24) [24] or the t(10;11)(q22;q23) involving the *TET1* ("Ten-Eleven-Translocation", tet oncogene 1) gene [25]. With a combination of diverse methods, including cytogenetics, comparative genomic hybridization (CGH), and SNP (single nucleotide polymorphism) array analyses, Delhommeau et al. identified *TET2* as candidate tumor suppressor gene being relevant for AML, MDS, and MPNs. Sequencing studies in 320 patients with different myeloid disorders revealed *TET2* mutations in 19% of patients with MDS, 24% with secondary AML (s-AML), 22% with chronic myelomonocytic leukemia (CMML), and 12% with MPNs [19]. Similarly, Langemeijer et al. identified recurring deletions and copy-neutral loss of heterozygosity (LOH) involving 4q in MDS patients based on SNP microarray analyses. Sequencing analysis in 102 MDS patients revealed mutations of the *TET2* gene in 26% of cases [20]. Subsequent studies confirmed the presence of the *TET2* mutations in diverse myeloid disorders [26–28]. Thus, it became evident that this novel molecular marker is highly relevant for a variety of myeloid disorders.

Pattern of the *TET2* mutations

Thus far, observed *TET2* mutations are extremely heterogeneous: nonsense mutations, out-of-frame insertions, deletions, and splice site mutations have been described [24, 27, 29]. The mutations are spread over several exons (Fig. 1), mostly involving the largest exons 3 and 11. In most cases, they result in frameshift or stop codon alterations [30]. Such changes usually result in truncated translation, and therefore, inadequate production of a potential tumor suppressor protein [21, 30].

In the study of Delhommeau et al. analyzing patients with different myeloid disorders, both alleles were affected by two different *TET2* mutations in 25 of 55 mutation carriers (45%). Coexistence of two different *TET2* mutations each affecting a different allele of the same clone has also been reported by Saint-Martin et al. in patients with MPNs [29]. Thus, there seems no doubt that the *TET2* mutations can occur as hemizygous or compound heterozygous alterations [31].

Saint-Martin et al. retrospectively tracked the *TET2* and *JAK2*V617F mutation loads in a patient with MPN and demonstrated increase of the mutation burden concomitantly with the development of the disease [29]. Similarly, Delhommeau et al. performed analysis of the $CD34^+$ stem cells in one patient with MDS at the RAEB-1 (refractory anemia with excess blasts) and after progression to RAEB-2. In the RAEB-1 stage, *TET2* wild type and mutated alleles were found in parallel. Following the transformation to RAEB-2, only mutated cells were detected [19]. Therefore, the progression of MDS may be accompanied by increase of the *TET2* mutated cell population load. Furthermore, the mutations were shown to be present already in $CD34^+$ hematopoietic stem cells. Delhommeau et al. demonstrated that the proportion of *TET2* mutated cells was higher in the more mature progenitor cells ($CD34^+$ $CD38^+$) from MDS patients when compared to the very immature ($CD34^+$ $CD38^-$) cells [19]. Further, in samples from patients with myeloproliferative disorders who had both *TET2* and *JAK2* mutations, *TET2* mutations occurred first

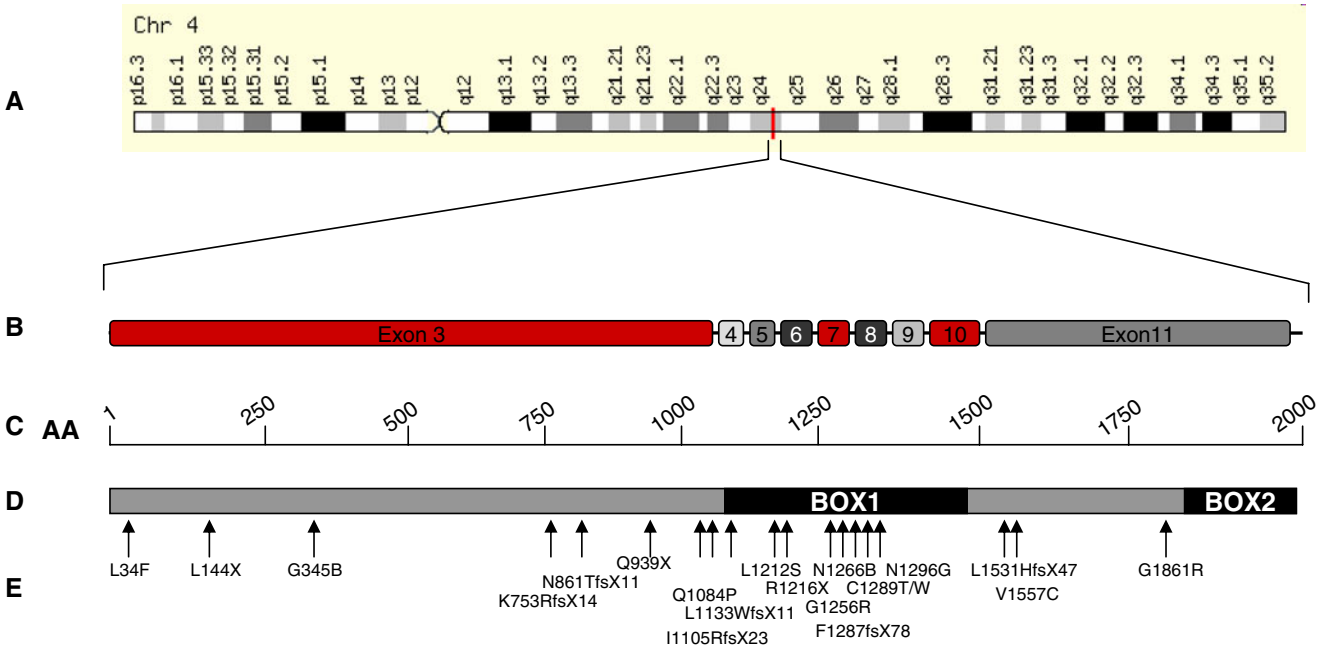

Fig. 1 **a** Localization of the *TET2* gene on chromosome 4q24. **b** Exon structure of *TET2*. **c** Amino acid (AA) positions. **d** Position of the conserved structures BOX1 and BOX2. **e** Distribution of mutations (own unpublished results). Mutations can be distributed across all 11 exons with some clustering in and around BOX1

in the course of the disease. Therefore, in the study of Delhommeau et al., the *TET2* defects seemed to precede the *JAK2* mutations during the evolution of the disease [19]. To examine the order of events, Schaub et al. genotyped *TET2* and *JAK2* in individual colonies in samples from eight patients with MPNs who were all carriers of both a *TET2* mutation and *JAK2* mutation in parallel. They found that colonies with mutated *TET2* could either carry *JAK2* wild type whereas others were *JAK2*V617F positive, indicating that the *TET2* mutation occurred before the *JAK2*V617F. However, in two other patients data were compatible with the opposite order of events (the *JAK2* mutation occurring before the *TET2* alteration). Finally, in two patients, the *TET2* and *JAK2*V617F mutations defined two separate clones. Thus, there was no strict temporal order of occurrence of these genetic events [32]. Abdel-Wahab et al. showed that the transformation of MPNs to s-AML was accompanied by acquisition of a *TET2* mutation in 6/14 cases (43%) when paired samples from different time points were analyzed [33]. This was giving further confirmation to the idea that the *TET2* mutations can as well be later events in myeloproliferative disorders.

TET2 mutations in MDS and AML

Based on SNP microarray profiling and genomic sequencing studies, Langemeijer et al. identified mutations of *TET2* in 26% of 102 MDS patients [20], Kosmider et al. in 23% of 96 MDS patients [27]. Therefore, *TET2* mutations seem to account for one of the most frequent molecular markers in MDS and might even exceed the frequency of the *RUNX1* mutations which can be identified in up to 24% of MDS patients [34]. Whether the presence of the *TET2* mutations is clinically relevant in MDS, has to be further determined. Preliminary results show that they may be prognostically favorable in MDS [27].

In s-AML following MDS, Tefferi et al. found the mutation in three of seven patients with s-AML [21], and Delhommeau et al. reported a mutation rate of 24% in 21 s-AML patients [19]. In de novo AML, Tefferi et al. found one mutated patient out of five cases analyzed. This patient had acute promyelocytic leukemia with a t(15;17)/*PML-RARA* rearrangement (fusion of the promyelocytic leukemia and retinoic acid receptor-alpha genes). Abdel-Wahab et al. found a mutation rate of 12% of *TET2* mutations in 91 patients with AML and described a significant negative impact on prognosis [26].

In CMML, a 15–22% frequency of *TET2* mutations was described by Tefferi et al. and Delhommeau et al. [19, 21]. Kosmider et al. found an even higher mutation rate of 50%, having identified the mutations in 44 of 88 patients with the disease [28] (Table 1). These data suggested that *TET2* mutations are especially frequent in this myeloid entity and allowed to speculate on a specific association of the *TET2* gene with regulation of the monocytic lineage. Furthermore, Kosmider et al. described a significant adverse effect of the

Table 1 Frequency of *TET2* mutations in different myeloid entities

Reference	Total cases	*N* mutated (%)
De novo AML		
Langemeijer et al. [20]	32	6 (19%)
Tefferi et al. [21]	5	1 (20%)
Abdel-Wahab et al. [26]	91	11 (12%)
s-AML		
Tefferi et al. [21]	7	3 (43%)
Delhommeau et al. [19]	21	5 (24%)
Couronne et al. [57]	19 (s-AML post MPN)	6 (32%)
MDS		
Kosmider et al. [27]	96	22 (23%)
Delhommeau et al. [19]	81	15 (19%)
Langemeijer et al. [20]	102	27 (26%)
CMML		
Tefferi et al. [21]	15	3 (20%)
Jankowska et al. [31]	17	6 (35%)
Delhommeau et al. [19]	9	2 (22%)
Kosmider et al. [28]	88	44 (50%)
Abdel-Wahab et al. [26]	69	29 (42%)
Kohlmann et al. [46]	81	41 (51%)
MPNs		
Delhommeau et al. [19]	198	24 (12%)
Saint-Martin et al. [29]	61 (familial)	12 (20%)
Tefferi et al. [30]	239	32 (13%)
Abdel-Wahab et al. [26]	354	27 (8%)
SM		
Tefferi et al. [35]	42	12 (29%)

AML acute myeloid leukemia, *s-AML* secondary AML, *MDS* myelodysplastic syndrome, *CMML* chronic myelomonocytic leukemia, *MPNs* myeloproliferative neoplasms, *SM* systemic mastocytosis

TET2 mutations on survival in CMML-1 patients (according to the WHO classification) [3, 28].

TET2 mutations in myeloproliferative neoplasms

Tefferi et al. evaluated 239 patients with different *BCR-ABL1* negative MPNs such as PV, ET, PMF, post-polycythemic myelofibrosis (PMF), or blast phase of MPN by high-throughput DNA sequence analysis and found an overall *TET2* mutation rate of ~13%. The frequency of *TET2* mutations was significantly higher in patients ≥60 years with 23% of all cases when compared to only 4% in younger patients. Mutation rates did not differ significantly between the different MPN entities or stages. The mutations showed occurrence in *JAK2*V617F positive as in wild type cases and had no significant influence on clinical outcomes [30].

Saint-Martin et al. performed investigation in 61 patients with familial MPNs. They detected *TET2* mutations in 7.7% of patients without hematological complications, which was significantly lower than the mutation rate in those with complications (29%). Patients with PMF, post-polycythemic myelofibrosis, or s-AML showed mutations in 10/12 cases (83%), which suggested a trend to more advanced stages in mutation carriers. Distribution and types of the *TET2* mutations did not differ from sporadic MPN cases. In this study, there was no trend to higher age in the *TET2* mutated patients [29].

Investigating 42 patients with systemic mastocytosis (SM), Tefferi et al. found a 29% *TET2* mutation rate. *KIT*D816V mutations were detected in patients with or without *TET2* mutations [35]. Presence of *TET2* mutations did not affect survival in the SM patients, and the mutation occurred in both indolent and aggressive cases. In addition, the authors investigated six patients with chronic eosinophilic leukemia (CEL) and evidence of a *FIP1L1-PDGFRA* rearrangement (fusion of the homologue of FIP1 like 1 (S. cerevisiae) and the platelet-derived growth factor receptor, alpha polypeptide genes), but found no coincidental *TET2* mutation.

Identification of mutations of the *CBL* gene

The Casitas B-cell lymphoma (*CBL*) gene on chromosome 11q23.3 contains several functional domains. One of these domains, the C-terminal domain, gives rise to the Cbl protein which has ubiquitin ligase activity that targets a variety of tyrosine kinases for degradation by ubiquitination, i.e., meaning the process of attaching ubiquitin monomers to a protein. Cbl proteins further associate with the endocytic machinery and thus are important for the termination of signaling of receptor tyrosine kinases. Three different homologs in mammalians are known— *c-CBL*, *CBL-b*, and *CBL-c* which differ in lengths of the C terminal domains. *CBL* oncogenes were initially identified in the murine system. The *CBL*-70Z mutation which carries an internal deletion of 167 amino acids was isolated from the 70Z/3 mouse pre-B-cell lymphoma cell line [36]. In human leukemia, the *CBL* gene was reported to be involved in recurrent translocations affecting, e.g., the mixed lineage leukemia (*MLL*) gene in AML [37–39].

Sargin et al. reported the first human *CBL* mutation in a patient with AML—one *c-CBL* point mutation (*CBL*-R420Q) in exon 9 following analysis of 150 patients with AML [23]. The occurrence of *c-CBL* mutations in human leukemia was confirmed by Caligiuri et al. who identified *c-CBL* mutations in 4 of 12 AML patients and demonstrated evidence of a *c-CBL* splice site mutation

with an 18 base pair deletion in the AML cell line MOLM-13 [40].

In vitro experiments confirmed constitutive activation of the FLT3 pathway by the *CBL* mutants, and the phenotype of the altered cells resembled the one of *FLT3* mutated receptor tyrosine kinases [39]. The *CBL* mutations were able to inhibit Flt3 internalization and ubiquitination in cell line experiments as shown by Sargin et al. The mutant Cbl proteins altered the amplitude and duration of FLT3 depending signaling events [23].

Structure of the *CBL* mutations

Mutations of the *c-CBL* gene are localized in the domain which functions as linker between the tyrosine kinase binding domain (TKB) and the RING finger domain (Fig. 2). This linker sequence is essential for the interaction between both domains and guarantees the negative regulatory function of *CBL* [40]. Disruption of this linker sequence renders *CBL* unable to degrade receptor tyrosine kinases and increases proliferation and survival signaling.

Diverse mutation subtypes exist. The R420Q missense (point) mutation was detected in patients with AML, MDS/MPN, and CMML [22, 23, 39]. Other missense mutations were reported to affect cysteine residues of the RING finger domain [22], but also many other residues in exons 8 and 9 can be affected [41]. Caligiuri et al. described a mutation affecting proper splicing of exon 8 [40]. Abbas et al. detected two cases expressing *CBL* mRNA splice variants which lacked exon 8. This mutation subtype was similar to the alteration which had been reported in the MOLM cell line by Caligiuri et al. [42].

The mutations additionally can affect the related *CBL-b* gene as shown by Caligiuri et al. who identified a *CBL-b* missense mutation due to change of a glutamate to a glycine residue [40].

CBL mutations and uniparental disomy

Somatic uniparental disomy (UPD) either results from mitotic recombination or as an attempt to correct loss of chromosomal material and can be detected more easily now using SNP microarray technology. Frequently, areas with acquired UPD harbor genes which are involved in the pathogenesis. In a cohort of 301 patients with different myeloid disorders, Dunbar et al. described the most frequent loss of heterozygosity (LOH) at 11q in 12 of 301 patients (4.0%), 6 of which had MDS/MPN, CMML, or

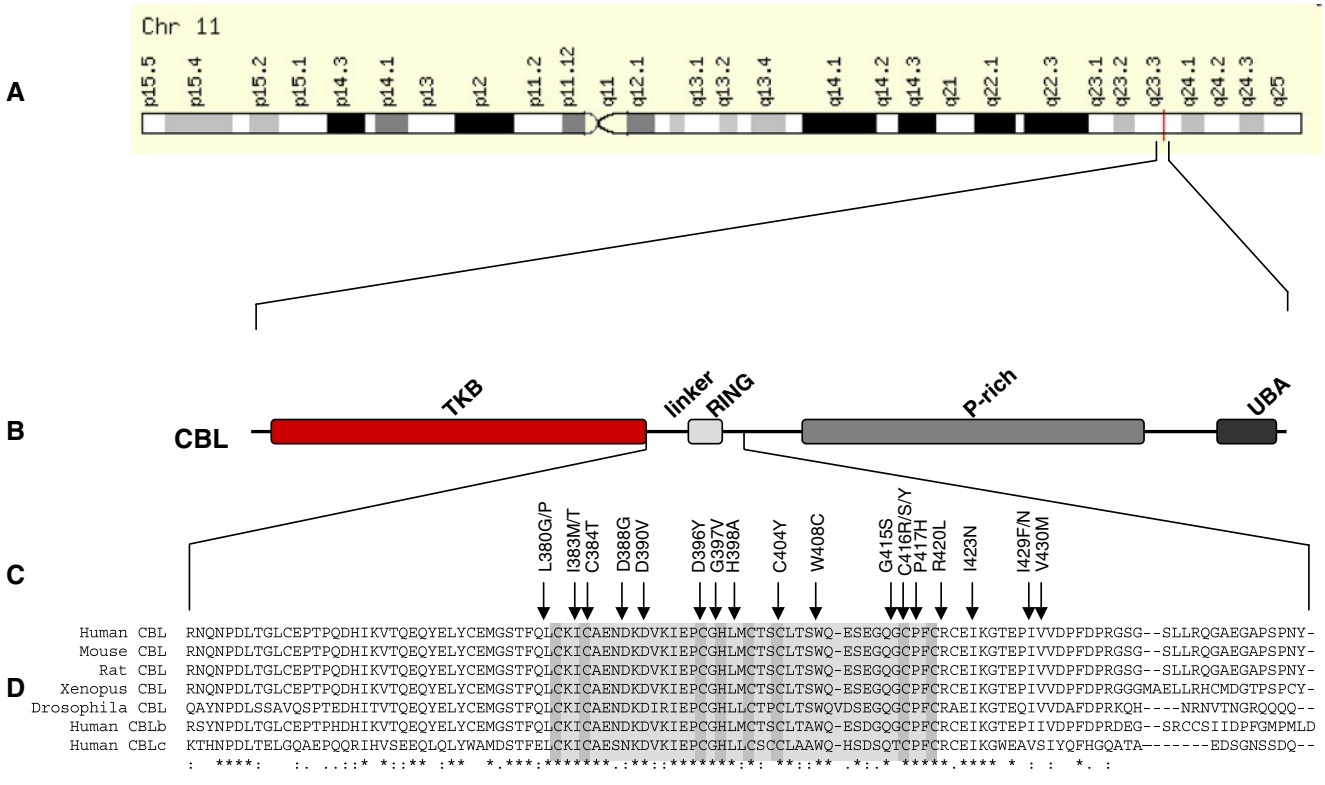

Fig. 2 **a** Localization of the *CBL* gene on chromosome 11q23.3. **b** Schematic presentation of functional domains. **c** Mutations in the RING and LINKER domain (own unpublished data). **d** Presentation of conserved domains in different species. All mutations were localized in the conserved RING and LINKER domain. *TKB* tyrosine kinase binding, *P-rich* proline rich, *UBA* ubiquitin-associated/leucine zipper

related disorders [22]. All patients harbored UPD11q in the region of the *c-CBL* gene. Mutations of the *c-CBL* gene were identified in 7 of those 12 patients with UPD in the chromosome 11q region. This demonstrated the close association of UPD11q and *c-CBL* mutations. A SNP microarray study by Grand et al. in 58 patients with *JAK2*V617F negative MPNs demonstrated evidence of *c-CBL* mutations in all three cases with acquired UPD11q. Analysis of 574 additional MPNs including myelofibrosis and CMML revealed a total of 27 *c-CBL* mutations in 26 patients which was corresponding to a 4.5% mutation rate [41]. A significant association of UPD11q and the *c-CBL* mutations was further observed in a large study including 222 patients with different myeloid disorders with myeloproliferative features (e.g., MDS/MPN overlap, and CMML) being performed by Sanada et al. There was a high rate of c-*CBL* mutations in cases with UPD11q (15/17; 88%), while only 3 out of 205 cases (1.5%) without UPD11q had *c-CBL* mutations ($p<0.001$) [43].

Table 2 Frequency of *c-CBL* mutations in different myeloid malignancies

Reference	Total cases	*N* mutated (%)
AML		
Sargin et al. [23]	150	1 (0.7%)
Abbas et al. [42]	319	2 (0.6%)
Reindl et al. [39]	279	3 (1.1%)
Chronic myeloid disorders (MPNs, MDS/MPN, CMML)		
Sanada et al. [43]	222	18 (8.1%)
Grand et al. [41]	574	26 (4.5%)
Dunbar et al. [22]	301	7 (2.3%)
Kohlmann et al. [46]	81	20 (24.7%)
Makishima et al. [45]	38 (CMML)	2 (5.3%)
JMML		
Loh et al. [48]	159	27 (17%)
Muramatsu et al. [47]	49	5 (10.2%)

AML acute myeloid leukemia, *MDS/MPN* myelodysplastic syndrome/myeloproliferative neoplasms overlap category, *CMML* chronic myelomonocytic leukemia, *JMML* juvenile myelomonocytic leukemia

CBL mutations in AML

In the analysis from Sargin et al., the *CBL*-R420Q mutation was detected in 1 of 150 patients with AML (0.7%) [23]. In the study from Abbas et al. in AML patients, there was a similar low mutation rate of 0.6% (2/319). As both identified cases with a *c-CBL* mutation carried an inv(16)/*CBFB-MYH11* (fusion of the core-binding factor beta subunit and myosin heavy chain 11 smooth muscle genes), the authors evaluated another 79 patients with core binding factor (CBF) leukemias. In this new cohort, they found three additional *c-CBL* mutated cases, 2/40 patients with a t(8;21) (5%) and 1/39 (3%) with an inv(16) [42]. Reindl et al. identified *c-CBL* exon 8/9 deletion mutants in 1.1% of 279 patients with AML/MDS. All patients with *CBL* mutants belonged to the CBF and 11q deletion subtypes [39]. In a series of 37 patients with newly diagnosed inv (16) AML, we detected a frequency of 16% of *CBL* splicing mutations [44]. These reports give further confirmation to the assumption of a specific association between *c-CBL* mutations and CBF leukemias.

CBL mutations in other myeloid malignancies

Dunbar et al. identified UPD11q in 12/301 patients with different chronic myeloid disorders (MDS/MPN and CMML). Of these 12 patients, 7 were found to carry *c-CBL* mutations. This resulted in a 2.3% frequency of *c-CBL* mutations in these chronic myeloid malignancies [22]. Sanada et al. identified *c-CBL* mutations in 18/222 patients with MDS/MPN and CMML [43], which accounted for an 8.1% frequency, and Grand et al. revealed 26 mutated cases in 574 patients with diverse MPNs, including myelofibrosis and CMML [41]. This resulted in a 4.5% mutation rate. Makishima et al. detected *CBL* mutants in 2 of 38 CMML cases (5.3%) [45]. In our own series of 81 patients with CMML, we detected *CBL* mutations in a frequency of 24.7% performing next generation sequencing [46]. Therefore, although studies in this entity are limited so far, it seems that the *c-CBL* mutations might have higher frequencies in chronic myeloid malignancies when compared to AML (Table 2).

Attention was focused as well on juvenile myelomonocytic leukemia (JMML); Muramatsu et al. investigated the frequency of *CBL* mutations in a series of 49 children with this disorder. In a high proportion of patients, they detected presence of *NF1* (neurofibromatosis type 1), *PTPN11* (protein tyrosine phosphatase, nonreceptor type 11), *NRAS* (neuroblastoma RAS viral (v-ras) oncogene homolog), and *KRAS* (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) mutations, but in 37% of all patients, they were unable to identify any of the known genetic lesions. With SNP arrays, they identified somatic UPD of 11q in 4/49 patients who all were found to harbor c-*CBL* mutations. Subsequent direct genomic sequencing studies identified a frequency of 5/49 *c-CBL* mutants in this cohort (10.2%) [47]. Similarly, Loh et al. identified *c-CBL* mutations in 17% of 159 patients with JMML performing SNP microarray and sequencing studies. Most of these mutations involved the codon Y371. Interestingly, there was no coincidence with the finding of *RAS*/*PTPN11* mutations, which are also known to be frequent in JMML. Both

mutation types seemed to be mutually exclusive. Therefore, it can be assumed that the components of the *RAS* signaling network and *CBL* mutations do not coexist [48]. In addition, this study suggested that *c-CBL* mutations have a higher frequency in JMML when compared to other so far investigated myeloid entities.

Conclusion and perspectives

The recent detection of mutations both of the *TET2* [19, 20] and *CBL* [23, 40] genes in diverse myeloid disorders has shed new light on the genetic complexity of these malignancies. The *TET2* mutations were detected in approximately 15% of patients with diverse acute and chronic myeloid malignancies [19, 49], ranging from de novo and secondary AML to MDS and CMML/JMML and were also detected in various MPNs. Even in SM, a considerable proportion of patients was shown to carry the mutation [35]. Especially in CMML, the mutation was observed with high frequency according to a report from Kosmider et al. who revealed a 50% mutation rate [28].

In the MPNs, the occurrence of the *TET2* mutations in the *JAK2*V617F-negative as well as in the *JAK2*-mutated clones from the same individual suggests that the mutation is an early event during leukemogenesis [19, 50]. Also, the mutation had been shown to exist on the hematopoietic stem cell level. The *TET2* mutations were demonstrated to precede the *JAK2* mutations in MPN patients in one study [19], while others observed occurrence before or after the *JAK2*V617F mutation [29]. The occurrence of *TET2* mutations thus underlines the genetic complexity of the MPNs [35]. Observations that the mutation coexists with the *JAK2*V617F (in MPNs) or alterations of *KIT* (in SM) but does not occur combined with *FIP1L1-PDGRFA* (in CEL), suggests that there are specific pathways of interaction [35].

Many questions remain open with respect to this novel molecular aberration. First, the function of the *TET2* gene still has to be clearly determined [49]. There is no doubt regarding the association of the respective mutation with loss of heterozygosity (LOH) of chromosome 4q [51]. Considering that most *TET2* mutations are predicted to truncate the proteins, the mutation could result in partial or total loss of function of the *TET2* proteins [19], which were suggested to have a role in epigenetic regulation [50, 52]. Also, a tumor suppressor function of the *TET2* gene is being discussed [19]. The broad range of myeloid disorders linked to the *TET2* mutations suggests a pleiotropic role in myeloid transformation, indicating a genetic link between these apparently disparate myeloid diseases [49] and an interaction with other genetic or epigenetic cofactors [21]. Recently, mutations of the additional sex comb-like 1 gene (*ASXL1*) were described in 17.5% of selected patients with AML and were as well suggested to interfere with a hypothesized tumor suppressor function of the respective gene [53].

Secondly, the clinical impact of the *TET2* mutations still has to be ascertained for the respective malignant entities. Thus far, published studies suggested that in different entities, occurrence of the mutation might have different influences regarding its clinical importance [26–28]. However, it is too early for definite conclusions with respect to an influence of the *TET2* mutations on future therapeutic strategies in AML or chronic myeloid malignancies. Considering the diagnostic uncertainty which frequently remains in cases being suspicious for MDS or *JAK2*V617F negative MPN, a potential diagnostic utility of this molecular marker should be further explored. Finally, it remains to be clarified whether the *TET2* gene has a role in neoplasms other than myeloid malignancies [26].

Regarding mutations of the *CBL* gene, low frequencies of *c-CBL* and *CBL-b* mutations were described in human AML [23, 40]. Previously, it has been demonstrated that *FLT3* overexpression in AML is not necessarily associated with presence of a *FLT3* mutation [9]. Recently, cell line experiments have clearly shown the inhibition of Flt3 internalization and ubiquitination by *CBL* mutations [23]. Therefore, mutations in the *CBL* gene seem to represent a novel mechanism to explain overexpression of *FLT3* in a subgroup of AML patients, and it was hypothesized that *CBL* mutant AML patients might benefit from FLT3 and PTK inhibitor treatment [39]. Although the number of studies is still very limited, it seems that the *c-CBL* mutations can as well be found in the MPNs and in CMML with even slightly higher frequency when compared to AML [41].

It further can be speculated that *CBL* mutants interfere not only with *FLT3,* but also with other receptor tyrosine kinases: retroviral transduction of primary murine bone marrow with *c-CBL* mutants and transplantation into mice was shown to lead to generalized mastocytosis, myeloproliferative disease, and in rare cases to AML in an experimental study from Bandi et al. [54]. Overexpression of the *CBL* mutants inhibited ubiquitination and internalization of the activated KIT receptor. This suggests that mutations of *c-CBL* inhibit its negative regulatory function for *KIT* [54].

Specific mechanisms of interaction were suggested for the *c-CBL* mutations. In AML, Abbas et al. and Reindl et al. both demonstrated an association of the *c-CBL* mutations with the CBF leukemias [39, 42]. In contrast, in JMML, Loh et al. described mutual exclusiveness of the *RAS* and *CBL* mutations [48]. Definite conclusions regarding the potential use of *c-CBL* mutations for the diagnostic work-up of myeloid malignancies cannot yet be drawn. However, they might contribute to differential diagnosis in cases with

monocytosis and suspicion of CMML/JMML, MPN, or the overlap MDS/MPN category.

In conclusion, the detection of mutations of both the *TET2* and *CBL* genes highly contributes to a further understanding of genetic networks and aberrant signaling in various acute and chronic myeloid malignancies. As being illustrated by the interactions of *CBL* mutants and *FLT3* signaling, the identification of such novel targets might lead to new options of targeted therapy [39]. With the occurrence of *TET2* mutations, a new recurrent molecular marker has been identified in a considerably high proportion of MDS patients [20, 27]. This might demonstrate clinical utility for differential diagnosis in cases being suspicious for MDS. Both the *TET2* and *CBL* mutations were detected by applying novel genomic technologies, e.g., SNP microarrays, underlining their potential in the identification of molecular genetic targets in myeloid malignancies. The application of massively parallel high-throughput sequencing [55, 56], so called next-generation sequencing, will further catalyze these steps aiming to improve diagnosis and therapy in leukemia.

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