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New avenues for genetics guided therapeutic approaches in AML

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ABSTRACT

The development of single nucleotide polymorphism (SNP) microarray analysis and next generation sequencing (NGS) has significantly contributed to comprehensively characterize the genetic changes underlying acute myeloid leukemia (AML). These genomics technologies have led to the identification of an increasing number of genomic aberrations and gene mutations that cause epigenetic changes and lead to deregulated gene expression. In accordance, AML patients present with a distinct and almost individual combination of somatically acquired genetic alterations reflecting the molecular heterogeneity of the disease. Some of these are known driver mutations perturbing self-renewal, proliferation, and hematopoietic differentiation, whereas many mutations also represent mere passenger events, which do not significantly contribute to AML. In the future, we will have to discriminate driver from passenger mutations and in addition it will be crucial to evaluate the prognostic and predictive values of the respective driver mutations, especially in the context of the overall genetic background. While first genetic markers have already been translated into the daily clinical routine by impacting treatment decisions, novel biomarkers are needed especially to improve the effectiveness of molecular targeted therapies, which have to be put into the perspective of mutational networks to further "precision medicine" by personalized combination treatment approaches.

Introduction

The risk stratification of acute myeloid leukemia (AML) has significantly improved by the identification of cytogenetic markers [1], and the development of single nucleotide polymorphism (SNP) microarray analysis [2] and next generation sequencing (NGS) [3], two novel genomics technologies, has provided a tremendous contribution to decipher the genomic landscape of AML. Individual patients present with a distinct and almost unique combination of somatically acquired genetic aberrations; however, not all identified gene mutations perturb cellular processes such as self-renewal, proliferation, differentiation, and epigenetic regulation, thereby contributing to leukemic transformation. For example, several genomic aberrations and gene mutations were found to cause epigenetic changes and to deregulate gene expression in AML, such as genomic losses and/or

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mutations of the *TET2* gene [4] as well as *IDH1* and *DNMT3A* mutations [5, 6], whereas other mutation most likely represent less important passenger and no driver mutations.

Over the last years novel genetic information has started to translate into updated classification schemes such as the World Health Organization (WHO) classification [7], as genetic changes represent powerful prognostic and predictive markers for novel therapeutic approaches, such as tyrosine kinase inhibitors (TKIs) and demethylating agents [8]. These might significantly contribute to an improvement in the treatment of AML, which was slow in recent decades [9]. However, some genetic mutations already impact diagnosis and guide therapeutic decisions in adult AML, and in this brief review we will discuss the clinical value of the established as well as novel investigational genomic markers.

AML with recurrent fusions genes

AML patients who present with a *translocation or inversion of the core-binding-factor (CBF) complex*, characterized by either a t(8;21)(q22;q22) [leading to a *RUNX1-RUNX1T1* fusion] or an inv(16)(p13.1q22)/t(16;16)(p13.1;q22) [leading to a *CBFB-MYH11* fusion], are categorized into a favorable-risk genetic group [6, 7] that benefits from consolidation therapy with repetitive cycles of high-dose cytarabine [8, 10]. Recent evidence points also to a benefit from an antibody-directed chemotherapeutic approach using the anti-CD33 immunoconjugate gemtuzumab ozogamicin (GO), as a subgroup analysis showed a significant survival benefit for patients with CBF-AML [11], and other studies showed also a similar beneficial effect [12, 13].

Frequently observed secondary genetic changes in CBF-AML associated with inferior outcome comprise mutations of *KIT* (*v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog*) and *FLT3* (*FMS-like tyrosine kinase 3*) [10, 14–16]. In accordance, recent efforts combining conventional induction and consolidation therapy with dasatinib, a potent inhibitor of mutated and wild-type KIT, provided promising results (www.ClinicalTrials.gov identifier NCT00850382 and NCT01238211) that might lead to additional molecular targeted approaches [17, 18]. Similarly, the CBF fusion genes are good markers for MRD monitoring as molecular disease persistence is a highly predictive factor for relapse-free survival (RFS) and overall survival (OS) [15, 19–21]. In accordance, copy ratios of the fusion transcripts should be monitored in CBF-AML.

Over 60 different fusion partners are involved in the *rearrangements of the mixed lineage leukemia (MLL) gene*, and the translocation t(9;11)(p22;q23), leading to a *MLLT3-MLL* fusion (also known as *MLL-AF9*), forming a unique WHO classification entity [7]. Notably, *MLL*-rearranged leukemias display remarkable genomic stability [22] and seem to be largely driven by epigenetic deregulation as several DNA or histones modifying epigenetic regulators were implicated in MLL-fusion driven leukemogenesis [23]. In accordance, modifying the epigenetic state might have therapeutic efficacy in *MLL*-rearranged leukemia, and epigenetic treatment approaches now focus on mediators of MLL-fusion mediated leukemic transformation, such as the histone methyltransferase DOT1L, which modifies histone H3 on lysine 79 (H3K79) [24]. While DOT1L plays an active role in the maintenance of the MLL-fusion mediated transformation and leukemogenesis programs [24], a first specific small-molecule DOT1L inhibitor EPZ-5676 showed promising antiproliferative activity [25], and a first phase 1 trial testing EPZ-5676 was initiated (www.ClinicalTrials.gov identifier NCT01684150).

AML with recurrent mutations

Mutations of *nucleophosmin 1* (*NPM1*) are found in 25%–35% of adult AML, especially in cytogenetically normal (CN-)AML (45%-64%) (Fig. 1) [8], and blast cells typically show high CD33-antigen, but low or absent CD34-antigen expression [26]. Associated with *FLT3* internal tandem duplications (*FLT3*-ITDs), more recently *NPM1* mutations were also linked with *IDH* and *DNMT3A* mutations [6, 27–31]. The genotype "mutated *NPM1* without *FLT3*-ITD" confers a superior outcome in CN-AML only [1, 32], and has been incorporated into the genetic favorable-risk category of the current AML recommendations [8]. However, the prognostic value of this genotype has to be revisited in the context of *IDH* and *DNMT3A* mutations [33]. Younger adult patients with mutated *NPM1* without *FLT3*-ITD AML might not benefit from allogeneic HSCT in first complete remission (CR) [32]; however, it may be considered in patients with molecular disease persistence [34], especially in those with low transplantation-related risk. The favorable prognostic impact of mutated *NPM1* without *FLT3*-ITD is also seen in older adults [35, 36], and these patients might also benefit from intensive conventional chemotherapy [37]. Furthermore, anti-CD33 antibody GO appears to be an attractive therapeutic strategy in *NPM1* mutant AML due to high CD33 expression levels,

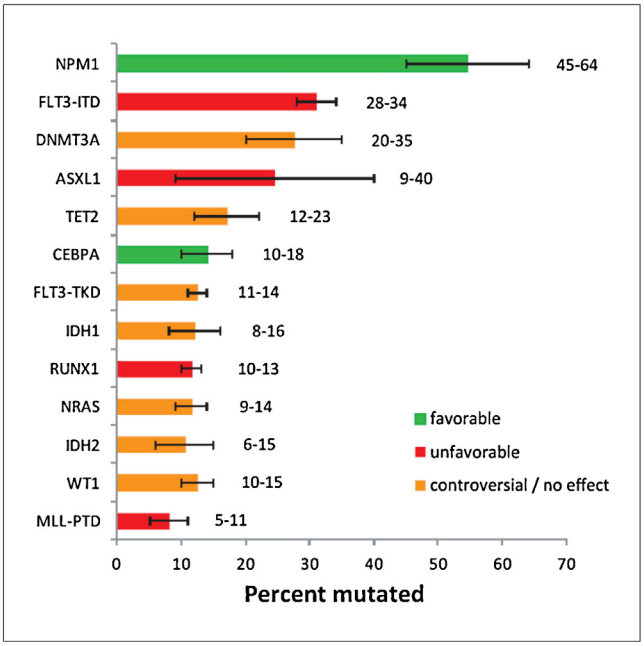

Fig. 1 – Incidence and prognostic impact of aberrant gene mutations in CN-AML
Adopted from Marcucci et al. [1]

Table I – Prognostic value and impact on treatment decision of selected molecular markers in adult AML

Biomarker	Prognostic significance	Clinical relevance
AML with *RUNX1-RUNX1T1* and *CBFB-MYH11*	Favorable prognosis in younger and older patients [additional trisomy 22 predicts superior RFS in AML with inv(16)] High relapse probability in patients with molecular disease persistence Secondary *KIT* and possibly also *FLT3* mutations associated with inferior outcome in most but not all studies	"3 + 7" induction followed by repetitive cycles of high-dose cytarabine = widely accepted standard therapy (older patients with CBF-AML do also benefit from intensive conventional chemotherapy) Allogeneic HSCT may be only considered in individual patients with high-risk factors (e.g. elevated WBC counts, molecular disease persistence) and low transplantation-related mortality KIT inhibitor dasatinib in combination with intensive induction and consolidation therapy in phase II clinical trials Addition of GO significantly improved OS in the MRC15 trial
AML with *MLL* fusions	Unfavorable prognosis, except for AML with t(9;11)	Allogeneic HSCT appears to improve outcome in younger adult patients Experimental therapeutic strategies within clinical trials (e.g. hypomethylating agents, DOT1L inhibitors)
NPM1	Genotype "mutated *NPM1* without *FLT3*-ITD" (in CN-AML) associated with favorable outcome *NPM1* mutations in older patients associated with CR achievement and better outcome, even in patients above the age of 70 years Impact of concurrent gene mutations e.g. in *IDH1*, *IDH2*, *DNMT3A*, and *TET2* currently under investigation	Standard induction therapy followed by repetitive cycles of high-dose cytarabine = reasonable first-line treatment option in patients with the genotype "mutated *NPM1* without *FLT3*-ITD" (CN-AML) Favorable-risk "mutated *NPM1* without *FLT3*-ITD" CN-AML may not benefit from allogeneic HSCT in first CR, except in individual cases (e.g. those with molecular disease persistence) with low transplantation-related risk Older patients with NPM1-mutated AML benefit from intensive conventional chemotherapy Concurrent gene mutations other than *FLT3* (*IDH1*, *IDH2*, *DNMT3A*, etc.) should not yet be used for making treatment decisions
CEBPA	Only *CEBPA*^{dm} cases define this AML entity *CEBPA*^{dm} (CN-AML) associated with favorable outcome Impact on older patients under investigation	Standard induction and consolidation therapy = reasonable first-line treatment option Patients may not benefit from allogeneic HSCT in first CR
FLT3-ITD	Unfavorable prognosis Particular poor outcome in AML with high burden of mutated *FLT3*-ITD allele (high mutant to wild-type allelic ratio as assessed by DNA fragment analysis) AML with *FLT3*-ITD located outside the JM (non-JM ITD, approximately 30% of cases) appears to do significantly worse than AML with JM-ITD	Allogeneic HSCT appears to improve outcome in younger adult patients (no data available for elderly patients) Patients should be entered on clinical trials with FLT3 tyrosine kinase inhibitors whenever possible; first-generation (e.g. midostaurin, lestaurtinib, sorafenib) and second-generation TKI (quizartinib) are currently being evaluated in phase II and III clinical trials
TP53	Unfavorable prognosis Mutations/deletions mostly in AML with complex karyotype (56%-78%)	Allogeneic HSCT does not seem to improve outcome; experimental therapeutic approaches within clinical trials warranted
WT1	Prognostic significance somewhat controversial; most studies report a negative prognostic impact Additional studies, preferentially large intra-individual patient meta-analyses, needed to explore the prognostic impact by different post-remission therapies *WT1* SNP rs16754 located in mutational hot spot in exon 7 found to be associated with favorable prognosis in patients with CN-AML	Unknown
RUNX1	Unfavorable prognosis; all studies showed an association of RUNX1 mutations with lower CR rate and adverse outcome	Unknown One study (AMLSG) suggested that allogeneic HSCT may improve outcome; finding needs to be confirmed

Table I (*Continued*)

Biomarker	Prognostic significance	Clinical relevance
TET2	Prognostic significance unclear CALGB study found a negative impact in the subset of molecular favorable-risk (mutated NPM1 without FLT3-ITD) AML; AMLSG study found no impact	Unknown
IDH1	*IDH1* mutations appear to confer higher risk of relapse and inferior OS in CN-AML; however, the effect in the various molecular subsets of CN-AML is controversial *IDH1* SNP rs11554137 (located in the same exon as the R132 mutation) in one study found to be associated with inferior outcome in molecular high-risk CN-AML (either *NPM1* wild-type or *FLT3*-ITD positive)	Unknown IDH inhibitors in preclinical development
IDH2	IDH2R172 mutations are only rarely found in concert with other known recurring gene mutations (i.e. *NPM1*, *CEBPA*, *FLT3*-ITD); they are associated with inferior CR rate; impact on outcome unclear Prognostic impact of IDH2R140 mutations controversial, although some studies reported an association with a better prognosis	(see above)
DNMT3A	Associated with intermediate-risk cytogenetics (in particular CN-AML) and with *FLT3*, *NPM1*, and *IDH* mutations Prognostic significance under investigation	Unknown
ASXL1	Unfavorable prognosis; Mutation incidence increases with age	Unknown

Adopted from Bullinger and Döhner [79]

and some trials showed a benefit for GO in the low and intermediate risk groups [38]. Similarly, all-*trans* retinoic acid (ATRA) in patients with mutated *NPM1* without *FLT3*-ITD remains elusive [36, 39], but a recent AMLSG study in younger AML patients confirms a beneficial effect (Table I) [40].

In CN-AML mutations of the *CCAAT/enhancer binding protein alpha* (*CEBPA*) gene are found in 10%–18% of cases, either as single mutation, $CEBPA^{sm}$ (one-third of cases), or double mutation, $CEBPA^{dm}$ (two-thirds of cases), affecting both alleles, one showing an N-terminal and one a C-terminal mutation.[1] Based on recent studies, only $CEBPA^{dm}$ AML is an independent prognostic factor for favorable outcome [41], and in consequence only $CEBPA^{dm}$ AML should be considered as a distinct entity and prognostic category, which can be associated with additional genomic aberrations such as *GATA2* mutations [42]. As allogeneic HSCT may not improve outcome in favorable-risk AML [8], novel treatment strategies using e.g. lenalidomide might improve patient outcome [43].

FMS-like tyrosine kinase 3 (*FLT3*)-ITDs are found in approximately 20% of all AML cases and in 28%–34% of CN-AML, in whom the presence of *FLT3*-ITDs confers a significantly worse prognosis [8], especially in cases with a high mutant-to-wild-type allelic ratio [1]. In addition, point mutations in the activation loop of the tyrosine kinase domain (TKD) are found in 11%–14% of CN-AML, but the prognostic relevance of *FLT3*-TKD mutations remains controversial [1]. Currently, therapeutic inhibitors of FLT3 such as midostaurin (PKC412), lestaurtinib (CEP-701), sunitinib (SU-11248), sorafenib (BAY-43-9006), and the second-generation compound quizartinib (AC220) have shown promising anti-leukemic activity [44]. In addition to tyrosine kinase inhibitor (TKI) based targeted treatment approaches, CN-AML and other unfavorable genotypes with *FLT3*-ITD might also benefit from allogeneic HSCT, especially [32, 45, 46].

Finally, while *tumor protein p53* (*TP53*) mutations are rarely seen in AML (2.1%), mutations and/or loss of the *TP53* allele is found in 69%–78% of AML cases with a complex karyotype (CK-AML) [47, 48]. Characterized by a higher degree of genomic complexity, *TP53*-altered CK-AMLs more frequently exhibit a monosomal karyotype (MK) [49], and are associated with older age, specific DNA copy number alterations, and dismal outcome [48, 50]. Thus, treatment approaches in *TP53*-altered CK-AML aim at early allogeneic HSCT in *TP53* altered AML cases, although there might be limited benefit in patients with 17p abnormal AML [51]. In accordance, novel therapeutic approaches are needed such as combinations of hypomethylating agents, mTOR (mammalian target of rapamycin) inhibitors, and tosedostat, an orally available aminopeptidase inhibitor [52], which demonstrated significant clinical activity in relapsed or refractory AML [53, 54].

"Novel" genomic markers

Based on SNP array analysis [4, 55] and NGS studies [3, 56], novel biomarkers have been discovered that include mutations in transcription factors (*WT1*, *RUNX1*, and *GATA2*), in genes influencing transcriptional regulation (*NRAS*, *KRAS*, *CBL*, *KIT*, and *RAD21*), and in epigenetic modifiers (*TET2*, *IDH1*, *IDH2*, *DNMT3A*, *ASXL1*, *MLL*, *TET1*, *BCOR*, *NSD1*, *PHF6*, *DNMT1*, *NSD1*, *EZH2*, and *MLL3*) [1, 33, 57]. Most of these

genes remain mainly investigational and are still controversially discussed, but for some mutations an important role in AML was recently supported.

Wilms' Tumor 1 (*WT1*) mutations occur primarily in CN-AML with a frequency of 10%–15% [58]. However, the prognostic relevance of the mutations remains still inconclusive as several studies show a negative impact on OS, whereas no impact was found in a large AMLSG CN-AML study [58]. Notably, an SNP (rs16754) located in the *WT1* exon 7 mutational hot spot was associated with favorable outcome in CN-AML [59], a finding that warrants further validation. In addition, the intragenic mutations of the *runt-related transcription factor 1* (*RUNX1*) have been associated with inferior outcome [60–63]. Present in 6% of AML cases, *RUNX1* mutations cluster in the intermediate-risk cytogenetic group and predict for resistance to chemotherapy as well as inferior outcome, which might be overcome by allogeneic HSCT [60].

Among the genes impacting epigenetic regulation *tet oncogene family member 2* (*TET2*) mutations are found in 12–27% of patients with AML and in other myeloid diseases [1]. TET2 converts 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), an α-KG-dependent reaction playing an important role in DNA demethylation [64, 65], and *TET2*-mutated AML displays uniformly low levels of 5hmC. However, the prognostic impact of *TET2* mutations is still inconclusive [66, 67], although a recent study reported reduced OS in *TET2* mutant patients with intermediate-risk AML [68]. Similarly, *isocitrate dehydrogenase 1* (*IDH1*) [5] and *IDH2* are found mutated in AML between 15% and 22% [27, 29, 69], and mutations typically affect *IDH1* at codon R132 and *IDH2* at codons R140 or R172 [1]. Mutant IDH proteins acquire an enzymatic activity that converts α-ketoglutarate (α-KG) to a putative oncogenic metabolite 2-hydroxyglutarate (2-HG), a competitive inhibitor of α-KG-dependent histone demethylases and TET family 5-methylcytosine hydroxylases [70]. In accordance, there is a mutual exclusivity between *IDH* and *TET2* mutations in AML [71], and similar to *TET2* mutations the prognostic effect of *IDH* mutations in CN-AML remains still controversial [29, 69]. *DNA (cytosine-5-)-methyltransferase 3 alpha* (*DNMT3A*) mutations affect a de novo methyltransferase that methylates cytosines in CpG dinucleotides, thereby further supporting the importance of altered epigenetic patterns in AML [6, 72, 73]. Found in ~20% of AML cases, *DNMT3A* mutations are associated with *FLT3*-ITD, *NPM1*, and *IDH* mutations [6]. An association with inferior survival could only be found in selected studies [30, 73], while analysis of 1770 younger AMLSG AML patients showed no clear correlation with outcome [74]. This might be in part explained by a selection bias as well as differences in treatment strategies, as high dose daunorubicin can improve outcome [68]. Loss of function mutations of the polycomb family gene *additional sex combs like 1* (*ASXL1*) results in impaired polycomb repressive complex 2 (PRC2)-mediated histone H3 lysine 27 tri-methylation (H3K27me3) [75], which is a common theme in myeloid pathogenesis reflected by additional loss-of-function mutations in other PRC2 complex members such *EZH2*, *SUZ12*, *EED* and *JARID2* [57]. Despite the fact that *ASXL1* mutations are relatively uncommon, it could be shown that *ASXL1* mutations confer a poor impact on OS [68, 76–78]. Finally, additional studies are needed to understand the mechanisms underlying mutations in other epigenetic modifiers such as *PHF6*, *TET1*, *BCOR*, *NSD1*, *DNMT1*, *NSD1*, and *MLL3*, in order to develop novel therapeutic strategies to restore epigenetic regulation in AML.

Conclusions

Improved understanding of AML pathogenesis has started to translate into the clinical setting, but a growing number of molecular markers identified by genomics and NGS based approaches challenge us to determine biomarkers of prognostic significance in the context of the overall mutation spectrum. This demands the analysis of large numbers of homogeneously treated patients within international collaborations that will allow to control the effects of treatment on outcome. In addition, for ''personalized'' treatment approaches not only will complex genotypes have to be considered, but we will also have to examine the evolution and impact of subclonal mutations. Thus, studying the effects of treatment on clonal evolution may help to target not only the most prevalent drivers, but also the evolutionary landscape. In that regard personalized combination therapies should be the goal that ultimately will lead to an improvement in AML patient survival.

Authors' contributions/Wkład autorów

According to order.

Conflict of interest/Konflikt interesu

None declared.

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None declared.

Ethics/Etyka

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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