

Review

Novel Prognostic and Therapeutic Mutations in Acute Myeloid Leukemia

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Abstract. *Acute myeloid leukemia (AML) is a biologically complex and molecularly and clinically heterogeneous disease, and its incidence increases with age. Cytogenetics and mutation testing remain important prognostic tools for treatment after induction therapy. The post-induction treatment is dependent on risk stratification. Despite rapid advances in determination of gene mutations involved in the pathophysiology and biology of AML, and the rapid development of new drugs, treatment improvements changed slowly over the past 30 years, with the majority of patients eventually experiencing relapse and dying of their disease. Allogenic hematopoietic stem cell transplantation remains the best chance of cure for patients with intermediate- or high-risk disease. This review gives an overview about advances in prognostic markers and novel treatment options for AML, focusing on new prognostic and probably therapeutic mutations, and novel drug therapies such as tyrosine kinase inhibitors.*

Acute myeloid leukaemia (AML) is a heterogeneous disorder characterized by clonal expansion of myeloid progenitors (blasts) in the bone marrow and peripheral blood. Formerly, AML had a very poor prognosis. Due to improvements in therapeutic regimens and supportive care (*e.g.* anti-infective agents, and transfusion support), AML is now cured in approximately 35-40% of patients younger than 60 years (1). For those over 60 years old, the prognosis has improved but

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remains poor (2). Chromosomal abnormalities (*e.g.* deletions, translocations) are identified in approximately 50% of all adult patients with primary AML and have long been recognized as the genetic events that cause and promote this disease (1, 3). Certain cytogenetic abnormalities, including t(8;21)(q22;q22), t(15;17)(q22;q12) and inv(16)(p13.1;q22), are associated with longer remission and survival, while alterations of chromosomes 5, 7, complex karyotype (described as >3 chromosomal abnormalities) and 11q23 are associated with poor response to therapy and shorter overall survival (1). In contrast, about 40-50% of all AML cases are cytogenetically normal (CN-AML) (3). Although, this group has an intermediate risk of relapse, a substantial heterogeneity is found in this population regarding clinical outcome. Molecular screening of this AML category is important in risk stratification and therapy decisions (4). The identification of new mutations in AML has raised prognostic and probably therapeutic implications for patients with AML.

Prognosis/Risk Stratification

Age and performance status in addition to chromosomal and molecular aberrations remain the most important tools for outcome prediction in AML (3). In 2010, the European Leukaemia Net classification scheme was created in an effort to standardize risk stratification in adult patients with AML by incorporating cytogenetic and known molecular abnormalities (4). Patients are classified into one of four risk groups: favorable, intermediate 1, intermediate 2 and adverse (Table I).

Standard Therapy for AML

Intensive induction chemotherapy has remained unchanged for more than four decades and is the backbone of therapy. For young adults (age <60 years) and fit elderly patients the intensive anthracycline and cytarabine regimen '7+3' induction therapy is the standard of care (5, 6). The aim of

Table I. *European Leukaemia Net risk group [adapted from (4)].*

Genetic group	Subsets
Favorable	t(8;21), inv(16) Mutated <i>NPM1</i> without <i>FLT3</i> ITD (normal karyotype) Mutated <i>CEBPA</i> (normal karyotype)
Intermediate I	Wild-type <i>NPM1</i> (normal karyotypes) <i>FLT3</i> -ITD (normal karyotype)
Intermediate II	t(9;11); <i>MLL3-MLL</i> Cytogenetic abnormality not classified as favorable or adverse
Adverse	inv(3) or t(3;3) t(6;9) t(v;11) -5 or del (5q); -7; abnl (17p); complex karyotype

abnl, Abnormalities; *CEBPA*, CCAAT/enhancer-binding protein alpha; del, deletion; *FLT3* ITD, Fms-related tyrosine kinase 3 internal tandem duplications; *MLL3-MLL*, mixed lineage leukemia; *NPM1*, nucleophosmin 1.

induction chemotherapy is to achieve morphological complete remission (CR) (5). Thus, patients with *de novo* AML achieve CR in 65-73% of cases using standard induction with 7+3, while only 38-62% of patients over 60 years of age with AML achieve CR (5, 6). Consolidation or post-induction therapy is given to prevent relapse and eradicate minimal residual leukemia in the bone marrow after induction as a bridge to transplant or to achieve cure. Assessment of minimal residual disease using real-time polymerase chain reaction or next-generation sequencing is increasingly being used to help track treatment response and has been shown to be superior than morphology alone in predicting impending relapse (7, 8).

In general, there are two main strategies for consolidation; chemotherapy (including targeted agents) and hematopoietic stem cell transplantation (HSCT) (9, 10). Both strategies can be used alone or most commonly in combination depending on the type of leukemia, the fitness of the patient and the availability of a stem cell donor. One of the most important treatment decisions in AML is to estimate the benefit and risk associated with allogeneic HSCT in first remission for a given patient.

Transplantation offers the best means of preventing AML recurrence, but remains associated with higher treatment-related morbidity and mortality, especially in older patients (11). In patients with favorable-risk AML, the relapse risk may be low enough and the salvage rate high enough to postpone HSCT to second remission. This strategy has been validated in several donor *versus* no-donor studies (12, 13). In these studies, favorable patients (*i.e.* those with core binding factor AML (CBF-AML)) from the no-donor group performed, as well as those from the donor group, whereas all other patients

appeared to benefit from undergoing HSCT. A study by the European Society for Blood and Marrow Transplantation examined the role of reduced intensity (RIC) conditioning *versus* myeloablative regimens (MAC) to younger patients aged 40-60 years in first CR1 (14). Among 2,974 patients, 1,638 had MAC and 1,336 RIC transplants. Overall survival was higher in patients with RIC with low-risk cytogenetics but not in the intermediate- or poor-risk AML. Relapse incidence was lower with MAC in poor- and intermediate-risk AML. Non-relapse mortality was higher in MAC in all cytogenetic risk groups. They confirmed lower relapse but higher non-relapse mortality risks with MAC. MAC is not superior in patients with higher risk cytogenetics, but is inferior to RIC in the small cohort of patients with AML with low-risk cytogenetics (14).

New Mutations with Prognostic and Therapeutic Implications in AML

During the past decade, several studies have shown that the presence or absence of specific gene mutations or changes in gene expression can further classify AML cases and have an effect on patient prognosis (3, 4, 15-17). This is particularly relevant for patients with CN-AML. With the technique of next-generation sequencing, the genetic landscape of CN-AML has been more defined with each case having an average of 13 mutations, eight of which are random ‘passenger’ mutations and five of which are recurrent ‘driver’ mutations (15, 16). Key molecular abnormalities have been identified and are now used to predict outcome and help guide treatment for patients with AML. In a very recent article by Papaemmanuil *et al.* the role of mutations and their correlation with pathophysiology was examined in a large cohort of 1,540 patients with AML (17). They identified 5,234 driver mutations across 76 genes or genomic regions, with two or more drivers being identified in 86% of the patients. Patterns of co-mutation compartmentalized the cohort into 11 classes, each with distinct diagnostic features and clinical outcomes. In addition to currently defined AML sub-groups, three heterogeneous genomic categories emerged: AML with mutations in genes encoding chromatin, RNA-splicing regulators, or both (in 18% of patients); AML with *TP53* mutations, chromosomal aneuploidies, or both (in 13%); and, provisionally, AML with isocitrate dehydrogenase (*IDH2*) R172 mutations (in 1%). Patients with chromatin-spliceosome and *TP53*-aneuploidy AML had poor outcomes, with the various class-defining mutations contributing independently and additively to the outcome. They found gene-gene interactions that were especially pronounced for nucleophosmin 1 (*NPM1*)-mutated AML, in which patterns of co-mutation identified groups with a favorable or adverse prognosis (17). In our review, we focus on the most relevant AML mutations (Table II).

Table II. *New relevant mutations in acute myeloid leukaemia.*

Mutation	Frequency in CN-AML	Mode of action	Prognosis	Target druggable	Drug
<i>NPM1</i>	30-45%	Nucleolar component	Favorable	-	-
<i>DNMT3A</i>	34%	<i>De novo</i> DNA methylation	Inconclusive	-	-
<i>FLT3</i> -ITD	28-34%	Receptor tyrosine kinase for <i>FLT3</i> ligand	Unfavorable	Tyrosine kinase inhibitors	<i>e.g.</i> sorafenib, midostaurin, quizartinib
<i>FLT3</i> -TKD	11-14%	Receptor tyrosine kinase for <i>FLT3</i> ligand	Neutral	-	-
<i>IDH1</i> and <i>IDH2</i>	15-30%	Conversion of isocitrate to α -ketoglutarate	Favorable	Inhibitors of mutant <i>IDH1</i> and <i>IDH2</i>	<i>e.g.</i> AG-221
TET2	10%	Conversion of 5 methylcytosine to 5-hydroxy-methylcytosine, mediating demethylation	Inconclusive	-	-
<i>ASXL1</i>	5-16%	Epigenetic regulation by interaction with PRC2	Unfavorable	-	-
<i>CEBPA</i>	10-18%	Hemopoietic transcription factor	Favorable	-	-
<i>RAS</i>	25% <i>NRAS</i> , 15% <i>KRAS</i>	G-Protein associated with receptor tyrosine kinases	Neutral	RAS, downstream, inhibitors	-
<i>KIT</i>	20-30% of CBF AML	Receptor tyrosine kinase for stem cell factor	Unfavorable	Kinase inhibitors	<i>e.g.</i> imatinib, dasatinib
<i>MLL</i> -PTD	5-10%	20-30% of CBF AML	Unfavorable	-	-
<i>RUNX1</i>	5-13%	Hemopoietic transcription factor	Unfavorable	-	-

ASXL1, Additional sex comb-like 1; CBF, core-binding factor; *CEBPA*, CCAAT/enhancer-binding protein alpha; CN-AML; cytogenetically normal acute myeloid leukaemia; *DNMT3A*, DNA methyl-transferase 3A; *FLT3* ITD, Fms-related tyrosine kinase 3-internal tandem duplications; *FLT3* TKD, Fms-related tyrosine kinase 3-tyrosine kinase domain; *IDH1* and *IDH2*, Isocitrate dehydrogenase 1,2; *KIT*, CD117; *MLL* PTD, mixed lineage leukaemia partial tandem duplication; *NPM1*, nucleophosmin 1; *PRC2*, polycomb repressive complex 2; *RAS*, RAS viral (v-ras) oncogene homologue; *RUNX1*, Runt-related transcription factor 1; *TET2*, ten-11 translocation 2.

Fms-like Tyrosine Kinase 3 (*FLT3*) Mutations

FLT3 is a class III family receptor tyrosine kinase acting as a cytokine receptor for *FLT3* ligand. *FLT3* was found to be strongly expressed in hematopoietic stem cells with important roles in cell survival and proliferation (18, 19). *FLT3* mutations are among the most frequent mutations observed in AML and two types are distinguished. Internal tandem duplications (ITD) of *FLT3* can be identified in about 20% of patients with AML, and in 28-34% of those with CN-AML; in the latter, they predict poor outcome (1, 3, 4, 19, 20). These mutations are mostly located in the juxtamembrane domain. In 28% of cases, they are found in the tyrosine kinase domain (TKD), and predict a particularly poor prognosis. The ITDs in *FLT3* constitutively activate the tyrosine kinase by interfering with the auto-inhibitory function of the juxtamembrane domain and lead to enhanced rat sarcoma (RAS), mitogen-activated protein kinases (MAPK), and signal transducer and activator of transcription 5 (STAT5) signaling (19-22). They predict increased frequency of relapse, and shorter overall survival. Both types of mutations constitutively activate *FLT3* signaling, promoting blast proliferation (19-21). This effect on prognosis is modulated by the ratio of mutated to wild-type alleles, with inferior outcome in the presence of a higher load of internal tandem duplications in *FLT3*. Evidence is emerging

that patients with AML with these mutations benefit from allogeneic HSCT in first CR, that is recommended for this group (4). Furthermore, *FLT3* ITD mutations have been associated with increased risk of relapse, while the prognostic relevance of *FLT3* TKD mutations is controversial (22). The degree to which *FLT3* ITD is a biomarker associated with poor outcome is determined by the binding site and *FLT3* ITD allelic burden (20, 22, 23). Studies have shown that non-juxtamembrane (*JM*) ITD are worse than *JM* domain ITD mutation and higher mutant to wild-type allelic ratios were significantly associated with lower CR rates (22, 23). Currently, tyrosine kinase inhibitors (TKI) are being tested in patients with *FLT3*-mutated AML. Unfortunately, when used alone, TKIs showed only a transient reduction of blasts, and even if initially effective, subsequent acquisition of secondary mutations induces resistance over time (24). Several small-molecule inhibitors of *FLT3* have been developed with mixed results. First-generation drugs include multi-kinase inhibitors such as midostaurin, lestaurtinib, tandutinib, sunitinib and sorafenib. When used as single agents they have limited anti-leukemia activity, mostly leading only to transient reduction of blood and bone marrow blasts, and increased toxicity (25). In a randomized trial of 224 patients with *FLT3*-mutated AML, in first relapse, lestaurtinib did not increase the response rate or prolong survival (26). Single-agent use with

midostaurin, tandutinib and KW2449 in phase I/II trials were also not clinically effective (27-29). Combination therapy using FLT3 inhibitors with chemotherapy have also been conducted. Serve *et al.* reported a randomized trial of 201 newly diagnosed older patients with AML, using the addition of sorafenib to induction and consolidation therapy. Unfortunately, sorafenib did not improve outcomes and patients did worse in the sorafenib arm due to higher treatment-related mortality and lower CR rates (30). A recent phase II study of sorafenib in combination with 5-azacitidine in relapsed/refractory FLT3 ITD-mutant AML demonstrated a response rate of 46%, mostly consisting of CR or CR with incomplete count recovery (31). Sunitinib added to induction and consolidation chemotherapy in older patients with AML and FLT3-activating mutations showed some effectiveness, with CR rates of 53% (8/15) and 71% (5/7) for patients with FLT3 ITD and FLT3 TKD mutations, respectively. The 13 patients who achieved CR went on to be consolidated with high-dose cytarabine and 7/13 received sunitinib maintenance. The median overall survival in this study was 18.8 months (32). In a recent randomized, double-blind, placebo-controlled, phase II study by Röllig *et al.*, the efficacy and tolerability of sorafenib *versus* placebo in addition to standard chemotherapy in patients with AML aged 60 years or younger was examined (33). A total of 267 patients were included in the primary analysis (placebo, n=133; sorafenib, n=134). With a median follow-up of 36 months, median event-free survival was 9 months in the placebo group *versus* 21 months in the sorafenib group, corresponding to a 3-year event-free survival of 22% *versus* 40% respectively.

Second-generation agents, promising to have better potency and fewer side-effects, include quizartinib and crenolanib, which are still undergoing clinical investigation. Drug resistance has become the major challenge in treating patients with a single FLT3 inhibitor. The point mutations identified which lead to resistance include N676, F691, and D835 within the kinase domain of FLT3 ITD (34). The novel FLT3 inhibitors, G-749 and ASP2215 (gilteritinib; active against both FLT3 ITD and D835 mutations), have recently been shown to provide sustained inhibition of FLT3 phosphorylation and increased ability to overcome drug resistance in pre-clinical trials but further studies are needed to determine if it will have clinical efficacy (35, 36). Some drugs are discussed in detail below.

Midostaurin. Midostaurin (PKC-412) is a moderately potent inhibitor of FLT3-ITD and FLT3 TKD mutations and inhibits other kinases such as stem cell growth factor receptor (c-KIT), platelet-derived growth factor receptor B (PDGFRB), vascular endothelial growth factor receptor 2 (VEGFR2), and protein kinase C. A phase II study of midostaurin monotherapy for patients with relapsed and refractory FLT3-positive AML showed minimal clinical activity; only one patient achieved a

partial remission (37). A follow-up phase IB study combined midostaurin with induction chemotherapy (7+3) without regard to FLT3 status and demonstrated that the combination was safe and well tolerated; complete remissions were seen in 92% of patients with FLT3 ITD compared with 74% of patients with wild-type FLT3 (38). On the basis of these data, the RATIFY study, an international randomized phase III study of midostaurin or placebo in combination with induction and consolidation chemotherapy, was designed. The outcomes, reported at the 2015 American Society of Hematology (ASH) Annual Meeting, showed improved 5-year overall survival in the midostaurin arm (51.4% *versus* 44.2%), regardless of whether patients were censored at the time of stem cell transplant, despite no difference in the rates of complete remission at 60 days (39). The superiority of midostaurin/chemotherapy over placebo/chemotherapy was consistent regardless of allelic burden (high *vs.* low), FLT3 ITD, and FLT3 TKD. Patients receiving midostaurin had an increased frequency of grade 3-4 desquamating rash. The overall survival benefit in combination with the favorable toxicity profile makes midostaurin in combination with induction and consolidation chemotherapy the new standard of care for patients with FLT3-mutated AML.

Quizartinib. More potent inhibitors of FLT3 ITD, and FLT3 TKD are in development. Quizartinib is a selective inhibitor of FLT3 ITD, but lacks activity against FLT3 TKD. The composite CR rate in phase II studies of quizartinib as a single agent in relapsed and refractory FLT3-mutated AML ranged between 44% and 54% (40-43). The median duration of response was between 11.3 and 12.7 weeks. Many patients who received quizartinib acquire mutations in the TKD of the FLT3 gene (D835 and F691) and resistance to ongoing treatment. Because of this, quizartinib may be best used as monotherapy 'bridge' to a potentially curative allogeneic bone marrow transplant. A randomized open-label phase III study of quizartinib *versus* chemotherapy (NCT02039726) with a primary endpoint of overall survival is currently accruing patients, and a randomized double-blind study of quizartinib or placebo in combination with induction and consolidation chemotherapy is in development (NCT02668653).

Gilteritinib. Gilteritinib (ASP-2215), also a potent inhibitor of FLT3, differs from quizartinib in its ability to inhibit both FLT3 ITD and FLT3 TKD mutations. Results of a phase I/II study among 165 patients receiving 80 mg or greater of gilteritinib were reported at the 2015 ASH Annual Meeting (36). Among patients with FLT3 ITD, the composite CR rate was 46%, and was 9% for those with FLT3 TKD. Responses were similar regardless of whether patients had received prior FLT3-directed therapy. The median duration of response was 15.9 weeks, similar to what is seen in clinical studies with quizartinib. A phase I study of ASP2215 in combination with

induction and consolidation chemotherapy is ongoing (NCT02236013), and a randomized phase III study of ASP2215 *versus* salvage chemotherapy is accruing patients (NCT02421939).

Nucleophosmin 1 (*NPM1*) Mutations

The nucleolar protein NPM1 is involved in many cellular functions such as ribosome biogenesis, DNA repair, and regulation of apoptosis. Mutations result in aberrant localization of the protein to the cytosol; an *N*-terminal nucleolar localization signal is disrupted and an export signal created instead. Mutations in the *NPM1* gene are among the most common genetic changes in AML (occurring in 25-35% of patients), especially CN-AML (present in 45-64% of cytogenetically normal patients) (16, 44, 45). In the absence of *FLT3* ITD mutations, *NPM1* mutations are associated with improved outcome for patients with CN-AML, even in those older than 60 years. Current ELN recommendations for diagnosis and treatment of AML class *NPM1*-mutated, *FLT3*-wild-type CN-AML as a favorable risk condition and discourage allogeneic HSCT in first CR (4). *NPM1* mutations result in the aberrant expression of the NPM1 protein in the cytoplasm rather than the nucleus, stimulating myeloid proliferation and leukemia development (45-47). Clinically, the mutation is associated with monocytic morphology and in absence of *FLT3* or *FLT3* ITD predicts favorable overall survival. The reason for improved survival remains unclear, however, it *NPM1* mutations have been associated with chemosensitivity to intensive chemotherapy in both young and old patients, which may account for improved outcome (48). *NPM1* mutations are associated with other recurrent genetic abnormalities such as +8, DNA methyl-transferase 3A (*DNMT3A*) mutations, *FLT3* ITD (40% of the time), *FLT3* TKD (10-15%) and *IDH* mutations (25% of the time) (16, 49, 50).

DNA Methyltransferase 3A (*DNMT3A*) Mutations

DNMT3A methyltransferase 3A is an epigenetic regulator mediating *de novo* DNA methylation of cytosine residues. Recurrent *DNMT3A* mutations were first identified at residue R882 by candidate gene next-generation sequencing in 2010, accounting for about 50% of *DNMT3A* mutations; subsequent whole-genome and whole-exome sequencing approaches revealed additional mutations throughout the complete *DNMT3A* coding sequence (51). *DNMT3A* mutations are the most frequent recurrent gene mutations in AML after *NPM1* and *FLT3* mutations. This trio of *FLT3*, *NPM1*, and *DNMT3A* mutations was commonly found occurring together in the 200 AML genomes analyzed by the Cancer Genome Atlas project (15). Mutations in *DNMT3A* gene occurs in 18-22% of all AML cases and in about 34% of CN-AML (51). Missense

mutations affecting arginine codon 882 (R882-*DNMT3A*) are more common than those affecting other codons (non-R882-*DNMT3A*) causing a defect in normal hematopoiesis and proper methylation (19, 51). Recently, *DNMT3A* mutations were identified as pre-leukemic mutations, arising early in AML evolution and persisting in times of remission (52). The prognostic significance of *DNMT3A* mutations is therefore thought to be adverse. Initial studies showed unfavorable impact on outcome in CN-AML (49). However, these effects were age-related. Younger patients with non-R882-*DNMT3A* mutations had shorter disease-free and overall survival, whereas older patients with R882-*DNMT3A* mutations had shorter disease-free and overall survival after adjustment for other clinical and molecular prognosticators (49). A larger study involving more than 1700 AML cases found no significant impact of *DNMT3A* mutation on survival endpoints (53). Recently, it was reported that patients with *DNMT3A*-mutated AML have an inferior survival when treated with standard-dose anthracycline induction therapy. Sehgal *et al.* concluded that this group should be considered for high-dose induction therapy (54). High-dose daunorubicin, compared to standard-dose daunorubicin, improved the rate of survival among patients with *DNMT3A* or *NPM1* mutation or mixed lineage leukaemia (*MLL*) translocations but not among patients with wild-type *DNMT3A*, *NPM1*, and *MLL* (55).

IDH Mutations

Mutations of *IDH1* and *IDH2* gene are gain-of-function mutations which cause loss of the physiological enzyme function and create a novel ability of the enzymes to convert α -ketoglutarate into 2-hydroxyglutarate. Specifically recurrent mutations affecting the highly conserved arginine (R) residue at codon 132 (R132) of *IDH1* and at codons R140 and R172 of *IDH2* have been identified in 15-20% of all AML and 25% to 30% of patients with CN-AML (16, 55, 56). *IDH* mutations are oncogenic. They are found more frequently in older patients (50). *IDH*, the enzyme that converts isocitrate to alpha-ketoglutarate in the mitochondria (*IDH2*) or the cytoplasm (*IDH1*) as part of the citric acid cycle, is mutated in 15% and 10% of patients with *de novo* AML, respectively (57). The prevalence of *IDH* mutations increases with age (50). *IDH* mutations, in particular *IDH1*, are associated with lower disease-free and overall survival in CN-AML cases with *NPM1* mutations and wild-type *FLT3* (50, 56). Orally available, selective, potent inhibitors of mutated *IDH* are currently being tested in phase I and II studies in AML with promising results (58). Mutant *IDH* enzymes acquire neomorphic activity and catalyze the conversion of alpha-ketoglutarate into beta-hydroxyglutarate (2-HG). Increased intracellular 2-HG causes inhibition of ten-eleven translocation (TET) enzymes and subsequent arrest in myeloblast maturation (59-61). Inhibitors of mutant *IDH1* and

mutant IDH2 are currently in phase I clinical trials (NCT02381886, NCT01915498, and NCT02074839). Interim results of a phase I/II study of the IDH2 inhibitor AG-221 (Agiros/Celgene), presented at the 2015 ASH Annual Meeting, demonstrated an overall response rate of 37% among 159 patients with relapsed/refractory AML with a composite CR of 27%. The duration of therapy response was 6.9 months (62). Similarly, a phase I study of the IDH1 inhibitor AG-120 demonstrated an overall response rate of 35%, with a composite CR rate of 33% (63). Accrual has started on a phase I study exploring the safety of combining AG-120 and AG-221 with both induction and consolidation chemotherapy and with 5-azacitidine (NCT02632708 and NCT0267792).

TET2 Mutations

TET2 mutations are found throughout the range of myeloid malignancies, including myelodysplastic syndrome and myeloproliferative neoplasms. *TET2* mutations are detected in about 10% of patients with AML, but the prognostic relevance remains controversial (64, 65). *TET2* catalytic activity converts 5-methylcytosine to 5-hydroxymethyl cytosine in an α -ketoglutarate-dependent reaction. 5-hydroxymethyl cytosine is an intermediate to DNA demethylation and could serve as a new epigenetic mark associated with transcriptional regulation of promoter regions (64). Accordingly, *TET2* mutant AML displays increased promoter methylation. In general, *TET2* mutations are loss-of-function mutations. Overall, despite several studies, their prognostic significance remains unclear. Metzeler *et al.* reported *TET2* mutations as being an adverse factor for CR and overall survival (65). However Gaidzik *et al.* did not find a prognostic effect of *TET2* mutations (66).

Runt-Related Transcription Factor (RUNX1) Mutations

The *RUNX1* gene is part of the t(8;21) fusion gene in core-binding factor (CBF) leukemia and is also affected by recurrent gene mutations in AML (67). Reported *RUNX1* mutation frequencies vary between 5% and 90% (in AML associated with trisomy 21), probably owing to differing study populations. *RUNX1* mutations in AML are associated with poor outcomes, which contrasts with the favorable prognostic effect of gene fusions involving *RUNX1* (68, 69). The differential prognostic impact of chromosomal and mutational lesions in *RUNX1* emphasizes the importance of a complete assessment of genetic factors in AML pathogenesis. *RUNX1* has been shown to be essential in normal hematopoiesis (67). Also known as AML1 protein or CBF subunit α -2 (CBFA2), *RUNX1* is located at chromosome 21 and is frequently translocated with the *RUNX1T1* (*ETO/MTG8*) gene located on chromosome 8q22, creating a fusion protein *RUNX1-*

RUNX1T1 (AML-ETO) or t(8;21)(q22;q22) AML (68). In addition to chromosome translocations, *RUNX1* mutations are commonly associated with trisomy 13, trisomy 21, absence of *NPM1* and CN-AML in older patients (16). In general, studies have shown *RUNX1* mutations are associated with resistance to standard induction therapy, leading to inferior overall survival for both younger and older patients (69).

CCAAT Enhancer Binding Protein α (CEBPA) Mutations

CEBPA is a transcription factor involved in lineage specification; it is crucial for the development of myeloid progenitors to differentiated neutrophils. Mutations in *CEBPA* are specific to AML, and are not reported in other cancers; however, overexpression of wild-type *CEBPA* is seen in acute lymphoblastic leukemia (ALL) with translocation t(4;19). *CEBPA* mutations are found in 6-10% of all AML and 15-19% of CN-AML, commonly in association with del(9q) (1, 70). *CEBPA* is a critical transcription factor that controls gene expression during hematopoiesis (71). Importantly, only bi-allelic mutation, not single, *CEBPA* mutations predicted a higher CR and favorable overall survival, occurring in 4-5% of AML (72). AML with a single *CEBPA* mutation is associated with survival similar to that of AML with wild-type *CEBPA* (16, 73).

Additional Sex Comb-like 1 (ASXL1) Mutations

ASXL1 is a transcriptional regulator which can either repress or activate transcription. *ASXL1* mutations were first identified in 2009 by copy number analysis of epigenetic regulators using comparative genome hybridisation. They have since been found throughout myeloid malignancies, mainly chronic myelomonocytic leukaemia, myelodysplastic syndrome, and myeloproliferative neoplasms (74). *ASXL1* mutations are five times more common in older (≥ 60 years) patients (16.2%) than those younger than 60 years (74); these mutations are associated with poor outcome in all studies reported to date (74). *ASXL1* mutations are loss-of-function mutations that occur in 5-11% of all AML cases (74). The function of *ASXL1* protein is not fully understood but it is suggested that it may be involved in epigenetic regulation (DNA and histone modifications) (19, 66). Among older patients, *ASXL1* mutations are associated with t(8;21), wild-type *NPM1*, absence of *FLT3* ITD, mutated *CEBPA*, and overall inferior complete remission and overall survival (75, 76).

MLL Mutations

The *MLL* gene at chromosome 11q23 encodes for a protein which has histone methyltransferase activity that coordinates chromatin modification as part of a regulatory complex (77).

Translocations affecting the *MLL* gene lead to aggressive acute lymphoblastic and myeloid leukemia with poor prognosis. A duplication of the region between exon 5 and 11 or between exons 5 and 12, which include the DNA binding motifs of *MLL*, is inserted in frame into intron 4, and the histone methyltransferase activity of *MLL* is preserved. Partial tandem duplication in *MLL* was the first mutation identified to confer an adverse prognosis for AML patients with normal cytogenetics: most patients with this genotype relapsed within a year. In addition to translocations, partial in tandem duplications of the *MLL* gene have been demonstrated most often in adult *de novo* CN-AML and in trisomy 11 AML cases (78, 79). In adult CN-AML, the frequency of *MLL* rearrangement is 11% with the presence of the *MLL* partial in tandem duplications associated with a worse prognosis (*i.e.* shorter duration of remission) when compared to CN-AML without such duplication (80).

Tumor Protein p53 (*TP53*) Mutations

TP53 is known as a prototypic tumor-suppressor gene because of its crucial role in cell cycle control. *TP53* mutations are the commonest genetic changes implicated in human cancer (81). In AML, *TP53* changes are rare and closely related to a complex karyotype, in which they are the strongest prognostic factor. Mutations and deletions of the tumor-suppressor gene *TP53* are primarily associated in AML with complex karyotype and are rare in patients without chromosomal deletions (81). *TP53* mutation is found in 8-14% of AML cases. In general, *TP53* mutations confer a very adverse prognosis with documented chemoresistance (81).

c-KIT Mutations

The *KIT* tyrosine kinase receptor is a 145 kDa transmembrane protein critical to normal hematopoiesis (82). This mutation is rare in AML (<5%) however it is present approximately 22-29% of the time in CBF mutations (*i.e.* AML harboring t(8;21)(q22;q22) or inv(16)(p13.1q22) or corresponding respective fusion genes (*RUNX1-RUNX1T1* and *CBFβ-myosin heavy chain 11 (MYH11)*). *KIT* mutations have been shown to confer higher relapse risk and lower overall survival (83). The *KIT* mutation in the codon D816 in particular has been associated with unfavorable disease-free and overall survival, particularly in patients with t(8;21)(q22;q22) (83). Prospective studies later confirmed that patients with CBF AML harboring *KIT* mutations have shorter overall survival than patients with wild type *KIT* for those with t(8;21)(q22;q22) but not for patients with inv(16)(p13.1q22) (84). Remarkably *KIT* can be targeted pharmacologically by using tyrosine kinase inhibitors, such as dasatinib (82). Preliminary results were presented recently at the American Society of Hematology Annual Meeting

from a phase II trial that combined the *KIT* inhibitor, dasatinib with standard chemotherapy for newly diagnosed patients with CBF AML. After a median follow-up of 21 months, patients with *KIT* mutations who received dasatinib with standard chemotherapy showed similar outcomes to those with wild-type *KIT* (85).

A phase II study by Boissel *et al.* aimed to evaluate dasatinib as maintenance therapy in patients with CBF AML in first hematologic CR, but at higher risk of relapse due to molecular disease persistence or recurrence. A total of 26 patients aged 18-60 years old previously included in the CBF-2006 trial were eligible to receive dasatinib 140 mg daily if they had a poor initial molecular response (n=18) or molecular recurrence (n=8). The tolerance of dasatinib as maintenance therapy was satisfactory. The 2-year disease-free survival in this high-risk population of patients was 25.7%. All but one patient with molecular recurrence presented subsequent hematological relapse. Patients with slow initial molecular response had a similar disease-free survival when treated with dasatinib (40.2% at 2 years) and without any maintenance (50.0% at 2 years). The disappearance of *KIT* gene mutations at relapse suggests that clonal devolution may in part explain the absence of efficacy observed with single-agent dasatinib in these patients (86). More studies are needed to evaluate the long-term outcomes of *KIT* inhibitors in CBF AML.

Novel Targets and Drugs

B-Cell lymphoma 2 (BCL2) inhibitors. *BCL2* overexpression has been implicated in the maintenance and survival of AML cells *in vitro* and is associated with resistance to chemotherapy and poor survival among patients with AML. The *BCL2* inhibitor venetoclax (ABT-199) led to a CR/CR with incomplete blood count recovery in five of 32 patients in a phase II clinical study (87). Preclinical models suggest that the combination of venetoclax and hypomethylating agents are synergistic. On the basis of preclinical data, a phase I study of the combination of venetoclax and decitabine or 5-azacitidine was initiated. The overall response rate (34 patients) was 76%, with 71% of patients having a CR or CR with incomplete blood count recovery, without differences in response between patients who received decitabine or patients who received 5-azacitidine (88). In a study by Jacque *et al.*, cancer cells were found to require glutamine in order to adapt to increased biosynthetic activity (89). The limiting step in intracellular glutamine catabolism involves its conversion to glutamate by glutaminase. Different glutaminase isoforms are encoded by the genes *GLS1* and *GLS2* in humans. Glutaminolysis inhibition activated mitochondrial apoptosis and synergistically sensitized leukemic cells to priming with the *BCL2* inhibitor ABT-199. These findings show that targeting glutamine addiction *via* *GLS1* inhibition offers a potential novel therapeutic strategy for AML.

Nuclear exporter inhibitors. The anti-leukemic efficacy of reversible inhibitors of the major nuclear export receptor, chromosome region maintenance 1 (CRM1, also termed XPO1) has brought much excitement (19). CRM1 is a major nuclear exporter protein which mediates the export and inactivation of several tumor suppressors such as p53, p73, forkhead box protein O1 (FOXO1), RB1 and p21 cyclin-dependent kinase inhibitor 1A (CDKN1A) among others (90). *CRM1* has been shown to be up-regulated in a range of solid tumors and hematological malignancies, including AML (91, 92). Preclinical studies indicate that treatment of AML cell lines, patient samples and AML xenografts with novel CRM1 inhibitors (Selinexor) induces strong anti-leukemic effects (93, 94). Based on these studies, phase I/II clinical trials are currently ongoing to assess the safety, tolerability and activity of selinexor in AML patients (NCT02212561).

Immune therapies. Novel antibody therapies are revolutionary in the treatment of leukemia and currently under development in AML. Monoclonal antibodies being explored include CD33 (gemtuzumab ozogamicin) (95) and bispecific antibodies such as AMG 330 (anti-CD33 and CD3) (96). In a study by Amadori *et al.*, single-agent gemtuzumab ozogamicin (GO) was compared with best supportive care (BSC) including hydroxyurea as first-line therapy in older patients with AML unsuitable for intensive chemotherapy (97). A total of 237 patients were randomly assigned (118 to GO and 119 to BSC). The median OS was 4.9 months in the GO group and 3.6 months in the BSC group; the 1-year OS rate was 24.3% with GO and 9.7% with BSC. The overall survival benefit with GO was consistent across most subgroups, and was especially apparent in patients with high CD33 expression status, in those with favorable/intermediate cytogenetic risk profile, and in women. Overall, CR plus CR with incomplete recovery of peripheral blood counts occurred in 30 out of 111 (27%) GO recipients. The rates of serious adverse events (AEs) were similar in the two groups, and no excess mortality was observed with GO.

Chimeric antigen receptor (CAR)-transduced T-cells (CARTs) are T-cells engineered to express a specific antigen receptor target designed against a specific cell-surface antigen. CD123 has been found to be expressed on the majority of AML blasts but also on normal hematopoietic cells. Preclinical data show that targeting CD123 *via* CARTs results in rejection of human AML and myeloablation in mouse models (98-100).

In a recent study by Davids *et al.*, the immune checkpoint blockade established by targeting cytotoxic T-lymphocyte-associated protein 4 with ipilimumab was examined in patients after allogeneic hematopoietic stem cell transplantation. Their early-phase data showed that administration of ipilimumab was feasible in patients with recurrent hematological cancers after allogeneic HSCT, although immune-mediated toxic effects and GvHD occurred. Durable responses were observed in

association with several histological subtypes of these cancers, including extramedullary acute myeloid leukemia (101).

Conclusion

AML is complex disease with a diverse genetic landscape. The field is rapidly expanding with increased understanding of the pathophysiology and potential new drug targets. Despite our improvements in targeted therapy, it has become apparent that single-drug options may be less likely to succeed over multiple-drug targets. Although allogeneic stem cell transplant has traditionally been considered to be the best strategy in this setting, the available data suggest that it may not be the most effective strategy for eradicating minimal residual disease. Novel agents such as molecularly targeted drugs (FLT3 or IDH inhibitors) or monoclonal antibody-based agents, including antibody–drug conjugates and bispecific antibodies, and, potentially, checkpoint inhibitors and chimeric antigen receptor T-cells, may improve therapeutic strategies for eradicating persistent minimal residual disease after cytotoxic regimens.

Conflicts of Interest

No Author has any conflict of interest to report in regard to this study.

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