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Novel therapeutic options 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 is increasing as the population ages. Cytogenetic anomalies and mutation testing remain important prognostic tools for tailoring treatment after induction therapy. Despite major advances in understanding the genetic landscape of AML and its impact on the pathophysiology and biology of the disease, as well as the rapid development of new drugs, standard treatment options have not experienced major changes during the past three decades. Especially for patients with intermediate or high-risk AML, which often show relapse. Allogeneic hematopoietic stem cell transplantation (HSCT) remains the best chance for cure. Here we review the state of the art therapy of AML, with special focus on new developments in immunotherapies and cellular therapies including HSCT and particularly discuss the impact of new conditioning and haplo-identical donor regimens for HSCT, post-transplant strategies for preventing and treating relapse, and emerging novel therapeutic options.

1. Introduction

Acute myeloid leukemia (AML) is a heterogeneous disorder characterized by clonal expansion of blasts (myeloid progenitors) in the bone marrow and peripheral blood. Formerly, AML had a very poor prognosis; due to improvement in therapeutic regimens and supportive care (e.g. anti-infective drugs, blood transfusion support), AML is now cured in approximately 35–40% of patients younger with age younger than 60 years [1]. For elderly patients (> 60 years), the prognosis has also improved, but overall remains adverse. Molecular screening plays a major role in prognostic categorization and subsequent definition of treatment strategies in AML. Cytogenetic abnormalities (e.g. deletions, translocations), as detectable in approximately 50% of adult patients with primary AML have long been associated with and recognized cause [2]. Of these, for example alterations of chromosomes 5, 7, 11q23 and a complex karyotype (described as > 3 chromosomal abnormalities) were shown to associate with poor response to therapy and shorter overall survival (OS) while the presence of other cytogenetic abnormalities like t(15;17)(q22;q12), t(8;21)(q22;q22) or inv(16)(p13.1;q22) indicate longer disease remission and patient survival [1,3]. In contrast, about 40–50% of all AML cases are cytogenetically normal AML (CN-AML) [3]. CN-AMLs are considered to have an intermediate risk for relapse. However, with respect to clinical outcome substantial heterogeneity is observed in this group, which indicates that further prognostic markers to be evaluated. More recently, the

identification of mutations by gene sequencing has provided novel prognostic and potentially therapeutical tools for patients with AML.

2. Prognosis/risk stratification

Besides age and performance status, cytogenetic and molecular aberrations are the most important tools to predict outcome in AML [3]. In 2010, the European LeukemiaNet (ELN) classification scheme was created with the aim to standardize risk stratification in adult AML patients by including cytogenetic and known molecular abnormalities [4]. Patients are classified into one of four risk groups: favorable, intermediate 1, intermediate 2 and adverse (Table 1). Of note, acute promyelocytic leukemia (APL) is excluded from the ELN classification and also not discussed in this review, as APL requires highly specific prognostic, therapy and monitoring approaches that are largely different from those applied to other forms of AML.

3. AML therapies with curative intent

3.1. Induction Therapy

The backbone of anti-leukemic treatments with curative intent builts on intensive induction chemotherapy regimens. The composition of induction therapies has remained largely unchanged over more than 4 decades. For young adults (age < 60 years) and fit elderly patients

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Table 1
European LeukemiaNet (ELN) risk group classification (adapted from Ref. [4]).

Genetic group	Subsets
Favorable	t(8;21), inv(16) Mutated NPM1 without FLT3-ITD (normal karyotype) Mutated CEBPA (normal karyotype)
Intermediate I	Wild-type NPM1 (normal karyotypes) FLT3-ITD (normal karyotype)
Intermediate II	t(9;11); MLLT3-MLL 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

Abbreviations: abnl, abnormalities; CEBPA, CCAAT/enhancer-binding protein alpha; del, deletion; FLT3-ITD, Fms-related tyrosine kinase 3-internal tandem duplications; MLLT3-MLL, mixed lineage leukemia; NPM1, nucleophosmin 1.

(especially if belonging to favorable prognostic groups according to ELN criteria; see Table 1) the intensive anthracycline and cytarabine regimen, "3+7", induction therapy is standard of care [5]. The aim of induction chemotherapy is to achieve complete morphologic remission (CR) in curative intent [6]. Using standard induction with "3+7", higher morphologic CR rates are observed in young, de novo, versus elderly (> 60 years) AML patients (65–73% versus only 38–62%) [7,8]. The poorer CR rates observed in elderly versus younger patients are due to more often observed patient co-morbidities that limit they dose-intensity of applied therapies, but may also result from inherent differences in disease biology, since secondary, therapy related and high risk AML – according to molecular criteria – are overrepresented in this group. Several trials have now shown that further enhancing the dose of anthracycline (from 45 to 90 mg/m^2) augments CR rates and OS in both younger and 60–65 years old fit adults, reinforcing the notion that dose-intense chemotherapy is required to achieve cure [9]. However, enhanced toxicity is also observed with dose intensification. Thus, the grading of fitness is important when deciding which treatment strategy is most appropriate [10]. Besides assessment of the AML risk group (see Table 1) the choice of intensive therapy for elderly AML patients requires careful evaluation of the patient's fitness, vulnerability and frailty and should be assessed using standardized geriatric assessment tools rather than based on calendaric age (reviewed in detail in [11]).

3.2. Consolidation therapy

The main aim of consolidation therapy is to prevent relapse by eradicating minimal residual disease (MRD) still present in the bone marrow after induction therapy [2]. For AML patients presenting with molecular abnormalities, the depth of response after induction therapy and during the course of the disease can be assessed at minimal residual disease level using real-time polymerase chain reaction or Next Generation Sequencing (NGS). Recent studies suggest that adding molecular criteria to the morphological assessment is superior with respect to prediction of imminent relapse, and therefore may be a useful guide for treatment decisions [12,13]. There are two main options for consolidation therapy: chemotherapy (including targeted agents) and hematopoietic stem cell transplantation (HSCT) [14]. These strategies are used alone or most commonly in combination. In younger adults (< 60 years of age) with favorable risk AML, post induction chemotherapy using intermediate-dose cytarabine 1.5 g/m^2 twice daily on days 1, 3 and 5 given in three to four cycles is an effective and established regimen to prolong remission and improve survival [5,15]. These patients are thus usually treated with chemotherapy

alone and transplantation is reserved only at relapse [5,15]. In the HOVON/SAKK group, a third cycle of chemotherapy with mitoxantrone and etoposide is used as consolidation therapy [14]. In contrast, patients suffering of intermediate or high-risk AML commonly receive high-dose chemotherapy (as bridge to transplant) followed by HSCT; overall, therapy is tailored depending on the aggressiveness of the AML, but also the fitness of the patient and the availability of a stem cell donor.

3.2.1. Allogeneic hematopoietic stem cell transplantation

Particularly in fit patients that suffer of intermediate or high-risk AML and achieve CR after induction therapy, allogeneic HSCT remains the most effective long term treatment yielding cure in 50–60% of patients [14,16–18]. Nevertheless, several patients never become eligible for transplant because of co-morbidities, failure to reach CR or lack of a suitable donor [1,2,16]. While waiting for transplant, it is standard practice to give post induction chemotherapy to maintain CR and keep the leukemia burden low. Eligibility for transplant is decided upon based on pre-transplant performance status, co-morbidities and current remission. The most widely recognized and validated tool for assessing comorbidity includes the Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI) [19]. The higher the comorbidity index score, the worse the clinical outcome. Improvements in supportive care, increased donor options (haplo-identical donors and cord grafts) and reduced intensity preparation regimens for HSCT have increased the success of transplant in all age groups.

One of the most important treatment decisions in AML is to estimate the benefit/risk ratio of allogeneic HSCT for a given patient in first CR. Transplantation offers the best means of preventing AML recurrence, but remains associated with higher treatment-related morbidity and mortality (TRM), especially in elderly patients. 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 [18,20]. In these studies, favorable patients (i.e., those with CBF-AML) from the no-donor group did as well as those from the donor group, whereas all other patients appeared to benefit from undergoing allograft. One should keep in mind that patients in these studies mostly underwent sibling donor myeloablative conditioning (MAC) transplantation and as such, the benefit associated with HSCT was only demonstrated for patients < 40 years of age. Based on another donor versus no-donor analysis, patients with CN-AML and a favorable genotype defined as mutated CCAAT/enhancer-binding protein alpha *(CEBPA)* or nucleophosmin 1 *(NPM1)* without Fms-related tyrosine kinase 3-internal tandem duplications *(FLT3*-ITD) were recently categorized in the favorable subgroup [4]. Because the outcome after allogeneic HSCT from fully matched unrelated donors appears to be similar compared with allogeneic HSCT from matched related donors, all younger patients with intermediate- and unfavorable-risk AML are generally considered candidates for allogeneic HSCT from sibling or fully-matched unrelated donors in cases of first CR [16].

This HSCT benefit/risk assessment, based on the European LeukemiaNet (ELN) genetic classification only [4], needs however to be reconsidered in the near future. Alternative stem cell sources are more widely used and are safer, as illustrated by post-transplant administration of cyclophosphamide in haplo-identical HSCT [16,21].

In a recent survey of the EBMT (European Society for Blood and Marrow Transplantation) by Passweg et al., a record number of 40.829 HSCT in 36.469 patients (15.765 allogeneic (43%), 20.704 autologous (57%)) were reported by 656 centers in 47 countries. The trends in the report included a continued growth in transplant activity, more so in Eastern European countries than in the west; a continued increase in the use of haplo-identical family donors (by 25%) and slower growth for unrelated donor HSCT. The use of cord blood as a stem cell source has decreased again in 2014 [16].

A recent study by Versluis et al. showed that allo-cHSCT might be

the preferred treatment approach in patients 60 years of age and older with intermediate-risk and adverse-risk AML in first CR [17]. 5-year overall survival was 35% for patients who received an allo-HSCT, 21% for those who received no additional post-remission therapy, and 26% for patients who received either additional chemotherapy or auto-HSCT. OS at 5 years was strongly affected by the European LeukemiaNET risk score, with patients in the favorable-risk group (n=65) having better 5-year overall survival (56%) than those with intermediate-risk (n=131; 23%) or adverse-risk (n=444; 13%) AML [17].

In a further recent study of the EBMT, the outcomes of hypomethylating therapy (HMA) compared with conventional chemotherapy (CC) pre-HSCT in 209 patients with advanced MDS was analyzed. Median follow-up was 22.1 months and the median age of the group was 57.6 years with 37% of the population aged > 60 years. The majority of patients (59%) received reduced intensity conditioning and 34% and 27% had Int-2 and high IPSS scores, respectively. At time of HSCT, 32% of patients did not achieve CR and 13% had primary refractory disease. On univariate analysis outcomes at three years were not significantly different between HMA and CC for OS, relapse free survival (RFS), cumulative incidence of relapse (CIR) and non-relapse mortality (NRM); OS (42 vs 35%), RFS (29 versus 31%), CIR (45 versus 40%) and NRM (26 versus 28%), respectively [22]. The authors concluded that outcomes after HSCT are similar for patients receiving HMA compared with those receiving CC, despite the higher proportion of patients with primary refractory disease in the HMA group.

In a study by the HOVON/SAKK group, the best post-remission therapy in patients aged 40–60 years was examined. They examined the role of allo-HSCT (n=337) versus chemotherapy (n=271) or autologous HSCT (auto-HSCT) (n=152) in 760 patients aged 40–60 years with AML in first CR [14]. Patients receiving allo-HSCT showed improved OS as compared with chemotherapy (respectively, 57 ± 3% versus 40 ± 3% at 5 years, P < 0.001). Comparable OS was observed following allo-HSCT and auto-HSCT in patients with intermediate-risk AML (60 ± 4 versus 54 ± 5%). However, allo-HSCT was associated with less relapse (hazard ratio (HR) 0.51, P < 0.001) and better RFS (HR 0.74, P=0.029) as compared with auto-HSCT in intermediate-risk AMLs. Allo-HSCT was applied following myeloablative conditioning (n=157) or reduced intensity conditioning (n=180), resulting in less NRM, but comparable outcome with respect to OS, RFS and relapse. Taken together, these results show that allo-HSCT is to be preferred over chemotherapy as post-remission therapy in patients with intermediate- and poor-risk AML aged 40–60 years, whereas auto-HSCT remains a treatment option to be considered in patients with intermediate-risk AML.

In a further study by Versluis *et al.*, post-remission treatment in patients with CN-AML in CR1 was examined [23]. Therapy consisted of RIC allo-HSCT (n=68), MAC allo-HSCT (n=137), auto-HSCT (n=168) or chemotherapy (n=148). Favorable OS was found for patients with mutated NPM1 without FLT3-ITD (71 ± 4%). Outcome in patients with a high FLT3-ITD allelic ratio appeared to be very poor with OS and relapse-free survival (RFS) of 23 ± 8% and 12 ± 6%, respectively. Patients with wild-type NPM1 without FLT3-ITD or with a low allelic burden of FLT3-ITD were considered as intermediate-risk group because of similar OS and RFS at 5 years, in which post remission therapy by RIC allo-HSCT resulted in better OS and RFS as compared with chemotherapy (hazard ratio (HR) 0.56, P=0.022 and HR 0.50, P=0.004, respectively) or auto-HSCT (HR 0.60, P=0.046 and HR 0.60, P=0.043, respectively). The lowest cumulative incidence of relapse (23 ± 4%) was observed following MAC allo-HSCT. These results suggest that allo-HSCT may be preferred in patients with molecularly intermediate-risk CN-AML, while the choice of conditioning type may be personalized according to risk for non-relapse mortality.

Taken together, these results highlight how genetic analyses increasingly personalize the choice of anti-leukemic consolidation therapies. While favorable-risk AML should be treated with chemother-

apy alone, addition of allo-HSCT improves outcome in high- and most of the intermediate-risk AML, which overall are at higher risk for relapse. In patients with intermediate-risk AML, auto-HSCT remains a treatment option to be considered while further prognostic markers stratifying this heterogeneous population are awaited.

3.2.2. Reduced intensity conditioning (RIC) versus myeloablative conditioning (MAC)

A study by the EBMT examined the role of RIC conditioning versus MAC to younger patients aged 40–60 years in CR1 [24]. Among 2974 patients, 1638 had MAC and 1336 RIC transplants. OS 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. NRM was higher in MAC in all cytogenetic risk groups. Multivariate analysis confirmed a significant leukemia-free survival and OS advantage for RIC in low risk but no advantage of MAC in intermediate- and poor-risk leukemia. In patients aged 40–60 years, MAC has no advantage over RIC. They confirmed lower relapse but higher NRM risks with MAC. MAC is not superior in patients with higher risk cytogenetics, but is inferior to RIC in the small cohort of AML patients with low-risk cytogenetics. In sum, MAC conditioning shows lower relapse rates but higher TRM and overall has no advantage over RIC in patients aged 40–60 years, in spite of RIC transplant recipients being generally of poorer AML risk category. In the small cohort of patients with low-risk leukemia, RIC appears superior. Interestingly, MAC failed to show superiority over RIC even when AML patients of high-risk cytogenetics were analyzed separately.

3.2.3. Alternative donor transplantations

Haplo-identical HSCT provides an opportunity for nearly all patients to benefit from HSCT when a HLA genotypically matched sibling is not available. Initial results with the use of mismatched allografts led to limited enthusiasm due to graft-versus-host disease (GvHD) and infectious complications resulting in unacceptable treatment-related morbidity and mortality [16,21]. Recent advances with effective T-cell depletion, the use of 'megadoses' of stem cells, better antimicrobial therapy and reduced intensity conditioning has significantly decreased the early transplant-related mortality and GvHD. These modifications also enabled robust and prompt engraftment and led to enhancing the therapeutic benefits of haplo-identical transplantation. Due to the high rates of graft failure and graft-versus-host disease, haplo-identical transplant was not considered a feasible option up until the late 20th century, when strategies such as "megadose stem cell infusions" and post-transplantation immunosuppression with cyclophosphamide showed the ability to overcome the HLA disparity barrier and significantly improve the rates of engraftment and reduce the incidence and severity of GvHD. Newer technologies of graft manipulation have also yielded the same effects in addition to preserving the anti-leukemic cells in the donor graft.

In a study by Ruggeri et al., the outcomes after unmanipulated haplo-identical stem cell transplantation (haplo-HSCT) and after unrelated cord blood transplantation (UCBT) were compared in patients with high-risk acute leukemia without HLA-matched donor [25]. UCBT was associated with delayed engraftment and higher graft failure in both AML and ALL recipients. In multivariate analysis, UCBT was associated with lower incidence of chronic GvHD both in the AML group (hazard ratio (HR)=0.63, P=0.008) and in the ALL group (HR=0.58, P=0.01). No significant differences were noted between haplo-HSCT and UCBT with respect to relapse incidence (HR=0.95, P=0.76 for AML and HR=0.82, P=0.31 for ALL), non-relapse mortality (HR=1.16, P=0.47 for AML and HR=1.23, P=0.23 for ALL) and leukemia-free survival (HR 0.78, P=0.78 for AML and HR=1.00, P=0.84 for ALL). There were no statistically differences on main outcomes after unmanipulated haplo-HSCT and UCBT, and both approaches are valid for acute leukemia patients lacking a HLA matched donor. Thus, both strategies expand the donor pool for

patients in need.

In a study by Mo et al., the impact of occurrence of chronic GvHD (cGvHD) and its severity on transplantation outcomes in a consecutive cohort of AML and MDS patients who received haplo-HSCT (n=324) was investigated [26]. The cumulative incidence of relapse was significantly decreased in patients with cGVHD compared with the non-cGvHD group (1 year: 3.2% versus 11.9%, P=0.002; 3 years: 6.0% versus 16.3%, P=0.002), particularly in those with mild cGvHD. The cumulative incidence of non-relapse mortality was comparable between patients with and without cGvHD. The probabilities of disease-free survival (DFS) were significantly better in patients with cGvHD than in those in the non-cGvHD group (1 year: 90.5% versus 78.5%, P=0.002; 3 years: 86.5% versus 71.5%, P < 0.001), particularly in those with mild or moderate cGvHD; however, no significant impact of severe cGvHD on DFS was seen. Their findings highlight the close relationship between cGvHD and the immune-mediated graft-versus-leukemia (GvL) effect in patients with AML and MDS receiving haplo-HSCT.

In a study by Kasamon et al., the effect of age on non-myeloablative (NMA) related HLA-haploidentical blood or marrow transplantation (haplo-BMT) on outcomes in patients age 50-75 years was examined [27]. A retrospective analysis was performed of 271 consecutive patients with hematologic malignancies, age 50–75 years, who received NMA, T-cell-replete haplo-BMT with high-dose post-transplantation cyclophosphamide. The median age was 61 years, with 115 patients (42%) age 50-59, 129 (48%) age 60–69, and 27 (10%) age 70–75 years. Overall, 84% of patients had intermediate- or high-/very high-risk disease. The 6-month probabilities of grade 3 or 4 acute GvHD and NRM were 3% and 8%, respectively. Three-year progression-free survival probabilities were 40% in acute myeloid leukemia (n=65), 39% in aggressive non-Hodgkin lymphoma (n=83), and 37% in indolent or mantle-cell lymphoma (n=65). Older patient age was associated with a significantly higher risk of grade 2–4 acute GvHD but not grade 3–4 acute or chronic GvHD. No statistically significant associations were found between older age (relative to age 50–59 years or as a continuous variable) and NRM, relapse, or survival. They concluded that NMA haplo-BMT with post-transplantation cyclophosphamide has encouraging safety and survival outcomes in patients age 50-75 years which support consideration of this approach in elderly patients.

Taken together, the emergence of new transplant strategies involving reduced conditioning regimens but also alternative stem cell sources and post-transplant in vivo graft modulation promise to make this therapy (and thus cure) applicable to a wider group of patients (including elderly patients with co-morbidities and/or high-risk disease).

4. Alternative treatment strategies

4.1. The use of hypomethylating agents

Hypomethylating agents including decitabine and azacitidine seems to be beneficial in older AML patients, not fit for intensive induction therapy, especially, those harboring complex karyotype without NPM1 mutations [28–32]. Both agents, commonly used to treat MDS, have activity in AML as initial induction therapy and in the relapsed setting. Several phase II and III studies using azacitidine and decitabine have been conducted [28–32].

In a randomized phase III trial by Dombret et al., azacitidine efficacy and safety was compared with conventional care regimens (CCRs; standard induction chemotherapy, low-dose ara-c, or supportive care only) in 488 patients age ≥65 years with newly diagnosed AML with > 30% bone marrow blasts [29]. Median OS was increased with azacitidine versus CCR: 10.4 months versus 6.5 months. One-year survival rates with azacitidine and CCR were 46.5% and 34.2%, respectively. Univariate analysis showed favorable trends for azacitidine compared with CCR across all subgroups defined by baseline demographic and disease features.

However, in the randomized AML-AZA trial by Müller-Tidow et al., the efficacy of azacitidine applied before each cycle of intensive chemotherapy with chemotherapy alone in older patients with untreated AML was evaluated [30]. In total, 214 patients with a median age of 70 years were randomized to azacitidine/chemotherapy (arm-A) or chemotherapy (arm-B). Adverse events were more frequent in arm-A (15.44) versus 13.52 in arm-B, (P=0.26), but early death rates did not differ significantly (30-d mortality: 6% versus 5%, P=0.76). Median OS was 15 months for patients in arm-A compared with 21 months in arm-B (P=0.35). Azacitidine added to standard chemotherapy increases toxicity in older patients with AML, but provides no additional benefit for unselected patients.

Regarding older patients, a phase II study examined azacitidine in elderly or frail patients with AML [31]. Azacitidine was administered 100 mg/m^2, 5 of 28 days for up to six cycles. Altogether 45 patients were accrued. Best response was complete response/complete response with incomplete recovery of neutrophils and/or platelets (CR/CRi) in eight (18%), 0 (0%) partial response (PR), seven (16%) hematologic improvement, 17 (38%) stable disease. Three non-responding patients stopped treatment after six cycles, 31 patients stopped early and 11 patients continued treatment for 8–21 cycles. Adverse events (grade ≥ III) were infections (n =13), febrile neutropenia (n =8), thrombocytopenia (n =7), dyspnea (p=6), bleeding (n =5) and anemia (n =4). Median overall survival was 6 months. The authors concluded that azacitidine is feasible for elderly or frail patients with AML in an outpatient setting with moderate, mainly hematologic, toxicity and response in a proportion of patients, although the primary objective was not reached.

Recently, a new oral formulations of azacitidine was developed; CC-486. The randomized placebo-controlled phase III trial (AZA-MDS-003) examines efficacy and safety of CC-486 in patients with low-risk MDS (following IPSS; international prognostic scoring system) [32].

5. Targeted therapies: the new kids on the block

The recognition of specific mutations as genetic drivers or facilitators of AML has led to the development of new inhibitors and targeted treatment options. New promising drug candidates have been tested in clinical studies, for their ability to control disease as single agents or to improve cure rates and overall survival when combined with standard chemotherapy regimens (see Table 2). During the last decade, several studies have shown that the presence or absence of specific gene mutations and/or changes in gene expression can further classify AML cases and have an effect on the patients' prognosis [2–5,33–35]. This is particularly relevant for patients with cytogenetically normal AML (CN-AML). In a very recent article by Papaemmanuil et al. the role of mutations and its correlation with pathophysiology was examined in a large cohort of 1540 AML patients [35]. They identified 5234 driver mutations across 76 genes or genomic regions, with 2 or more drivers 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 subgroups, three heterogeneous genomic categories emerged: AML with mutations in genes encoding chromatin, RNAsplicing regulators, or both (in 18% of patients); AML with TP53 mutations, chromosomal aneuploidies, or both (in 13%); and, provisionally, AML with IDH2R172 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 which were especially pronounced for NPM1-mutated AML, in which patterns of co-mutation identified groups with a favorable or adverse prognosis [35]. In our review, we will focus on three relevant AML mutations.

Table 2
Mutational landscape in Acute myeloid leukemia.

Mutation	Frequency in CN-AML	Mode of action	Prognosis	Target "drugable"	Drugs
NPM1	30–45%	Nucleolar component	favorable	–	–
DNMT3A	34%	De no DNA methylation	inconclusive	–	–
FLT3-ITD	28–34%	Receptor tyrosine kinase for Flt3 ligand	Unfavorable	tyrosine kinase inhibitors	e.g. sorafenib, midostaurin quizartinib
FLT3-TKD	11–14%	Receptor tyrosine kinase for Flt3 ligand	Neutral	–	–
IDH1 and IDH2	15–30%	Conversion of isocitrate to α-ketoglutarate	Favorable		e.g. AG–221
TET2	10%	Conversion of 5 methylcytosine to 5-hydroxy-methylcytosine, mediating demethylation	Inconclusive	–	–
ASXL1	5–16%	Epigenetic regulation by interaction with PRC2	Unfavorable	–	–
CEBPA	10–18%	Haemopoietic transcription factor	Favorable	–	–
RAS	25% NRAS, 15% KRAS	G-protein associated with receptor tyrosine kinases	Neutral	RAS downstream Inhibitors	
KIT	20–30% of CBF AML	Receptor tyrosine kinase for stem cell factor	Unfavorable	Kinase inhibitors	E.g. imatinib dasatinib
MLL-PTD	5–10%	20–30% of CBF AML	Unfavorable	–	–
RUNX1	5–13%	Haemopoietic transcription factor	Unavorable		

Abbreviations: ASXL1, Additional sex comb like 1; CBF, core-binding factor; CEBPA, CCAAT/enhancer-binding protein alpha; CN-AML; cytogenetically normal acute myeloid leukemia; DNMT3A, DNA methyl-transferase 3 A; 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 leukemia 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.

5.1. 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 [5,36]. FLT3 mutations are among the most frequent mutations observed in AML and two types are distinguished. Internal tandem duplications of FLT3 are identified in about 20% of patients with AML, and in 28–34% of those with CN-AML; in the latter instance they predict poor outcome [2–5,37]. These mutations are mostly located in the juxtamembrane domain. In 28% of cases, they are found in the tyrosine kinase domain, and predict a particularly poor prognosis. The internal tandem duplications in FLT3 constitutively activate the tyrosine kinase by interfering with the auto-inhibitory function of the juxtamembrane domain and lead to enhanced RAS, MAPK, and STAT5 signaling [5,37–39]. Both types of mutations constitutively activate FLT3 signaling, promoting blast proliferation [5,37,38]. This effect on prognosis is modulated by the mutated to wildtype allele ratio, with inferior outcome in the presence of a higher load of internal tandem duplications in FLT3. Evidence is emerging that AML patients with these mutations benefit from allo- HSCT in CR1, which 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 [39]. The degree to which FLT3-ITD is a biomarker associated with poor outcome is determined by the binding site and FLT3-ITD allelic burden [37,39,40]. Studies have shown that non-JM ITD are worse than JM domain ITD and higher mutant to wild-type allelic ratios were significantly associated with lower CR rates [39,40]. Currently, tyrosine kinase inhibitors (TKI) are being tested in FLT3 mutated AML patients. 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 [41]. Several FLT3 small molecule inhibitors 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 showing only transient reduction of blood and bone marrow blasts and increased toxicity [42]. In a randomized trial of 224 patients with FLT3 mutated AML in first relapse lestaurtinib did not increase the response rate or prolong survival [43]. Single agent use with midostantrum, tandutinib and KW2449 in phase I/II trials were also not clinically effective [44–46]. Combination therapy using FLT3 inhibitors with chemotherapy have also been conducted. Serve et al. reported a randomized trial of 201 newly diagnosed older AML patients, 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 [47]. 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 [48]. Sunitinib added to induction and consolidation chemotherapy in older patients with AML and FLT3 activating mutations showed some effectiveness with CR rates 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 OS in this study was 18.8 months [49]. In a recent randomized, double-blind, placebo-controlled, phase 2 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 [50]. 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% in the placebo group versus 40% in the sorafenib group. Midostaurin (PKC-412) is a moderately potent inhibitor of FLT3-ITD and FLT3 tyrosine kinase domain (TKD) mutations and inhibits other kinases such as c-KIT, PDGFR-b, VEGFR-2, and protein kinase C [51]. At the 2015 American Society of Hematology (ASH) Annual Meeting, the RATIFY study, an international randomized phase III study of midostaurin or placebo in

combination with induction and consolidation chemotherapy, was presented. The outcomes showed improved 5-year OS in the mid-ostaurin arm (51.4% vs. 44.2%), regardless of whether patients were censored at the time of stem cell transplant, despite no difference in the rates of CR at 60 days [52]. The superiority of midostaurin/chemotherapy over placebo/chemotherapy was consistent regardless of allelic burden (high versus low), FLT3-ITD, or 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.

Second generation agents, promising to have better potency and less side effects include quizartinib and crenolanib 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 [53]. 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 [54,55].

5.2. Nucleophosmin 1 (NPM1) mutations

The nucleolar protein nucleophosmin 1 is involved in many cellular functions such as ribosome biogenesis, DNA repair, and regulation of apoptosis. Mutations result in aberrant localisation of the protein to the cytosol; an N-terminal nucleolar localisation signal is disrupted and an export signal created instead. Mutations in the gene NPM1 are among the most common genetic changes in AML (occurring in 25–35% of patients), especially in CN-AML (present in 45–64%) [56,57]. 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 European LeukemiaNet 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 CR1 [4]. The reason for improved survival remains unclear however it has been found that NPM1 mutations have been associated with chemosensitivity to intensive chemotherapy in both young and old patients, which may account for improved outcome [58]. NPM1 mutations are associated with other recurrent genetic abnormalities such as +8, DNMT3A mutations, FLT3-ITD (40% of the time), FLT3-TKD (10–15%) and IDH mutations (25% of time) [59,60].

5.3. Isocitrate Dehydrogenase (IDH) mutations

Mutations of the isocitrate dehydrogenase (IDH) 1 and 2 gene are gain-of-function mutations which cause loss of the physiologic 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 25–30% of patients with CN-AML [34,61,62]. IDH mutations are oncogenic. They are found more frequently in older patients [60]. IDH mutations, in particular IDH1, are associated with lower DFS and OS in CN-AML cases with NPM1 mutations and wild type FLT3 [60,62]. Orally available, selective, potent inhibitors of mutated IDH are currently being tested in Phase I and II studies in AML with promising results [63]. Mutant IDH enzymes acquire neomorphic activity and catalyze the conversion of alpha-ketoglutarate into beta-hydroxygutarate (2-HG). Increased intracellular 2-HG causes inhibition of TET enzymes and subsequent arrest in myeloblast maturation [64–66]. 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 (Agios/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 complete remission of 27%. Duration of response was 6.9 months [67]. Similarly, a phase I study of the IDH1 inhibitor AG-120 demonstrated an overall response rate of 35%, with a composite complete remission rate of 33% [68]. 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).

6. Strategies for prevention and treatment of relapse

6.1. Immunotherapy

Immunotherapy for AML involves two different therapeutic options: allo-HSCT (as reviewed above) and non-transplant treatments. Immunotherapy approaches are often first examined in patients who have received allo-HSCT either to prevent or treat relapsing leukemia [69]. While many immunotherapy strategies apply in or outside the context of HSCT, the alloimmune graft-versus-leukemia (GvL) effect is important mediating cure. Indeed, allo-HSCT is still the only undoubted immune-based approach to cure leukemias resistant to chemotherapy. The GvL effect is mediated by both T cells and NK cells [70,71]. While the stem-cell source (bone marrow, peripheral blood or cord blood), degree of compatibility (identical twin, matched related, matched unrelated and haplotype matched related donors) and transplant approach (conditioning regimen and method of GvHD prevention) can all influence outcome, the major factor determining the survival and cure of AML is the remission status of the leukemia before HSCT. Patients of standard-risk AML transplanted in CR1 have a relapse rate of 20% or less; those in CR2 or subsequent remission have an intermediate relapse risk of around 40%, while patients transplanted with overt disease either because of refractoriness to induction therapy or uncontrolled relapse had a significantly higher relapse risk around 60% [69,70]. Despite numerous strategies manipulating the transplant schedule to decrease relapse rates, these statistics have not changed significantly for over 40 years. This is in part due to the fact that approaches that boost the GvL effect are constrained by the increase in morbidity and mortality from GvHD, and increased ablation of leukemia by radiation and chemotherapy is limited by increased mortality from nonrelapse causes. In addition, despite many innovative attempts to control relapsed disease by boosting GvL, AML cells can display or develop resistance to immune control. Relapse of AML after HSCT is largely incurable and still carries a dismal prognosis. The standard treatment of AML relapsed after HSCT is chemotherapy, targeted drugs or hypomethylating agents in combination or followed by donor lymphocytes infusion (DLI).

6.1.1. Immunotherapy after HSCT: GvL, GvHD and Donor lymphocytes infusion (DLI)

The full realization that GvL was a major contributor to the curative effect of allo-HSCT in the 1980 s led logically to the use of DLI to prevent or treat relapse after transplant [72]. Unfortunately, the first demonstrations that DLI could achieve durable remissions in relapsed CML were not borne out in AML and MDS where the therapeutic benefit of DLI is at best modest.

New developments in immunological understanding and ability to manipulate immune cells are beginning to break the impass of GvL linked to GvHD and improve the curative potential of allo-HSCT. Relapse after HSCT with its extremely poor prognosis has become a proving ground for the most innovative and experimental immunotherapy, appropriate for such desperate circumstances. Thus many of the strategies described below were initially developed or have only been applied in the context of HSCT. However, the application of novel

immunotherapy outside the context of HSCT is now ongoing. These new strategies can be categorized as: (1) lymphocyte products to deliver enhanced and specific anti-leukemic cytotoxic (cell therapy); (2) immunomodulatory drugs; and (3) treatments to boost immunity and enhance leukemia's susceptibility to immune system [69].

For example, Treg-depleted DLI has been used to augment GvL [69]. Manipulation of Tregs in the context of HSCT (both in the graft and in the host after transplantation) has two implications: depletion of Tregs may enhance GvL, but enrichment of Tregs is efficacious for prevention and treatment of GvHD [73]. Further studies are required to identify the optimal therapeutic approach involving Treg-mediated pathways to achieve a fine balance between GvL and GvHD.

6.1.1.1. Modulation of post-transplant immunological responses to prevent or treat AML relapse. With improvement in transplant-related mortality over the past decades, the prevention of post-transplant relapse becomes important. The early post-transplant setting is uniquely advantageous for immunotherapy to work, where the leukemic burden is low and anti-leukemia lymphocytes have the chance to expand in the post-transplant immune milieu and lymphodepleted environment [74]. Furthermore, it is possible to adoptively transfer donor cells that are not exhausted or tolerated as a part of transplant course (as DLI, see above).

However, leukemic cells may perform immune escape and patients thus suffer from severe GvHD without benefiting from corresponding GvL effects. The investigation of mechanisms of immune-sensitization of leukemic cells and drugs provoking such responses has thus emerged as a promising research avenue. For example, post-transplant treatment with sorafenib was shown to potentiate GvL in AML patients treated with HSCT [75]. Furthermore, azacitidine has been reported to either prevent or delay hematological relapse when applied to MRD positive post-HSCT patients [76,77]. Prophylactic usage of low-dose azacitidine for patients with high-risk MDS/AML post-transplant is safe and may improve event-free survival and overall survival [76,78].

6.1.2. Cellular therapies

6.1.2.1. Chimeric antigen receptor modified T cells (CAR-T). The remarkable therapeutic successes using CAR-modified T cells to treat leukemia have attracted wide enthusiasm for CAR cell therapy and T-cell therapy in general. The principle is to insert a T-cell receptor (TCR)/costimulatory molecule/linker/antigen binding domain derived from the fusion of the variable regions of the heavy and light chains of immunoglobulins specific for a leukemia surface antigen (e.g. anti CD19) construct into T cells. The antibody binds surface molecules on the leukemia, triggers TCR activation and directs T-cell cytotoxicity to the leukemia. Initial reports of remissions achieved in ALL and chronic lymphocytic leukemia (CLL) by Porter and colleagues [79,80] have led many investigators to adopt and further refine this technology.

There has been considerable interest in using CAR technology to target myeloid leukemias. CD123 is overexpressed by AML but it is also well-expressed on normal myeloid cells, although expression on hematopoietic stem and progenitor cells is weaker [81–83]. Nevertheless the risk of marrow ablation limits the application of anti-myeloid leukemia CAR cells to pretransplant conditioning where healthy stem cells can be infused after ablation of the CAR cells. While CAR cells have alerted the hematology community to the potency of T-cell-based therapy and stimulated considerable interest of pharmaceutical companies in developing cell therapy, they have some important limitations. Most importantly, their use in myeloid leukemia is restricted by the lack of antigens that are uniquely expressed by the leukemic cell as compared to their counterparts, the hematopoietic stem/progenitor cells.

6.1.2.2. NK cell therapies. It is now feasible to expand NK cells *ex vivo* for cell therapy. NK cells can be selected with magnetic beads coated with CD56 from an apheresis collection and can be expanded with Epstein–Barr virus (EBV) transformed B cell lines or K562 cell lines expressing costimulatory molecules and membrane-bound IL-15 or IL-21 [84,85].

In a study by Meyer-Monard et al., the production of NK cells under good manufacturing practice (GMP) conditions in a sufficient number was examined [86]. Twenty-four apheresis procedures and subsequent NK-cell enrichment from 14 haplo-identical donors were performed. NK-cell enrichment was performed using a GMP suitable immuno-magnetic procedure. Factors influencing the NK-cell recovery, purity, and NK-cell dose were analyzed. A median number of 4.9×10^8 NK cells were obtained and median NK-cell recovery was 58%. Median T-cell depletion was 4.32 log. The absolute NK-cell number in the final product after processing significantly correlated with the preharvest NK-cell content of the peripheral blood (p=0.002; r =0.867). Early trials show that NK cell infusions are without the risk of GvHD. In a pilot study by Passweg et al., the feasibility of natural killer cell purification and infusion (NK-DLI) in patients after haplo-identical HSCT was examined [87]. The aim was to obtain ≥1.0×10^7/kg CD56+/ CD3- NK cells and < 1.0×10^5/kg CD3+ T cells. Mononuclear cells were collected by 10 l leukapheresis. A two-step ex vivo procedure was used to purify NK cells, using an immunomagnetic T-cell depletion, followed by NK-cell enrichment. Five patients with high-risk myeloid malignancies were included, presenting 3–12 months after a haplo-identical HSCT with mixed chimerism (3), impending graft failure (1) or early relapse (1). The purified product contained a median of 1.61×10^7/kg (range 0.21–2.2) NK cells and 0.29×10^5/kg (0.11–1.1) T cells. A purity of NK cells of 97% (78–99), a recovery of 35.5% (13–75), and a T-cell depletion of 3.55 log (2.9–4.5) was achieved. Infusions were well tolerated and none of the patients developed GvHD. They observed an increase in donor chimerism in 2/5, stable mixed chimerism, decreasing chimerism and relapse of AML in one patient each. Selection of NK-DLI is technically feasible. NK cells are well tolerated when used as adoptive immunotherapy in recipients of haplo-identical HSCT. Two recent uncontrolled studies suggest that NK cells may be efficacious in preventing relapse or treating refractory AML [88,89]. After lymphodepleting treatment with cyclophosphamide and fludarabine, haploi-dentical NK cells were administered to children with AML in first CR. The 2 year event-free survival was 100% [89]. A similar lymphodepletion/haploidentical NK cell infusion study in 19 adults with refractory AML achieved 5 complete remissions [88]. These results are preliminary and need further validation.

6.1.2.3. Immune checkpoint blockade. Programmed cell death protein 1 (PD-L1) blockade on the tumor cell or PD1 blockade targeting the T cell is emerging as one of the most powerful strategies to enhance T-cell effectiveness against cancer [90]. Antibodies or blocking molecules have been developed targeting the key checkpoint inhibitors. Most experience with these agents comes from the field of solid tumor immunotherapy [91–93]. However, anti-cytotoxic T lymphocyte antigen-4 (CTLA-4) has been used in a preclinical AML model [51] and also for treatment of patients with post-HSCT relapse [94]. This study demonstrated that CTLA-4 inhibition with ipilimumab does not increase the risk of GvHD, even in patients who received DLI in conjunction [95]. While promising responses were achieved, the study was too small to clearly identify efficacy of the agent in AML. Ongoing trials are testing the safety and efficacy of CTLA-4 blockade in both post-transplant relapse settings and nontransplant settings [ClinicalTrials. gov identifier: NCT00060372, NCT01757639, NCT01822509]. Similarly, preclinical data for PD-1/ PD-L1 blockade have shown that the target has therapeutic potential in leukemia [96]. PD-1/PD-L1 blockade has been tested and actively investigated in

various hematologic malignancies [97] [ClinicalTrials.gov identifier: NCT02117219, NCT01953692, NCT02332980], but clinical experiences in AML remain limited. In a phase I study of anti-PD-1 antibody for hematologic malignancies that enrolled total 17 patients, 8 patients had AML [98]. None of the patients experienced toxicity attributable to the agent. Majority of them did not show any response, except for one patient who demonstrated marked reduction in peripheral blood blasts burden. The result suggests that PD-1/PD-L1 blockade can be effective in a selected setting. While checkpoint inhibition is a promising novel treatment, further studies are required to understand the most efficacious methods of administration to exploit the possible clinical benefit. In a recent study by Davids et al., the immune checkpoint blockade established by targeting CTLA-4 with ipilimumab was examined in patients after allo-HSCT. Their early-phase data showed that administration of ipilimumab was feasible in patients with recurrent hematologic cancers after allo-HSCT, although immune-mediated toxic effects and GvHD occurred. Durable responses were observed in association with several histologic subtypes of these cancers, including extramedullary acute myeloid leukemia [99].

6.1.2.4. Immunomodulatory drugs (IMiDs). Because lenalidomide induces morphological and cytogenetic remissions in MDS patients, including those with excess of blasts, efficacy in AML was expected and evaluated. In a phase II trial by Chen *et al.,* the efficacy and safety of lenalidomide in patients with relapsed/refractory AML (n =18) and high-risk MDS (n =9) with chromosome 5 abnormalities was assessed [100]. Eighteen adults with AML and 9 with high-risk MDS were enrolled. Lenalidomide was given orally at doses 5–25 mg daily for 21 days of a 28-d cycle until disease progression or unacceptable adverse event. Median age for all 27 patients was 64 years. Two patients (7%) with AML and 5q deletion and +8 cytogenetic abnormalities in 2 separate clones achieved CR or CR without platelet recovery (CRp). Response durations were 4 and 6 months, respectively. No responses were seen in patients with chromosome 5 abnormality in a complex cytogenetic background. Thus, activity of lenalidomide was limited to patients with noncomplex cytogenetics.

Recently, it was shown that increased miR-181a expression was associated with improved outcomes in CN-AML. Lenalidomide treatment enhances the C/EBPα-p30 protein levels (which correlates with a favorable chemotherapy response) and in turn miR-181a which appears to sensitize AML blasts to chemotherapy [101].

7. Conclusions

AML is a complex disease with a diverse genetic landscape. The field is rapidly expanding with increased understanding of the pathophysiology. Although allogeneic stem cell transplant has been traditionally considered to be the best strategy in this setting, the available data suggest that it may not be the most effective strategy to eradicate 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 to eradicate persistent minimal residual disease remaining after cytotoxic regimens or will be used to "bridge" to transplant.

Conflict of interest

All of the authors have no conflict of interest to report.

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