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REVIEW ARTICLE

Post-remission strategies for the prevention of relapse following allogeneic hematopoietic cell transplantation for high-risk acute myeloid leukemia: expert review from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation

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Abstract

Acute myeloid leukemia (AML) is an aggressive hematopoietic malignancy generally associated with poor prognosis. Allogeneic hematopoietic cell transplantation (alloHCT) continues to be the most potent anti-leukemia treatment for adult patients with intermediate and high-risk AML. However, disease relapse after alloHCT remains unacceptably high and is the primary cause of treatment failure and mortality following alloHCT. It is therefore that post-transplant early cellular or pharmacologic maintenance or preemptive strategies to enhance the graft-versus-leukemia effect or to eradicate persistent minimal residual disease have been of renewed interest, particularly with the availability of more sensitive technologies to measure residual AML. Although preliminary studies have demonstrated improved outcomes with the use of post-alloHCT remission therapies, prospective randomized trials are required to determine their clinical efficacy and role in the treatment of AML. On behalf of the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation, we summarize the available evidence on the use and efficacy of available pharmacologic post-remission therapies, including hypomethylating agents, deacetylase inhibitors, and tyrosine kinase inhibitors, as well as cellular therapies, including preemptive and prophylactic donor lymphocyte infusions for the prevention of relapse of AML.

Introduction

Over the past decade, the international blood and marrow transplantation (BMT) community have witnessed significant therapeutic advances in allogeneic hematopoietic cell transplantation (alloHCT) for acute myeloid leukemia (AML). Development of improved supportive care, reduced-intensity conditioning (RIC) regimens, thereby extending transplants to adults age 60 and older and/or with co-morbidities, and transplant procedures using alternative donors and stem cell sources have resulted in 3–5-year overall survival (OS) rates ranging from 20 to 90%,

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depending on the AML risk profile and stage and the presence or absence of minimal residual disease (MRD) [1–3]. However, disease relapse accounts for ~40% of treatment failures, with a preponderance of failures occurring in the first year following alloHCT [4]. Therapeutic options for post-transplant relapse include best supportive care, rapid withdrawal of immunosuppression, low or high-intensity chemotherapy followed by cellular immunotherapy, such as a second transplantation in selected cases or donor lymphocyte infusions (DLI). However, outcomes following these salvage treatments are generally poor [4, 5]. Several studies have also shown worse survival among patients with early relapse (< 6 months) from time after alloHCT [4–6], leading to stronger rationale for early prevention strategies.

Increasing understanding of the immune-biology and molecular landscape of AML has resulted in targeted and other biologically directed therapies against minimal residual tumor burden while providing immunomodulatory support for the graft-versus-leukemia (GVL) effect by enhancing allogeneic immune responses. In addition, knowledge of pre-transplant parameters associated with a high relapse risk such as high-risk karyotype and/or mutations and molecular markers, advanced disease stage, or the presence of MRD has allowed selection of patients who would benefit most from post-remission prevention strategies. As older age in itself may be a risk factor for relapse, patients age ≥ 60 may also qualify for post-transplantation maintenance therapy. Other factors associated with a greater risk of AML relapse in the post-transplantation setting are related to the presence of pre-transplant or post-transplant MRD, RIC regimens, the absence of chronic GVHD (cGVHD), loss of donor chimerism, use of in vivo or ex vivo T-cell-depleting mechanisms, and although controversial, high-dose post-transplant cyclophosphamide (PTCy) for the prevention of GVHD [7, 8]. The on-going study BMT CTN 1301 (NCT02345850) that randomizes recipients of HLA-matched related and unrelated donor transplantation to three prophylaxis strategies for GVHD, two being infusion of bone marrow grafts followed by either PTCy or standard calcineurin inhibitor-based regimen, may better elucidate the effect of PTCy on disease relapse. At last, the development of more sensitive technologies continues to change our ability to measure remaining disease burden following alloHCT and allows direct evaluation of the efficacy of a specific post-remission treatment. This evolution has led to a new wave of active investigation into the role of post-remission maintenance or preemptive therapies to mitigate the risk of AML relapse following alloHCT. As several of these agents also have activity in myelodysplastic syndrome (MDS), we discuss the available and potential candidate therapies for prevention of relapse of AML and MDS in the post- transplantation setting.

Maintenance vs. preemptive strategies to prevent relapse

Maintenance treatments may be an effective strategy to provide early tumor control and immunomodulatory support for the GVL effect provided by allogeneic immune responses in patients with high-risk AML. However, for patients with intermediate-risk AML, maintenance therapies may represent over-treatment and expose patients who are cured of their disease to adverse long-term and late effects of therapy. Therefore, preemptive treatment strategies that employ monitoring for MRD after transplantation by use of flow cytometry, cytogenetic, or molecular assays, may avoid unnecessary treatment exposures while achieving similar success in preventing morphologic relapse. Prior to the advent of novel pharmacologic and cellular treatments, early withdrawal of immune suppression with or without prophylactic DLI were considered. However, these interventions have been limited by the development of significant GVHD and marrow aplasia, and in the setting of DLI, has limited activity outside of chronic myeloid leukemia (CML) [9, 10]. The development of epigenetic therapies that have the ability to expand T regulatory cells has led to renewed interest in evaluating the efficacy of concurrent administration with DLI for prevention of relapse in AML [11].

Hypomethylating agents

The hypomethylating agents, azacitidine and decitabine, reverse DNA hypermethylation employed by tumor cells to inhibit genes responsible for growth inhibition, differentiation, and apoptosis of cancer cells. Other mechanisms of hypomethylating agents include cytoreductive and pro-differentiation effects on leukemic cells [12]. Both azacitidine and decitabine have demonstrated meaningful clinical activity in front-line therapy for AML and MDS in older patients unable to tolerate intensive chemotherapy [13, 14]. In the transplant setting, azacitidine and decitabine may augment the GVL response through upregulation and re-expression of epigenetically silenced genes related to major histocompatibility complex class I, human leukocyte antigen DR-1 and tumor-associated antigens [15]. CD8 T-cell responses against tumor antigens are augmented by these changes [16] and also in part by increased expression of killer-cell immunoglobulin-like receptor on T cells [17]. In the setting of relapsed AML following allogeneic transplantation, low-dose azacitidine has shown favorable anti-leukemia activity and toxicity profile without exacerbating GVHD [18–22]. Subsequent research using murine models showed that immunomodulation of GVHD without the loss of GVL may result from expansion of regulatory T cells

exerted by azacitidine and decitabine [15, 16, 23, 24]. Collectively, these studies have laid rationale to examine the safety and efficacy of hypomethylating agents following alloHCT for the prevention of AML/MDS recurrence.

Azacitidine

Azacitidine has been the most extensively studied for this indication. Investigators at MD Anderson Cancer Center were the first to suggest efficacy for post-transplant azacitidine based on a retrospective case series of patients treated at their center [18].

A follow-up phase I dose-finding study of low-dose azacitidine following RIC alloHCT for 45 patients with AML/high-risk MDS in CR1 or greater demonstrated that azacitidine can be safely administered to heavily pretreated post-transplant patients [25]. A total of 45 patients (AML, $n = 37$; MDS, $n = 8$) with median age of 60 years who were in complete remission (CR) by day 30 following alloHCT received subcutaneous azacitidine starting at day + 40 post transplantation. The investigators defined a maintenance regimen of 32 mg/m^2/day, days 1–5 every 30 days, for four cycles as optimal. With a median follow-up time of 20.5 months (range, 7.7–39.6 months), 24 patients (53%) developed disease recurrence, with 7 recurrences occurring while on azacitidine. Nineteen (42%) patients died at a median of 30.8 months (95% confidence interval (CI), 14.3 months-upper limit not estimable) and 15 of these deaths were attributed to disease recurrence. One-year event-free survival and OS were 58 and 77%, and whereas definite associations between acute GVHD (aGVHD) and azacitidine administration could not be determined, cGVHD may have been diminished with longer schedules of azacitidine [25]. At present, a phase III randomized controlled study comparing 1-year of azacitidine maintenance therapy (32 mg/m^2/d for 5 days every 28 days) vs. no maintenance for prevention of relapse after alloHCT in patients with intermediate or high-risk AML/MDS is ongoing (NCT00887068).

In a single-arm, non-randomized, phase II study of maintenance azacitidine following RIC alloHCT for AML (RICAZA), Craddock et al. [26] reported on the safety and tolerability of subcutaneous azacitidine 36 mg/m^2 on days 1–5 every 28 days beginning on day + 42 after alloHCT for up to 12 months after transplantation in 37 patients. A preliminary analysis focused on biological parameters in the first 27 enrolled patients was reported by Goodyear et al. [16]. In the updated analysis, the median follow-up time was 24 months and 31 patients completed at least three cycles of azacitidine, whereas 16 (50%) patients completed 10 cycles. Sixteen patients relapsed at a median time of 8 months after transplantation, and a total of 19 (51%) deaths occurred with disease relapse as the cause in 16 (84%) cases. The 1-year and 2-year OS were 81% (95% CI, 69–95%) and 49% (95% CI, 35–68%), respectively. Grade 3–4 aGVHD did not occur during treatment with azacitidine and there were no cases of extensive cGVHD. Azacitidine was generally well-tolerated in the majority of patients. Correlative studies showed an association between induction of peripheral blood antitumor CD8$^+$ T-cell responses to one or more tumor-specific peptides in azacitidine-treated patients with a decrease in relapse risk (HR, 0.30; 95% CI, 0.10–0.85; $P = 0.02$) and improved relapse-free survival (RFS) (HR, 0.30; 95% CI, 0.1–0.94; $P = 0.039$). The ability of azacitidine to induce CD8 + tumor-specific T-cell responses, coupled with the expansion of T regulatory cells, support the notion that adjunctive administration of therapies that epigenetically modify the GVL effect without enhancing GVHD following transplantation is a feasible and rationale therapy.

Owing to the potential immunomodulatory effects of azacitidine in the post-transplantation setting, Platzbecker et al. [27] prospectively administered azacitidine to patients as preemptive treatment for prevention of AML/MDS relapse based on MRD as determined by decreasing CD34$^+$ donor chimerism (< 80%) analysis (RELAZA study). Twenty patients with MRD$^+$ hematologic remission following alloHCT received four cycles of azacitidine at 75 mg/m^2/day for 7 days. An improvement in donor chimerism to ≥ 80% or stabilization of chimerism was observed in 80% of patients. Eleven patients with stable chimerism or subsequent decrease to < 80% after the initial response received a median of four additional cycles. Although hematologic relapse occurred in 13 patients (65%), this was delayed until a median of 231 days (range, 56–558) from the initial decrease of CD34$^+$ donor chimerism. Similarly, the German Cooperative Transplant Study Group also reported outcomes of their retrospective multicenter analysis of 154 patients with AML ($n = 124$), MDS ($n = 28$), and myeloproliferative syndromes ($n = 2$) who had hematologic or molecular relapse of disease and were treated with a median number of four cycles of azacitidine and planned DLI [21]. Although the complete remission rate was 27% and 2-year OS was 29% for the entire group, those who were treated with molecular-only relapse had better OS (HR, 0.14; 95% CI, 0.03–0.59; $P = 0.007$). Results from this study and RELAZA demonstrate the feasibility of preemptive use of azacitidine in the setting of MRD and warrant further prospective study.

Oral azacitidine

The epigenetic modifier CC-486 is an oral formulation of azacitidine with promising clinical activity in patients with high-risk AML/MDS in Phase I/II studies. Extended azacitidine dosing with sustained DNA hypomethylation over a

treatment course and long-term treatment with oral administration of azacitidine have the potential to enhance demethylation among cycling malignant cells, translating to improved clinical outcomes. Final results from the QUAZAR phase I/II multicenter dose and schedule finding study of oral azacitidine as maintenance therapy after reduced-intensity or myeloablative alloHCT for AML/MDS were reported [28]. Treatment with one of four daily dosing schedules (CC-486 200 mg or 300 mg for 7 days, 150 mg or 200 mg for 14 days) in repeated 28-day cycles was started between day + 42 and day + 84 after alloHCT. Thirty evaluable patients (AML, $n = 26$; MDS, $n = 4$) were included. The maximum tolerated dose (MTD) was not reached and the CC-486 200 mg daily for 14 days dosing was additionally assessed. Ten of nineteen patients (53%) completed all 12 treatment cycles, and with a median follow-up for all patients of 19.0 months (range 1.0–41.3), 8 of 30 patients (27%) had relapsed or progressive disease. The median relapse- and progression-free survival (RPFS) for all patients was 34.1 months (95% CI 15.3, 37.8), whereas median RPFS and median OS were not reached in the combined 14-day dosing groups. One-year RPFS and OS in the 14-day dosing cohorts were 72% and 81%, respectively. The most common grade 3–4 treatment emergent adverse events were related to gastrointestinal and hematologic parameters, and two of eight patients experienced severe cGVHD. A randomized, phase III trial evaluating CC-486 at the 200 mg 14-day dosing regimen as maintenance therapy following transplantation for high-risk MDS and intermediate- or high-risk AML is in development.

Decitabine

A dose-escalation and safety study of decitabine as maintenance therapy after reduced-intensity or myeloablative allogeneic transplantation for AML/MDS was reported by Pusic et al. [29]. Four decitabine doses (5, 7.5, 10, and 15 mg/m^2/day) given on days 1–5 in 6-week cycles for up to eight cycles were investigated in cohorts of four patients among 22 AML/MDS (AML, $n = 17$; MDS, $n = 5$) patients in CR after alloHCT to determine the MTD. Treatment began between day + 50 and day + 100 after transplantation. All four-dose levels were completed and the MTD was not reached; however, the authors concluded that a dose of 10 mg/m^2 for 5 days every 6 weeks was optimal owing to minimal hematological toxicity. Nine of 22 (41%) patients completed eight cycles (median number of cycles completed = 5) and all were alive at the time of the report, with eight of the nine patients remaining in CR. After a median follow-up of 26.7 months (range, 3.4–49.1), six patients relapsed and nine died (four from relapse, three from infections, and two owing to GVHD). The 2-year OS and disease-free survival were 56% (95% CI, 38–83%) and 48% (95% CI, 30–75%), respectively. The 2-year cumulative incidence (CI) of relapse was 28% (95% CI, 8–48%). Decitabine did not seem to influence the incidence of cGVHD and its effect on aGVHD was uncertain owing to the timing of its administration. A correlative study showed a trend for increased FOXP3 expression and Treg cells among the lymphocyte population, but it was not statistically significant.

Additional studies evaluating azacitidine or decitabine as monotherapy or in combination with other therapies for prevention of relapse in high-risk AML/MDS following alloHCT are summarized (Table 1).

Histone deacetylase and hedgehog inihibitors

The class I/II histone deacetylase inhibitors (HDACi) have been explored as potential therapeutic agents in AML/MDS owing to marked induction effects on cell-cycle arrest, cell differentiation, and pro-apoptotic effects on AML cells through epigenetic modifications of nucleosome histones. HDACis have also been reported to affect both immune-suppressive and immune-stimulating responses through the modulation of cytokine expression [30, 31].

Panobinostat (PAN) is an oral, highly potent, and non-selective HDACi reported to have moderate anti-leukemia activity in a small subset of patients with advanced AML and high-risk MDS in early phase studies [32]. It also inhibits the suppressive function of regulatory T cells at low doses while promoting its inhibitory function at higher doses [30], and therefore, may have potential in mitigating GVHD. Using this rationale, Bug et al. [33] investigated PAN as maintenance therapy for patients with high-risk MDS/AML in hematologic CR following RIC alloHCT (PANOBEST). In this phase I/II study of 42 patients (median age, 52), the majority of whom were transplanted with active disease (67%), PAN was administered within 60–150 days after alloHCT and continued for up to 1-year. The phase 1 portion of the trial identified the MTD and dose-limiting toxicity (DLT) of two sequentially tested dosing schedules. In phase II, patients were randomized 1:1 to either schedule at the respective MTD. Phase I results on 23 out of 24 evaluable patients showed a recommended phase 2 dosing of 20 mg TIW for schedule A and 30 mg TIW every other week for schedule B.

In the phase II study, safety analysis on all patients showed that 35 of 42 patients (83%) experienced at least one grade 3–4 adverse events, most of them related to hematologic toxicity, constitutional symptoms, and gastrointestinal symptoms. There were no patient deaths while on treatment or within 28 days of the last PAN dose. More

Table 1 Ongoing studies using epigenetic and immunomodulatory agents as maintenance or preemptive therapy for high-risk AML/MDS following allogeneic hematopoietic cell transplantation

Drug	Active clinical trials
Azacitidine	Phase II study to determine the 2-year PFS of SC or IV azacitidine when started on Day + 42 and given days 1–5 every 4 weeks for six cycles after RIC alloHCT conditioned with Fludarabine, Busulfan, and ATG. Secondary outcomes include rate of aGVHD and cGVHD; 100-day and 1-year TRM, 5- year OS (NCT01168219). Phase II study to determine the 2-year relapse rates of SC azacitidine 32 mg/m^2 daily for 5 days of a 28-day cycle starting between days 60 and 120 post TCD alloHCT and up to a year. Secondary outcomes include 2-year OS and safety) (NCT01995578). Phase II study combining SC or IV azacitidine 40 mg/m^2 daily for 5 days with valproic acid 15 mg/kg daily, days 6–28, for up to four total cycles to determine the 1-year OS. Secondary outcomes include PFS, relapse, and toxicity (NCT02124174). Phase II study of SC or IV azacitidine 40 mg/m^2 with and without DLI based upon disease risk to determine 2-year relapse rate, grade 3 or higher adverse events and aGVHD and cGVHD in pediatric and young adults with acute leukemia (NCT02458235). Randomized phase 3 trial to determine whether SC azacitidine 32 mg/m^2 daily for 5 days up to 12 cycles provides at minimum a 50% improvement in median RFS at 3, 6, and 12 months (NCT00887068).
Decitabine	Phase I study to determine dose and schedule finding of decitabine 5 mg/kg/day to 15 mg/kg/day IV daily for 5 days up to 12 cycles beginning at days 42–90 after alloHCT. Secondary outcomes include OS and RFS; donor chimerism, GVHD; biomarkers of immune recovery (NCT01277484).
Lenalidomide	Phase II study to determine RFS of oral lenalidomide starting at a dose of 10 mg daily, days 1–28, for high-risk AML in remission after alloHCT (NCT02126553).
Hedgehog inhibitor (PF-04449913)	Phase II study to determine 1-year RFS of oral PF-04449913 dosed at 100 mg daily, days 1–28, for up to 12 cycles. Secondary outcomes include OS, remission duration, and toxicity (NCT01841333).

aGVHD acute graft-versus-host disease, *alloHCT* allogeneic hematopoietic cell transplantation, *AML* acute myeloid leukemia, *ATG* anti-thymocyte globulins, *cGVHD* chronic graft-versus-host disease, *CI* cumulative incidence, *CR* complete remission, *DFS* disease-free survival, *DLI* donor lymphocyte infusion, *EFS* event-free survival, *IV* intravaneous, *LFS* leukemia-free survival, *MRD* minimal residual disease, *NGS* next-generation sequencing, *NRM* non-relapse mortality, *OS* overall survival, *PFS* progression-free survival, *RFS* relapse-free survival, *SC* subcutaneous, *SOC* standard-of-care, *TCD* T-cell depleted, *TRM* transplant-related mortality

than half of the patients completed 1-year of treatment with PAN ($n = 22$). The median treatment duration at the MTD was 52 days (range, 11–368) in schedule A vs. 228 days (range, 16–365) in schedule B. Although DLIs were permitted by the study protocol, this did not translate to a significantly higher incidence of GVHD. Only four of 42 patients developed aGVHD and the CI of moderate/severe cGVHD was 29% (95% CI, 16–42%) at 2 years after starting PAN. There was no difference in cGVHD between the two schedule cohorts and all patients with aGVHD received schedule A dosing. After a median follow-up of 22 months, the median OS and RFS had not been reached and the estimated 2-year OS and RFS were 81% (95% CI, 69–95%) and 75% (95% CI, 63–90%), respectively. Non-relapse mortality (NRM) was also low at 5% (95% CI, 0–11%). Owing to the favorable outcomes demonstrated in this study compared with reported survival and relapse rates of similar patient groups, a large European randomized trial to formally test maintenance PAN following alloHCT for high-risk myeloid malignancies is planned [33].

Aberrant hedgehog (Hh) pathway signaling has been hypothesized to contribute to the survival and expansion of leukemia stem cells, and accordingly, the use of an Hh inhibitor can eliminate these cells and decrease relapse rates [34]. The smoothened receptor inhibitor PF-04449913 (glasdegib), an Hh inhibitor, has recently shown promising results in a randomized phase II trial, prolonging median OS by ~3 months when combined with low-dose cytarabine (LDAC) as compared to LDAC alone in AML/MDS patients ineligible for intensive chemotherapy [35]. With this background, use of PF-04449913 is under investigation in a phase II study as a maintenance agent following alloHCT for patients at high-risk of relapse (NCT01841333).

Inhibitors of flt3 tyrosine kinase

Activating mutations of the FMS-like tyrosine kinase 3 (*FLT3*) receptor are present in ~30% of patients with newly diagnosed AML and are associated with an overall poor prognosis [36]. *FLT3*-internal tandem duplications (ITD) of exons 13–15, coding for the juxtamembrane of FLT3 receptor region are the most frequent genetic aberrations and are found in 23% of *de novo* AML [36]. Mutations in *FLT3-ITD* confer downstream signaling pathways involving *STAT5*, *PI-3-kinase/AKT*, and *RAS/MAPK*, leading to abnormal leukemia cell growth, differentiation, and survival [37, 38]. Other contributing factors to unfavorable outcomes are the presence of high *FLT3* mutant allelic burden as compared with patients with low or intermediate allelic ratios [39], long ITD length [40],

Table 2 Ongoing studies using FLT3 inhibitors as maintenance therapy for FLT3-ITD AML following allogeneic hematopoietic cell transplantation

FLT3 inhibitor	Active clinical trials
Sorafenib	Pilot study evaluating the safety of sorafenib before and after alloHCT for patients with FLT3-ITD AML. Secondary outcomes include CI NRM, and relapse, DFS, OS, change in FLT3 inhibition and MRD by flow cytometry (NCT01578109).
	Phase II study evaluating sorafenib vs. placebo as post-alloHCT maintenance therapy on 42-month RFS in patients with *FLT3-ITD* AML. Secondary outcomes include median OS, median RFS, and OS according to NPM1 status, median RFS, and OS according to baseline *FLT3-ITD* allelic burden, safety and toxicity, and biomarker assessments (EudraCT 2010-018539-16)
	Phase II/III study evaluating sorafenib maintenance therapy vs. usual SOC following alloHCT on the incidence of leukemia relapse in FLT3-ITD AML. Secondary outcomes include OS, LFS, and safety (NCT02474290).
Midostaurin	Phase II study evaluating SOC with midostaurin vs. SOC following alloHCT on RFS among patients with *FLT3-ITD* AML who undergo alloHCT. Secondary outcomes include DFS, NRM, OS, FLT3-ITD mutation status (i.e., allelic ratio), safety, and tolerability (NCT01883362).
	Phase II study evaluating midostaurin with intensive induction, consolidation including alloHCT, and single-agent maintenance therapy on EFS in patients with *FLT3-ITD* AML. Secondary outcomes include CR, RFS, OS, CI relapse, and death in CR, FLT3 inhibition, QoL, and safety (NCT01477606).
Quizartinib	Phase III study evaluating quizartinib vs. placebo with standard induction, consolidation chemotherapy with or without alloHCT, then as maintenance therapy on EFS in patients with *FLT3-ITD* AML. Secondary outcomes include 2-year OS, rates of CR and composite CR after the 1st induction cycle, and CR with no MRD (NCT02668653).
Crenolanib	Phase II study evaluating crenolanib maintenance therapy following alloHCT for patients with *FLT3-ITD* AML. Secondary outcomes include 2-year DFS, OS, and GVHD, and 100-day TRM (NCT02400255).
Gilteritinib	Phase III study evaluating gilteritinib maintenance therapy vs. placebo following alloHCT for patients with *FLT3-ITD* AML on RFS. Secondary outcomes include OS, EFS, MRD using a novel NGS assay, and safety (BMT CTN 1506; NCT02927262 and NCT02997202).

alloHCT allogeneic hematopoietic cell transplantation, *AML* acute myeloid leukemia, *CI* cumulative incidence, *CR* complete remission, *DFS* disease-free survival, *EFS* event-free survival, *LFS* leukemia-free survival, *MRD* minimal residual disease, *NGS* next-generation sequencing, NRM non-relapse mortality, *OS* overall survival, *QoL* quality-of-life, *RFS* relapse-free survival, *SOC* standard-of-care, *TRM* transplant-related mortality.

and the location of the insertion site within the tyrosine kinase domain [39].

As multiple studies indicate AML harboring a *FLT3-ITD* mutation is a poor prognostic marker for relapse and survival in patients receiving chemotherapy alone [41–43], alloHCT has been widely recommended by expert panels as post-remission consolidation treatment for patients in first CR [44]. Despite the reported benefit conferred by alloHCT for *FLT3-ITD* AML, the literature also suggests that early relapse after transplant remains a threat for patients with *FLT3-ITD* AML as compared with other AML subtypes [36, 45, 46]. Therefore, for many patients, the current standard-of care consisting of induction chemotherapy followed by post-remission consolidation with alloHCT is inadequate. It has been postulated that *FLT3-ITD* AML originates as a polyclonal disease at diagnosis and can be successfully suppressed by conventional chemotherapy and intense post-remission therapy, however, clonal evolution may produce a dominant *FLT3*-dependent clone at the time of relapse for, which targeted therapy is necessary [47]. With this rationale, several small molecules capable of inhibiting *FLT3* tyrosine kinase activity have been developed and evaluated for use in the induction-remission, relapsed/refractory, and post-alloHCT setting. The paradigm of maintenance therapy following alloHCT for AML has garnered renewed interest, as reports of decreased incidence of relapses and improved outcomes with prophylactic use of *BCR-ABL* tyrosine kinase inhibitors (TKIs) following alloHCT for patients with high-risk CML or Ph + acute lymphocytic leukemia [48, 49]. In addition, *FLT3* inhibitors may function similarly to *BCR-ABL* TKIs and not only directly target residual leukemia cells but also synergize with allo-reactive donor cells and augment the GVL response [50]. Therefore, we summarize current ongoing clinical trials of various *FLT3* inhibitors under evaluation as maintenance therapy following alloHCT (Table 2).

Sorafenib

Sorafenib is a small inhibitor of several tyrosine protein kinases, such as *FLT3-ITD*, *RAF* kinase, *PDGFR*, *VEGFR*, *c-KIT*, and others [51]. As sorafenib demonstrated some clinical activity in the post-transplant relapsed setting [52, 53], the research focus shifted to determining its value for prevention of relapse following alloHCT. The data supporting sorafenib as post-transplantation maintenance therapy are based upon single-arm studies or retrospective reports. Chen et al. [54] reported results of the first phase I trial using sorafenib as maintenance therapy following transplantation for any patient with *FLT3-ITD* AML in CR after transplantation. Sorafenib was administered to 22 patients beginning between days 45 and 120 after first

alloHCT with any donor source, conditioning regimen, and GVHD prophylaxis and was given continuously in 28-day cycles for up to 12 cycles. Following a 28-day DLT period, the MTD was set at 400 mg twice daily. With a median follow-up for surviving patients of 16.7 months after alloHCT (range, 8.1–35.0 months), 1-year progression-free survival (PFS) was 85% (90% CI, 66%-94%) and 1-year OS was 95% (90% CI, 79–99%). For the cohort of patients who were in CR1/CR2 prior to HCT ($n = 19$), 1-year PFS was 95% (90% CI, 76–99%) and 1-year OS was 100%. Sorafenib was reasonably well tolerated, with grade 2–3 rash (36%), diarrhea (32%), and anemia (27%) as the most commonly adverse events reported. Only one patient developed aGVHD (grade II disease of the skin) after starting sorafenib maintenance. The CI of cGVHD in the 12 months after starting sorafenib (38%, 90% CI, 21–56%) was similar to historical controls [54].

This group of investigators further performed a retrospective analysis comparing outcomes of consecutive patients with *FLT3-ITD* AML who received transplantation in first CR and received sorafenib maintenance ($n = 26$) vs. those who did not ($n = 43$) [55]. A landmark analysis was performed beginning at day 68 after transplantation, as this was the median time to initiation of sorafenib. Sorafenib maintenance was associated with a significantly superior 2-year OS (81% vs. 62%, $P = 0.029$), PFS (82% vs. 53%, $P = 0.0081$), and lower 2-year CI of relapse (8.2% vs. 37.7%, $P = 0.0077$). The rates of NRM or cGVHD were no different between groups. Meanwhile, other retrospective studies have also reported encouraging outcomes with the use of sorafenib as post-alloHCT maintenance therapy [56, 57].

Sorafenib as post-transplant maintenance therapy for *FLT3-ITD* AML continues under active investigation across the world. In the United States, a National Cancer Institute sponsored study (NCT01578109) has completed accrual for their prospective trial evaluating the safety and outcomes of sorafenib administered peri-transplant and as post-transplant maintenance therapy. Pratz et al. [58] reported preliminary data on 28 patients with *FLT3-ITD* AML who underwent transplantation in CR. With a median duration of post-transplant follow-up of 450 days (range, 107–1192) and a median duration of sorafenib therapy of 252 days (range, 52–1081), a total of five relapses and six deaths occurred (three due to relapse). Nine patients developed grade 2 or higher GVHD requiring escalation of immunosuppression therapy.

The ongoing multicenter European study, SORMAIN (EudraCT 2010-018539-16), is a phase II, double-blinded, placebo-controlled, randomized trial to assess the efficacy of sorafenib maintenance therapy in *FLT3-ITD* AML in complete hematological remission after alloHCT. Study treatment begins at day 60–100 after transplant. Clinical objectives include determination of RFS, and comparison of median RFS and OS in *FLT3-ITD* patients receiving sorafenib vs. placebo, accounting for NPM1 mutations and baseline *FLT3-ITD* allelic burden. The final results of these prospective studies are eagerly anticipated and should provide additional evidence regarding tolerability and efficacy of maintenance sorafenib following alloHCT in patients with *FLT3-ITD* AML.

Midostaurin

Midostaurin (PKC412) is a first-generation oral multikinase inhibitor of both wild-type and mutated *FLT3*, *VEGFR2*, *c-KIT*, and *PDGFR* [59, 60]. It was recently approved by the US Food and Drug Administration (FDA) for upfront treatment in combination with chemotherapy for patients with newly diagnosed *FLT3*-mutated AML based on the superior OS and EFS reported in the phase III randomized CALGB 10603/RATIFY trial [61]. Concurrently, midostaurin is also under investigation as a potential maintenance therapy following alloHCT to prevent AML relapse. The RADIUS trial (NCT01883362) is an ongoing phase II, randomized trial to determine whether the addition of post-transplant maintenance therapy with midostaurin 50 mg twice daily to standard-of care will reduce relapse following myeloablative alloHCT from a matched related or unrelated donor in patients with *FLT3-ITD* AML. In this study, midostaurin is administered beginning days 28–60 post transplantation, every 28 days for 12 cycles. The primary study endpoint is RFS determined at 18 months from date of transplant.

The German-Austrian AMLSG 16-10 trial (NCT01477606) is currently accruing to their phase II, single-arm study of midostaurin 50 mg twice daily in combination with intensive induction, peri-transplant, and as single-agent maintenance therapy after alloHCT for patients with *FLT3-ITD* AML. Preliminary results from 147 patients who underwent induction therapy with midostaurin and conventional chemotherapy revealed an overall CR of 75% and death rate of 7.5%. Maintenance therapy was started in 52 patients (40 patients after alloHCT and 12 patients after consolidative chemotherapy). Initial analyses showed a low CI of relapse irrespective of the *FLT3-ITD* allelic burden. Most frequent grade 3–4 adverse events (AEs) reported during the first induction cycle included gastrointestinal AEs ($n = 34$) and infections ($n = 81$) [62].

Quizartinib

Quizartinib (AC220) is a highly potent, second-generation receptor tyrosine kinase inhibitor (TKI) developed specifically as a FLT3 inhibitor. It addition to its specificity for

FLT3-ITD, quizartinib is associated with a significantly longer half-life in vivo and has greater duration of FLT3 inhibition than first-generation FLT3 inhibitors [63]. Phase II studies of patients with relapsed/refractory *FLT3-ITD* AML showed quizartinib to have a high level of single-agent activity [64, 65].

Sandmaier et al. [66] reported on the safety of quizartinib maintenance in a phase I, dose-escalation study of 13 patients who achieved CR after a matched alloHCT. Patients were enrolled into two dose cohorts; 40 mg daily ($n = 7$) and 60 mg daily ($n = 6$), and began maintenance therapy between days 30 and 60 after transplantation for up to 24 continuous treatment cycles (28-day cycles). At the time of the report, 10 patients had received quizartinib for more than a year, 6 were currently receiving treatment, and 2 subjects completed 24 cycles. One out of 13 patients relapsed (after 22 days on study) and three subjects discontinued due to AEs including grade 4 neutropenia, grade 2 corneal epithelium defects, and grade 3 autoimmune hemolysis. Otherwise, toxicities were manageable. Quizartinib maintenance did not appear to increase the rate of GVHD. At present, the global phase III, randomized and placebo-controlled study, QuANTUM-First (NCT02668653), is determining the efficacy of quizartinib as adjunctive therapy to standard induction and consolidation chemotherapy followed by single-agent maintenance therapy on survival outcomes in newly diagnosed patients with *FLT3-ITD* AML. Although the available studies show encouraging activity of single-agent quizartinib, others have reported emerging mechanisms of resistance to quizartinib [67, 68] and indicate the need for FLT3 inhibitors with activity against resistance-conferring point mutant forms of FLT3.

Crenolanib

Crenolanib besylate (CP-868-596) is a potent orally bioavailable type 1 FLT3 TKI with activity against *FLT3-ITD* and resistance-conferring *FLT3-D835 TKD* mutants [69]. A phase II study of crenolanib given to 38 relapsed/refractory patients (FLT3 TKI-naive: 13 patients; progressed on prior FLT3 TKI: 21 patients) showed an overall response rate of 47% at a median follow-up time of 14 weeks [70]. Commonly reported AEs included nausea and vomiting, and elevated liver enzymes [70, 71]. Crenolanib is also under investigation as maintenance therapy in AML patients with FLT3 mutations who have achieved CR after alloHCT (NCT02400255). In this phase II trial, crenolanib is given to a cohort of patients who achieved CR prior to alloHCT and to a second cohort of patients who underwent alloHCT with incomplete recovery but with ≤ 10% bone marrow blasts. Maintenance therapy starts between days 45 to 90 after HCT and for up to 2 years.

Gilteritinib

Gilteritinib (ASP2215) is a novel small molecule with potent inhibitory activity against both *FLT3-ITD* and *FLT3-TKD* mutations. Like crenolanib, gilteritinib is active against most resistance-conferring point mutant forms of *FLT3*, specifically the kinase domain mutations at residue D835 and the gatekeeper mutation at residue F691 [67]. Early phase I/II trials showed gilteritinib to be well-tolerated and have highly potent antileukemic activity in patients with heavily pretreated relapsed/refractory AML regardless of prior TKI treatment, and a current randomized phase III trial is analyzing the value of gilteritinib as first salvage therapy in relapsed/refractory AML patients with *FLT3* mutation [72–74]. Owing to its minimal side-effects and potent inhibition of FLT3 in vivo, the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) is conducting an international, randomized, double-blind, placebo-controlled phase III trial to evaluate the potential benefit of maintenance therapy with gilteritinib vs. placebo following transplant for patients with *FLT3-ITD* AML in first morphologic CR undergoing alloHCT (NCT02927262 and NCT02997202). Stratification of participants is based on the conditioning regimen intensity, time from transplantation to randomization, and the presence or absence of MRD from the pre-registration bone marrow aspirate. Patients randomized to gilteritinib will receive a dose of 120 mg and all participants will continue maintenance therapy for 2 years. The primary study objective is to compare RFS between the two arms. A significant component of this study will be the use of a next-generation sequencing (NGS) assay to assess pre-transplant and monitor post-transplant MRD status by detection of *FLT3-ITD*. Correlation between MRD status and clinical outcomes will be necessary to validate the use of a NGS platform for monitoring of MRD and furthermore, may allow for the design of future clinical trials in *FLT3-ITD* AML using MRD as an endpoint.

Cellular and targeted immunotherapies

Cellular therapies to enhance the GVL effect are currently under evaluation and have the potential to treat MRD and prevent recurrence of AML. DLI has become one standard therapeutic approach to treat AML/MDS relapse following alloHCT, however, its efficacy and toxicity varies across studies. Therefore, the use of DLI as a prophylactic and preemptive intervention to harness the GVL effect prior to relapse has been more recently explored. In a prospective study of 75 patients with high-risk AML/MDS in which sequential DLI was given to patients after day + 120 of RIC alloHCT and who were off immunosuppression and were

free of GVHD, outcomes compared favorably with previously reported results following standard conditioning, with 2-year OS and leukemia-free survival of 42% and 40%, respectively [75]. In another retrospective analysis of long-term outcomes following prophylactic or preemptive DLI after RIC alloHCT for high-risk AML, OS at 7 years after transplant was 67% as compared with 31% in the control group ($P < 0.001$) [76]. In a prospective phase II study incorporating maintenance therapy combining azacitadine and DLI following myeloablative or RIC alloHCT (VIDAZA-DLI) for the prevention of relapse in high-risk AML/MDS, preliminary data showed a cumulative incidence of relapse at 3 years of 28% and 3-year OS of 66%. Together, the data suggest that either prophylactic or preemptive DLI may have a role in the prevention of AML/MDS relapse, however, further investigation in regards to timing, dosing, product manipulation, and co-administration with other agents is required [77].

Adoptive cell therapy using haploidentical natural killer (NK) cell enrichment and adoptive transfer have resulted in AML remissions [78, 79]. Genetically engineered antigen-specific T cells targeting AML-specific antigens such as Wilms tumor-1 are under active investigation [80]. Other candidate antigens for construction of chimeric antigen receptor T cells include CD123 [81], CD33 [82], and folate receptor β [83]. Targeted immunotherapeutic approaches including monoclonal antibodies directed against AML antigenic targets (i.e., CD33, CD123, CLEC12A), immune checkpoint inhibitors against PD-1 and CTLA4, and bispecific T-cell engagers are currently in early clinical trials [84]. At last, the contribution of donor-derived B cells that produce cytotoxic antibodies directed against AML cell surface antigens may contribute to the GVL effect and antitumor responses [85].

Perspectives

Significant progress in the understanding of the key molecular, epigenetic, metabolic, and immunological processes in AML has led to novel and broad array of therapies for the treatment of AML. In addition to epigenetic modifiers and FLT3 inhibitors, other drugs such as liposomal cytarabine-daunorubicin (CXP-351), $CD33^{+}$-specific antibody-toxin conjugate (gemtuzumab ozogamicin, vadastuximab talirine), bcl-2 inhibitor (venetoclax), and XPO1 inhibitor (selinexor) show promise for treatment efficacy in patients with newly diagnosed or relapsed/refractory AML and thus provide rationale for investigation in the post-transplant period. Recently, the IDH2 inhibitor, enasidenib, was FDA-approved for the treatment of adult patients with relapsed/refractory AML with an IDH2 mutation and potentially may be appealing for the post-transplant setting given its activity and tolerability. In addition, ivosidenib, a first-in-class, oral, targeted inhibitor of mutant IDH, has also shown promising activity and safety in a phase I study of patients with IDH1-mutated relapsed/refractory AML. Just as importantly as clinical activity, however, potential drug-drug interactions, risk of acute/chronic GVHD, and side-effects of maintenance agents such as cytopenias and intolerable gastrointestinal symptoms must also be accounted for as these toxicities limit quality-of-life (QoL) and impede the maximal benefit of a drug. As such, QoL metrics and other patient-reported outcomes should be increasingly included in AML maintenance trials as these endpoints are informative in determining the success of a therapy.

The availability of more sensitive assays for MRD has implications in treatment decisions, response evaluations, and MRD status after therapy potentially represents a new endpoint in clinical research studies. However, current MRD platforms vary between institutions and standardization of assays is lacking. As new agents and technologies continue to develop, further questions on post-transplant strategies to prevent relapse need to be answered and include determining appropriate patient risk groups warranting maintenance vs. preemptive vs. no treatment following transplantation, and optimal timing and duration of treatment. The optimal approach to treating patients at high-risk of AML relapse following transplant remains controversial and owing to the lack of phase III clinical data, there is insufficient evidence to support the routine use of post-transplant maintenance therapies. We strongly support the development of prospective, randomized studies to determine the clinical efficacy of available therapies. Although early phase studies demonstrate safety and tolerability of various approaches in the post-transplantation period, the results of ongoing or planned prospective randomized studies are required to determine the clinical efficacy needed to move this approach from experimental to standard-of care.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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