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Biology-Driven Approaches to Prevent and Treat Relapse of Myeloid Neoplasia after Allogeneic Hematopoietic Stem Cell Transplantation

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A B S T R A C T

The curative potential of allogeneic hematopoietic cell transplantation (allo-HCT) in the treatment of acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS) relies mainly on the graft-versus-leukemia effect. Relapse after allo-HCT occurs in a considerable proportion of patients and has a dismal prognosis, with still very limited curative potential. This review provides an overview of the established and evolving approaches to preventing or treating relapse of AML and MDS after allo-HCT, in the context of novel insight into the biology of relapse. Established prophylactic measures to prevent relapse include optimized conditioning and graft-versus-host disease (GVHD) prophylaxis, as well as donor lymphocyte infusion (DLI) for high-risk patients; novel immunomodulatory interventions and maintenance approaches are still experimental. Improved diagnostics can detect persistent or recurring disease at a molecular level, enabling early preemptive interventions. Established options include hypomethylating agents and DLI. Standard treatments for hematologic relapse include chemotherapy, cessation of immunosuppressive treatment, and DLI. Experimental approaches include molecular targeted therapies, novel immunomodulatory treatments, and second allo-HCT. For all interventions, the potential risks, including occurrence of GVHD, must be weighed against the benefits individually in each patient. Concurrently, prevention and treatment of relapse after allo-HCT remain challenging and unmet medical needs.

BACKGROUND

Epidemiology and Prognostic Factors of Relapse

The incidence of relapse for acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS) within the first 2 to 3 years after first allogeneic hematopoietic cell transplantation (allo-HCT) ranges from 20% to 50%. As for AML and MDS in general, risk factors for relapse include cytogenetic and

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molecular alterations, such as a complex karyotype and TP53 mutations [1–3]. In a retrospective analysis. FMS-like tyrosine kinase 3 (FLT3)-internal tandem duplication (ITD) and the use of more than 1 course of chemotherapy to achieve a complete response (CR) were risk factors associated with inferior outcome, independent of *NPM1* mutational status [4]. Genetic profiling of MDS in patients undergoing allo-HCT identified subgroups of patients based on the molecular characteristics of the disease [5]. The connection between RAS pathway mutations and the risk of relapse, compared with the absence of RAS pathway mutations, was evident in patients age >40 years only with reduced-intensity conditioning (RIC) and not with myeloablative conditioning (MAC) [5]. In contrast, the unfavorable prognostic impact of TP53 mutations was comparable in patients who received an RIC regimen and those who received an MAC regimen [5]. This information could allow for prognostic stratification and selection of more intensive or less intensive conditioning regimens [5].

There is evidence that MRD positivity before allo-HCT has prognostic value in patients who receive induction chemotherapy [6–8]. Based on minimal residual disease (MRD) assessed by flow cytometry before allo-HCT, the 3-year incidence of relapsed AML after allo-HCT was similar for MRD-positive remission (67%) and for morphologically active disease (65%) but was significantly lower for MRD-negative remission (22%) [9]. In the future, more sensitive MRD detection may allow for better identification of patients at increased relapse risk [10].

These data argue to aim for negative MRD before allo-HCT. However, the toxicity of further therapy, the limited data on which therapy may be best to achieve this aim, as well as the delay in achieving a graft-versus-leukemia (GVL) effect argue against this. Moreover, data on MDS suggest that therapy before allo-HCT can select for resistant subclones [11] and thus may contribute to the risk of relapse after allo-HCT. Therefore, in AML as well as in MDS, currently no recommendation can be made to treat for negative MRD in any situation. According to the European LeukemiaNet recommendations for patients with newly diagnosed AML and an indication for allo-HCT, a CR without consideration of MRD should be achieved [12]. In MDS, cytoreductive therapy before allo-HCT is recommended if the bone marrow blast count exceeds 10%. In MDS, cytoreductive therapy before allo-HCT is recommended depending on the bone marrow blast count [13]. The blast count cutoff for nonmyeloablative allo-HCT is 5% in most institutional and Blood and Marrow Transplant Clinical Trials Network (BMT CTN) studies. Long-term survival in patients with relapse of AML and MDS after allo-HCT is poor, ranging from 10% to 30%. Factors associated with better survival include duration of remission after first allo-HCT of >6 months, absence of graft-versus-host disease (GVHD), and low blast count at relapse [14].

In cases of relapse after allo-HCT, up to 50% of patients have cytogenetic or molecular evolution of their disease [15]. A recent analysis in patients with MDS suggested that relapse after allo-HCT is driven by subclones evolving from founding clones existing before allo-HCT but harboring new aberrations [11], arguing for clonal evolution. Thus, a complete workup similar to that performed at initial diagnosis is recommended to allow detection of potentially new targetable aberrations.

Biology of Relapse

Given the proven GVL effect, relapse after allo-HCT in AML and MDS must occur from persisting leukemic cells, particularly leukemia stem cells [16] which have escaped the cytotoxicity of conditioning and allogeneic immune response. Along with immunogenic peptides, T cells recognize structural epitopes of non-self HLAs, which leads to leukemia elimination. In agreement with a role of HLA molecules in leukemia elimination, deletion of mismatched HLA has been found in leukemia cells on post-allo-HCT relapse [17]. HLA loss has been observed after haploidentical allo-HCT [18,19] and after allo-HCT from an unrelated donor [20,21]. This HLA deletion does not reduce the abundance of other HLA class I molecules. The reported incidence of HLA loss in relapse after allo-HCT is higher in the haploidentical setting compared with the matched unrelated donor setting (reviewed in [22]). Treating relapse based on HLA loss with an allograft with a new mismatched haplotype may improve outcomes after second allo-HCT for a relapsed hematologic malignancy [23].

Along with T cells, the natural killer (NK) cell compartment plays an important role for relapse prevention, because NK cells are activated owing to the absence of self-HLA class I molecules on malignant cells. Reduced expression of HLA class II molecules has been observed in, for example, chronic myelogenous leukemia [24], Hodgkin lymphoma, aggressive B cell lymphomas [25,26], and relapsed leukemia in patients without genomic HLA loss [27,28]. Reduced expression of HLA class II molecules has been shown to promote leukemia relapse, which could be counterbalanced by IFN-γ treatment to enhance HLA expression [29]. Leukemia cells that cause relapse also may up-regulate inhibitory immune checkpoint molecules to escape from the allogeneic T cells. Increased expression of PD-1, TIGIT, and KLRG-1 on tumor-reactive $CD8^+$ T cells has been found in relapsed patients [30], and PD-1/PD-L1 interactions have been shown to promote T cell impairment in patients who relapse after allo-HCT [31]. These observations provide a rationale for immunotherapy with blocking antibodies to treat relapse after allo-HCT. Clinical data show that CTLA4 blockade in AML relapse can lead to impressive responses connected to the presence of perforin-producing donor T cells [32–34].

In contrast to CTLA4 blockade, the role of PD-1 inhibition in AML relapse is unclear. Case studies have shown potential activity of anti-PD1 immunotherapy for AML relapse [35], but prospective trials are needed to validate these preliminary data. Myeloid neoplasms with specific mutations are potentially immunogenic diseases, because alloreactive T cell for mutated genes, such as *JAK2*, have been reported [36]. An additional mechanism may be immune escape induced by oncogenic mutation; for example, mutated $JAK2^{V617F}$ causes higher PD-L1 levels and consecutive vulnerability to anti-PD-1 immunotherapy [37]. Along with the action of immune checkpoint molecules as negative regulators of T cell activation, anti-inflammatory cytokine production can interfere with effective antileukemia immune responses [38]; for example, IL-4 and IL-10, which may interfere with immune responses, have been found to be produced by AML cells [39]. Furthermore, it has been shown that chronic myelogenous leukemia cells produce TGF-β [38], which reduces the expression of CIITA and also MHC-II [40]. The reduction in MHC-II expression will reduce the ability of the immune system to recognize leukemia cells. In agreement with an important role for MHC class II molecules, in one study several class II genes (HLA-DPA1, -DPB1, -DQB1, and -DRB1) were significantly reduced in patients with AML at relapse after allo-HCT compared with those seen in paired samples obtained at presentation [28].

In addition to the production of anti-inflammatory factors, myeloid leukemia cells may reduce the production of proinflammatory cytokines, such as IL-2, IFN-γ, and IL-15 (reviewed in [22]). Although healthy myeloid progenitor cells secrete IL-

15 [41], some myeloid leukemia subtypes are associated with down-regulated IL-15 production [42]. IL-15 promotes the survival and activation of alloreactive T cells [43], which can be a disadvantage for leukemia cells.

Previous studies have shown an association between decreased IL-15 in the blood and relapse [44]. Taking this into account, strategies have been developed to use IL-15 for the enhancement of GVL effects; for example, a clinical study that analyzed the effect of an IL-15 superagonist complex showed that this intervention led to clinical responses and NK cell expansion [45].

To escape the allogeneic immune response, some leukemia subtypes may produce enzymes that change the metabolism. Examples include indoleamine 2,3-dioxygenase-1 (IDO1) [46], arginase [47], CD39 [48], and CD73 [49]. The enzyme CD73 converts adenosine-monophosphate into adenosine, a metabolite that inhibits the function of T cells and dendritic cells. In agreement with this, the rejection of leukemia cells after allo-HCT was enhanced in mice with a genetic deficiency of CD73 [50]. Furthermore, it has been shown that T cell lymphoma cells are rejected in A2aR-null mice in a CD8-dependent manner but are not controlled in WT mice [51]. The effect of adenosine is mediated through the A2aR and A2b receptors on innate and adaptive immune cells. Inhibiting agents for A2aR currently in clinical trials include CPI-444 (ClinicalTrials.gov identifier NCT02655822), PBF-509 (NCT02403193), MK-3814 (NCT03099161), and AZD4635 (NCT02740985), among others. The A2aR inhibitors are mostly combined with immune checkpoint inhibitors. The enzyme CD39 can interfere with immune activation in AML at primary diagnosis [48].

Last but not least, accumulating evidence demonstrates a role of the bone marrow microenvironment in the biology of MDS and AML and relapse after allo-HCT. In animal models, specific alterations in cells of the bone marrow stroma particularly affecting miRNA metabolism can elicit an MDS-like malignancy with ensuing AML [52]. In addition, in humans, alterations in patient-derived bone marrow stroma cells that propagate MDS and AML by affecting miRNA content [53] as well as the expression of cytokines [54–56] have been described. These alterations of stroma cells are likely induced by their interaction with MDS and AML cells [54,55] and appear to be reversible [57]. Whether such alterations also contribute to relapse remains speculative; however, a specific role of bone marrow stromal cells in conferring resistance to AML cells through induction of antiapoptotic and pro-proliferative signals in AML cells [58,59] has been reported recently. In animal models, the engraftment of hematopoietic stem cells (HSCs) after transplantation is strongly supported by cotransplanted stroma cells, which compensate for the damage caused by the conditioning [60]. The finding that normal HSCs and leukemia stem cells (LSCs) occupy the same niche and that HSCs can outperform LSCs [61] supports the concept that modification of the bone marrow stroma can improve the efficacy of allo-HCT. In accordance with this idea, preclinical data have been published suggesting that interference with the interaction of leukemia and stroma cells may provide a new approach to improve treatment [62]. However, clinical data on the specific use to prevent or treat relapse after allo-HCT are lacking.

PREVENTION OF RELAPSE

Conditioning Intensity

Several studies have explored the effect of the intensity of the conditioning regimen on relapse rate and overall survival (OS) in patients with AML and MDS. A prospective randomized Phase III study by the European Society of Blood and Marrow Transplantation showed no significant differences in 2-year progression-free survival (PFS) or OS in patients with MDS and secondary AML after RIC compared with MAC [63]. In another prospective randomized Phase III study, relapse rate and 3-year PFS were not significantly different between patients with intermediate-risk AML and those with high-risk AML after RIC compared with MAC [64]. However, in both studies, a trend (albeit not a significant one) toward reduced PFS was seen with RIC.

In contrast, a randomized study of younger (<65 years) patients with AML and MDS showed a significantly increased relapse rate in the RIC group. Relapse-free survival was 47.3% with RIC versus 67.8% with MAC ($P < .01$) [65]. At 18 months, OS was 67.7% for patients in the RIC arm versus 77.5% for those in the MAC arm ($P = .07$). Transplantation-related mortality was 4.4% with RIC versus 15.8% with MAC ($P = .002$) [65].

Taken together, the findings of the foregoing studies suggest that the intensity of conditioning contributes to disease control in AML and MDS. This idea is supported by retrospective data in patients with AML with active disease at the time of HCT, in whom MAC yielded similar PFS and OS compared with sequential chemotherapy followed by RIC [66]. This suggests that total cytotoxic activity is relevant for disease control.

Therefore, based on the current data, intensity of conditioning should be chosen based on patient age, comorbidities, and disease status.

Intensity and Duration of Immunosuppression for GVHD Prophylaxis

The intensity of immunosuppression appears to have an impact on the GVL effect, as it has been shown for other entities that reduction of immunosuppression in patients with positive MRD reduces the risk of overt relapse [67,68]. Specifically, in patients with AML, an association between the dose and duration of cyclosporin A treatment with risk of relapse has been reported [69].

Data from 3 prospective randomized studies on the use of antithymocyte globulin (ATG) during conditioning have been reported. Two open-label randomized studies showed that in vivo T cell depletion with anti-T lymphocyte globulin (ATLG; formerly Fresenius, now Neovii) reduces chronic GVHD (cGVHD) in the setting of related and unrelated donors, respectively [70,71]. A third prospective, double-blind phase III trial also investigating ATG (Neovii) in MUD MAC reported reductions in grade II-IV acute GVHD (aGVHD; 23% versus 40%; $P = .004$) and moderate to severe cGVHD (12% versus 33%; $P < .001$) in ATLG recipients but no difference in moderate-severe cGVHD-free survival between ATLG and placebo [72]. Rather, 2-year PFS (47% versus 65%; $P = .04$) and OS (59% versus 74%; $P = .034$) were reduced in the ATG group, and ATG maintained a negative impact on PFS and OS in multivariate analysis [72]. The reasons for the discrepancies between these trials are not yet clear, and more data on the subgroups in the latter trial are needed.

Other methods of GVHD prophylaxis, including alemtuzumab, in vitro T cell depletion, and post-transplantation cyclophosphamide, may have an impact on the GVL effect; however, longer follow-up is needed to allow for valid conclusions.

Based on these data, immunosuppression including ATG generally should be chosen depending on the availability of a related or unrelated donor, HLA match, and relapse risk. Specifically, the use of ATG is still recommended; however, caution should be applied regarding a possible ATG-related

increased risk of relapse, weighed against the risk of GVHD-related complications.

Maintenance Therapy to Prevent Relapse

For patients with MRD-positive AML heading to transplantation, one option is to treat them into remission before allo-HCT, which may incur a higher toxicity and increase non-relapse mortality (NRM) after allo-HCT. Conversely, the concept of controlling or eradicating residual LSCs after allo-HCT with maintenance therapy is intriguing. However, such therapy also carries the risk of reducing the GVL effect. Moreover, drugs established in the pretransplantation setting may result in much more pronounced side effects in the post-transplantation situation. Thus, well-designed studies are needed to establish maintenance approaches in patients with AML/MDS.

Conclusive data are not yet available, and thus no standard maintenance therapy for AML or MDS after allo-HCT can be currently recommended as standard of care. Several maintenance approaches show promise, but further data from ongoing or future clinical trials are needed to assess their potential.

Immunomodulators

Maintenance therapy with lenalidomide for patients with AML or MDS who achieved remission after allo-HCT has been associated with a high rate of severe GVHD [73]. In a Phase I/II study, maintenance therapy with the deacetylase inhibitor panobinostat for patients with high-risk MDS/AML in CR after allo-HCT led to a 2-year survival of 81% and a relapse-free survival of 75% [74]. These findings are promising, and a larger follow-up study is currently underway. A synergistic effect of the combination of 5-azacytidine and lenalidomide as postremission maintenance outside of allo-HCT was reported in a phase I trial [75], and randomized trials in the prophylactic setting are ongoing (Clinical Trials.gov identifier NCT00887068).

Tyrosine Kinase Inhibitor Maintenance for FLT3-ITD AML

Clinical data on maintenance with midostaurin for FLT3-ITD AML show a low 1-year relapse rate of 9.2% [76]. Several early-phase studies and case reports have reported evidence for the efficacy of sorafenib maintenance therapy in patients with FLT3-ITD AML [77–80]. The results of a placebo-controlled randomized phase II trial (SORMAIN trial) indicated a higher relapse-free survival in the sorafenib group compared with the placebo group [81]. Phase I data on quizartinib show the feasibility of tyrosine kinase inhibitor (TKI) maintenance after allo-HCT [82]. A prospective Phase III randomized trial using gilteritinib versus placebo for patients with FLT3-ITD$^+$ AML as maintenance after allo-HCT is currently ongoing [83].

Prophylactic Donor Lymphocyte Infusion and Other Cellular Therapy

General Considerations

Donor lymphocyte infusion (DLI) is a cellular product of mononuclear donor cells with a defined CD3$^+$ T cell content. DLI is derived by apheresis from the original HSC donor either as aliquots from the original G-CSF-mobilized peripheral blood stem cell (PBSC) product or by an additional unstimulated leukapheresis. Several reports suggest that DLI harvested after G-CSF stimulation from the original stem cell product is effective in eliciting a GVL effect [84–86]. Data on direct comparison of G-CSF-mobilized DLI versus DLI from unstimulated leukapheresis are sparse, but a retrospective analysis in 67 patients suggested similar activity of both DLI sources [85]. Owing to logistical and feasibility considerations, DLIs are mostly cryopreserved and transfused after thawing. New approaches to enhance the efficacy of DLIs or reduce their toxicity have been explored [87,88], but data are insufficient for clinical recommendations.

Prophylactic DLI is applied in the presence of CR to induce a GVL effect and/or improve donor chimerism. Multiple studies indicate that AML and MDS are sensitive to a DLI-induced GVL effect [89]; however, convincing prospective randomized data are lacking.

Available Data on Efficacy, Timing, and Dosing

An alemtuzumab-based T cell-depleted (TCD) study reported an effect of prophylactic DLI to improve PFS and OS [90]. A trial by the National Institutes of Health using add-back donor T cell infusion following ex vivo TCD HCT reported an OS of 58%, DFS of 46%, relapse rate of 40%, and NRM of 20%, at a median follow up of 4 years [91]. The incidence of grade II-IV aGVHD was 39% and that of cGVHD was 36% [91]. In a prospective study, 19 children with an HLA-matched or -mismatched unrelated PBSC transplant received T cell add-back following TCD allo-HCT, and the risk of grade II-IV aGVHD and extensive cGVHD was 16% and 0%, respectively [92]. All patients engrafted, and NRM at 1 year was 6%, with a 1-year OS of 82% [92].

Less data are available for non-TCD settings. In an initial prospective study, repeated prophylactic DLI in escalating doses starting at day +120 post-HCT were shown to be feasible in patients with AML and MDS with high relapse risk [93,94]. Besides the presence of a complete response, criteria for application of prophylactic DLI were no immunosuppressive medication for >30 days, lack of infection, and lack of GVHD. Relapse and cGVHD occurred in 30% and 20% of DLI-treated patients, respectively. A subsequent retrospective analysis was performed in an extended cohort of patients with AML who met the same criteria and received escalating doses of prophylactic DLI (starting at $.5 \times 10^6$/kg for related donor graft recipients and 1×10^6 for unrelated donor graft recipients). Results were compared with those of a matched control cohort. In the DLI group, 9% of patients developed grade II-IV aGVHD and 11% developed extensive cGVHD. At 7 years, OS was 67% in the DLI group compared with 31% in controls ($P < .001$), owing to a lower relapse risk in the DLI group (22% versus 53% in controls; $P = .004$) [95].

In a prospective study, DLI was applied prophylactically concurrent with immunosuppression within 60 days in 50 patients with high-risk acute leukemia after related donor allo-HCT [96]. The incidence of cGVHD was higher in the DLI group compared with controls (n = 73) (38% versus 17%; $P = .021$). However, DLI was associated with a lower 2-year cumulative relapse rate (46% versus 66%; $P = .02$) and a higher 3-year OS (36% versus 11%; $P = .001$). Further prospective [97] and retrospective [98] studies have reported on the feasibility of prophylactic DLI in AML/MDS with high relapse risk.

Prophylactic DLI also has been reported to be feasible in haploidentical or mismatched donor HCT [99]. In a retrospective analysis, patients receiving DLI including continued immunosuppressive medication (n = 61) compared with controls without DLI (n = 27) had a lower 2-year cumulative relapse rate (36% versus 55%; $P = .017$) and better estimated 3-year OS (31% versus 11%; $P = .001$) [100].

An open issue is the optimal timing of prophylactic DLI. In a prospective analysis, DLI was started as early as day 21 with concurrent cyclosporine A administration. The different settings and schedules in the published studies preclude clear recommendations and call for further evaluation.

Another, not-yet widely explored approach in AML and MDS is to combine DLI with maintenance therapies, using donor lymphocytes that have been manipulated to enhance their GVL efficacy while reducing the risk of GVHD.

Based on these data, the following approach for prophylactic DLI in AML and MDS after T cell-replete allo-HCT can be recommended. DLI should be considered based on the expected risk of relapse and GVHD but should be applied only if dominant overall donor chimerism has been achieved (if recipient chimerism is dominant, DLI may be less effective), GVHD and severe infections are absent, and immunosuppression has been stopped for >4 weeks. Thus, assessment of remission status and chimerism as well as clinical evaluation is necessary before DLI. A starting dose of .1 or 1×10^6 $CD3^+$ cells/kg body weight in allo-HCT recipients with unrelated or related donor grafts, respectively, carries a low risk of GVHD. DLI can be repeated every 4 to 12 weeks in doses escalated by 5- to 10-fold as long as GVHD does not occur. Representative studies of prophylactic DLI in AML and MDS are summarized in Table 1. Given the limited data, no recommendations can be made for DLI in haploidentical allo-HCT or for early DLI with concurrent immunosuppression.

TREATMENT OF MRD AND OVERT RELAPSE

Relapse incidence is highest within the first 2 years after allo-HCT, and survival is better when incipient relapse is detected early. Therefore, regular monitoring for MRD markers, including chimerism, is recommended. In overt AML/MDS relapse, reduction of immunosuppression can result in a response and even CR in some cases [12–18]. Several other approaches to "actively" treat AML/MDS relapse after allo-HCT have been proposed. Data on different strategies for treatment of overt relapse are sparse. Strategies to treat relapse are summarized in Figure 1.

Hypomethylating Agents

Preclinical Evidence Supporting a Role of Hypomethylating Agents in Antitumor Immunotherapy

Pharmacologic inhibition of DNA methylation in malignant cells can activate the expression of endogenous retroviruses, leading to type II interferon responses [101,102]. In addition, treatment with 5-aza-2′-deoxycytidine was found to induce the expression of cancer/testis antigens (CTAs) in AML cell lines [103]. One CTA, NY-ESO-1, converted the cell lines susceptible to antigen-specific recognition by $CD8^+$ T cell clones [103]. These findings provide a therapeutic rationale for combining DNA methylation inhibitors with allogeneic immunity to treat relapse.

Preemptive Treatment (Treatment of Incipient Relapse)

In a prospective uncontrolled Phase II study, treatment with 5-azacytidine in patients with elevated MRD markers induced an increase in donor chimerism or a decline in MRD [104]. 5-azacytidine treatment may be combined with DLI [104]. Based on these data and the labeling of azacytidine in Europe, preemptive use of 5-azacytidine might be considered in patients with AML or MDS and decreasing donor chimerism.

Therapeutic Treatment (Treatment of Hematologic Relapse)

The combination of azacytidine and DLI to treat relapse after allo-HCT has been shown to have efficacy in clinical applications, including a Phase I study [105], a Phase II study [106], and several retrospective analyses [106–109]. A retrospective analysis in patients receiving azacytidine in combination with DLI reported an overall response rate of 33% and a 2-year OS of 29% [108]. GVHD was seen almost exclusively in patients receiving DLI. Molecular relapse only, diagnosis of MDS, and low marrow blast count at the time of relapse were associated with better OS. Which patients will require a second allo-HCT for long-term disease control after achieving a first remission postrelapse is currently unclear.

Less data are available for decitabine, but a retrospective study on the use of decitabine in combination with DLI showed a response rate of 25%, including patients with previous azacytidine failure, and a 2-year OS of 11% with low rates of aGVHD and cGVHD [110].

In a retrospective center analysis, a direct comparison of intensive chemotherapy with hypomethylating agents showed better OS with intensive chemotherapy [111]; however this finding needs confirmation in a prospective trial. Induction of PD-L1 expression by hypomethylating agents has been reported [112]; therefore, up-regulation of PD-L1 expression on blasts of patients with AML/MDS who have progressed after therapy with hypomethylating agents may be a cause of immune evasion promoted by the treatment itself. In overt relapse, hypomethylating agents, in combination with DLI if possible, may be considered in a subgroup of patients and can be recommended especially for patients who might not be eligible for more aggressive remission induction.

TKI for FLT3-ITD AML Relapse

Preclinical Evidence Supporting a Role of TKI in Antitumor Immunotherapy

Sorafenib may increase the immunogenicity of leukemia cells via induction of IL-15 production by the cells, thereby enhancing T cell activation, as shown in AML mouse models [42]. FLT3-ITD is associated with induction of Activating transcription factor 4 (ATF4), which blocks the expression of IL-15.

Table 1
Selected Studies on Prophylactic DLI in AML/MDS

Condition (Number of Patients) and Study Type	Strategy	Main Findings	Reference
AML/MDS (N = 46); retrospective, matched control group	Prophylactic DLI at ≥120 d post-allo-HCT (if IS stopped for >30 d, no GVHD, and no infections)	Improved survival with DLI compared with controls: 7-yr OS, 67% versus 31% ($P < .001$)	[95]
AML/ALL (N = 89); retrospective, matched-pair	Prophylactic DLI <1 yr after allo-HCT if no preceding GVHD	Improved survival with DLI compared with controls only for high-risk AML (cytogenetic risk or allo-HCT in active disease): 5-yr OS, 70% versus 40% ($P = .036$)	[135]

IS indicates immunosuppressive medication.

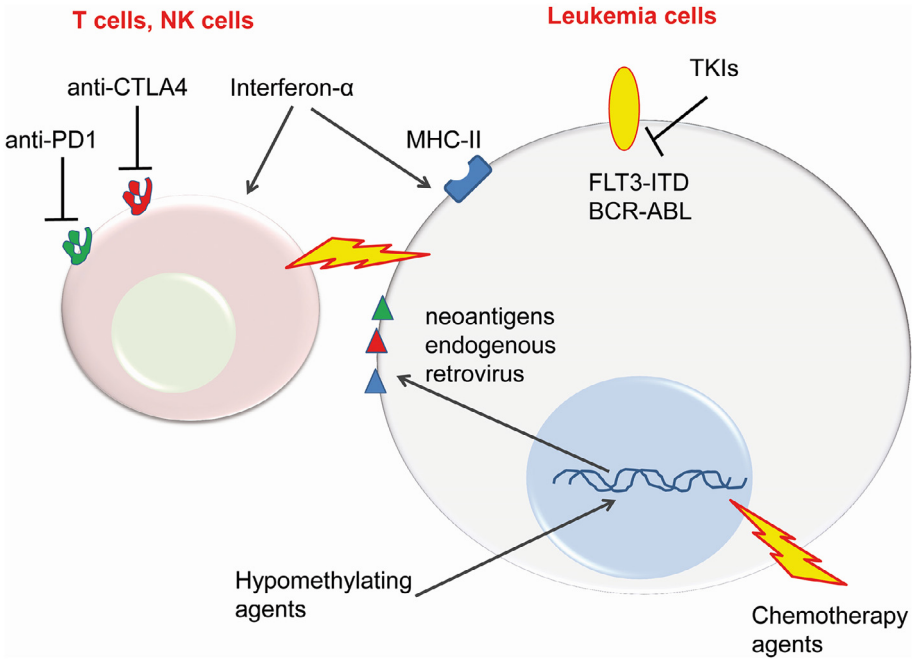

Figure 1. Currently known targets and drugs to enhance the GVL effect as an approach to prevent or treat relapse of AML/MDS after allo-HCT. Alloreactive T and NK cells can be activated via anti-CTLA4 and anti-PD1 immune checkpoint inhibition, as well as IFN-α. Leukemia cells can be rendered more immunogenic via (1) INF-α-induced up-regulation of MHC class II, (2) hypomethylating agents leading to the expression of neoantigens, and (3) TKIs like sorafenib inducing IL-15 production. Proliferation of leukemia cells can be inhibited by TKIs, hypomethylating agents, and chemotherapy.

TKI-based inhibition counterbalances the ATF4 effect and promotes IRF7-induced IL-15 transcription [42].

Therapeutic Treatment

Recent unprecedented evidence was provided from a retrospective cohort of 29 patients with FLT3-ITD$^+$ AML who relapsed after allo-HCT [113]. Six of the 29 patients (21%) achieved sustained CR with sorafenib monotherapy. Excluding 1 patient who underwent a second allo-HCT, 4 of these patients were in treatment-free remission for a median of 4.4 years. With a median follow-up after relapse of 7.5 years, these data suggest for the first time that FLT3-ITD inhibition alone can induce long-term disease control and conditional cure in patients who relapse after allo-HCT [113].

Several case series and retrospective studies in patients with FLT3-ITD$^+$ AML relapse after allo-HCT have shown impressive responses to sorafenib, including increased long-term survival [114–117]. Some patients have received sorafenib in combination with DLI or azacytidine [118]. In a retrospective analysis, treatment of FLT3-ITD$^+$ AML relapse after allo-HCT with sorafenib and DLI was found to be superior to treatment with DLI alone [42].

The clinical evidence of a synergism between FLT3 inhibition and alloimmunity against leukemia cells motivated studies in a mouse model, which showed that FLT3 inhibition combined with T cell infusion can lead to complete elimination of leukemia cells. Mechanistically, FLT3 inhibition reduced expression of the transcription factor ATF4. ATF4 normally blocks interferon regulatory factor 7 (IRF7) activation, and this effect was antagonized by the FLT3 inhibition. Removal of the ATF4-mediated blockade allowed IRF7 activation and caused IL-15 transcription in the leukemia cells, which were then eliminated by donor T cells [42]. Increased IL-15 was detected in the blood of responding patients and caused an increase in mitochondrial spare respiratory capacity in T cells, consistent with previous reports indicating that IL-15 causes mitochondrial reprogramming in T cells [119,120].

The FLT3 inhibitors sorafenib and midostaurin have broad inhibitory activity against multiple kinases, and whether this property has an advantage over selective FLT3 inhibition remains an open question.

Despite the promising data, however, no clear recommendation on the use of TKIs for post-allo-HCT relapse of FLT3-ITD$^+$ AML can be made owing to the lack of a randomized, placebo-controlled Phase III trial.

Immunomodulatory Treatment with Lenalidomide

Case studies have reported that lenalidomide treatment can eliminate leukemia cells in relapsed AML after allo-HCT [121]. However, data are sparse, and no recommendation can be made on the use of lenalidomide for relapsed AML/MDS after allo-HCT.

CTLA4/PD1/PD-L1 Inhibition

Based on the impressive success of immune checkpoint inhibition in solid cancers and the potential to enhance the GVL effect, the treatment of hematologic cancers with this immunotherapy is an attractive approach. A recent study of 28 patients with AML, MDS, or another hematologic malignancy who relapsed after allo-HCT and were treated with ipilimumab (anti-CTLA-4 antibody) found an overall response rate of 32%, with a 23% CR and 9% PR and a 14% rate of GVHD [34]. In addition, 27% of the nonresponding patients according to standard criteria had a decreased tumor burden. Intriguingly, CR occurred in 4 patients with extramedullary AML [34]. Similarly, 2 smaller studies reported responses in patients treated with ipilimumab for relapse of hematologic malignancy after allo-HCT [32,33].

In contrast to the findings for anti-CTLA-4 immunotherapy, higher rates of severe aGVHD and cGVHD rates (up to 55%) have been reported with anti-PD1 immunotherapy after allo-

Table 2
Selected Studies on the Use of Preemptive DLI

Condition (Number of Patients) and Study Type	Strategy	Main Findings	Reference
AML/MDS (n = 69) and ALL (total n = 101); retrospective, nonrandomized, single center	Preemptive CTx plus DLI, followed by IS for patients with positive MRD	Entire group: 3-yr PFS, 51.7%; aGVHD, 9%; moderate/severe cGVHD, 51%	[128]
AML/MDS (n = 529) and ALL (total n = 814); prospective, nonrandomized, monocenter	If MRD-positive and no GVHD: DLI (plus IL-2, partially with CTx before DLI; n = 56) or IL-2 only (n = 49); comparison with MRD-negative patients (n = 709)	Entire group: improved OS and reduced relapse rate with DLI compared with IL-2 only; 3-yr OS: MRD-negative, 66%; DLI, 58%; IL-2 only, 28%	[136]
Pediatric AML (n = 71); prospective, nonrandomized, multicenter	Preemptive DLI (n = 13) versus stop IS-only (n = 7) in patients with mixed chimerism (n = 20)	Entire group: long-term PFS only with DLI (46%; 6 of 13) but not in stop IS-only 0% (0 of 7)	[68]
AML/MDS (n = 44) and ALL (total n = 80); retrospective, matched-pair, single center	If MRD-positive, DLI (n = 11) versus CTx, followed by DLI (n = 33) with prophylactic IS after DLI	Entire group: similar 2-yr OS for DLI (69%) and CTx + DLI (78%; $P = .36$)	[124]

CTx indicates chemotherapy.

HCT [122]. For AML, a small case series reported efficacy of the anti-PD1 antibody nivolumab for relapse after allo-HCT [35]. The sparse data preclude any recommendation regarding the off-label use of ipilimumab or anti-PD-1/PD-L1 in AML/MDS relapse after allo-HCT, however.

Cellular Therapy

Given the strong GVL effect in myeloid neoplasia, DLI may be a viable approach in the preemptive setting—that is, in the presence of positive MRD or recipient chimerism—as well as in the therapeutic setting for overt relapse of AML and MDS. Several nonrandomized and retrospective studies have demonstrated the efficacy of preemptive DLI [123]; selected studies are summarized in Table 2. As for prophylactic DLI, immunosuppressive drugs were applied concurrently with DLI in some studies [124], but the data are heterogeneous, and further trials are needed.

A response of overt AML/MDS relapse to therapeutic DLI has been reported in a prospective study and several retrospective studies [94,125,126]. Selected studies are summarized in Table 3.

Available data suggest that DLI in the preemptive setting results in better response than DLI administered for overt relapse [127]. Given the biology of the GVL effect, preemptive or therapeutic DLI is indicated only if engraftment, as indicated by a predominant donor chimerism, has been achieved. In the majority of studies reported to date, DLI has been administered after discontinuation of immunosuppression and in the absence of GVHD or severe infections.

Although there is a lack of actual dose-finding studies, a higher starting dose of 5 to 10×10^6 $CD3^+$/kg is usually chosen for preemptive and therapeutic DLI. Based on the experience in numerous studies, this dose may yield higher efficacy but is also associated with a higher risk of GVHD. DLI can then be repeated in escalating doses based on response and the occurrence of GVHD.

Several studies have explored the combination of DLI with chemotherapy [128]. Here DLI is administered either at the time of the leukocyte nadir or after a response is achieved with regenerated bone marrow function. The former approach is attractive because it does not require sustained response, but it carries a higher risk of toxicity. The latter approach holds promise for better response and less GVHD risk but might not be appropriate in some patients owing to a lack of sustained response.

A promising approach is the repeated application of hypomethylating agents followed by DLI as described above [106,108]. Based on the rationale that hypomethylating agents

Table 3
Selected Studies on the Use of Therapeutic DLI

Condition (Number of Patients) and Study Type	Strategy	Main Findings	Reference
AML (n = 399) retrospective, multicenter	DLI (n = 171) versus no-DLI (n = 228) partially combined with CTx in both groups	2-yr OS: DLI, 20% versus CTx, 9% ($P < .01$); better results if disease controlled by CTx before DLI	[94]
AML (n = 263); retrospective, multicenter	Various strategies, including CTx, DLI, and second allo-HCT or their combination for relapse after RIC allo-HCT	Long-term OS almost exclusively in patients who achieved CR after CTx and received DLI or 2nd HCT	[14]
AML/CML/MDS (n = 65); prospective, nonrandomized, single center	Cytarabine-based CTx followed by DLI	56% GVHD; 2-yr OS, 19%; patients with CR, 1-yr OS, 51%	[137]
Pediatric AML (n = 123); ALL (n = 157), and biphenotypic AL (n = 8); retrospective, multicenter	CTx alone (n = 108) versus DLI alone (n = 13) versus BSC (n = 67) versus CTx followed by either DLI (n = 30) or second allo-HCT (n = 70)	Similar better 1-yr OS with CTx followed by DLI (53%) or by second allo-HCT (51%) compared with CTx alone (28%), DLI alone (15%), or BSC (5%)	[126]
AML (n = 30) and ALL (n = 16); retrospective, multicenter	DLI (n = 19) or second allo- HCT (n = 27) after cytoreductive CTx	Similar outcomes for DLI or 2nd allo-HCT; interval from 1st allo-HCT to relapse the only factor impacting OS	[138]
AML (n = 184), MDS (n = 69), and other malignancies (n = 161); retrospective, multicenter	DLI with or without preceding CTx	CR at day +100 post-DLI: AML, 17%; MDS, 30%; factors associated with response in entire group: GVHD, molecular/cytogenetic relapse compared with hematologic relapse	[127]
AML/MDS (n = 100); retrospective, monocenter	CTx (n = 73) versus HMA (n = 27); DLI (n = 41 in CTx group; n = 9 in the HMA group)	Median OS: 6 mo in CTx group versus 3.9 mo in HMA group ($P < .01$); CTx + DLI with best outcome: 1-yr OS, 44%; median OS, 9.8 mo	[111]

BSC indicates best supportive care; HMA, hypomethylating agents.

Table 4
Selected Studies on Prevention/Treatment of AML/MDS Relapse after Allo-HCT

ConditionNumber of Patients Study Type	Study Title	Identifier
AML 21 Phase I	Phase I Study of Single-Agent and Combined Checkpoint Inhibition after Allogeneic Hematopoietic Stem Cell Transplantation in Patients at High Risk for Post-Transplant Recurrence	ClinicalTrials.gov NCT02846376
AML, MDS, Hodgkin lymphoma, B-NHL 26 Pilot study	Pilot Study of Pembrolizumab Treatment for Disease Relapse after Allogeneic Stem Cell Transplantation	ClinicalTrials.gov NCT02981914
AML FLT3-ITD$^+$ 77 Phase II	Phase 2 Study of Ponatinib (Iclusig) for Prevention of Relapse after Allogeneic Stem Cell Transplantation (Allo-SCT) in FLT3-ITD AML Patients: the PONALLO Trial	ClinicalTrials.gov NCT03690115
AML 52 Phase I	A Phase I Study of the Tumor-Targeting Human F16IL2 Monoclonal Antibody-Cytokine Fusion Protein in Combination with the Anti-CD33 Antibody BI 836858 in Patients with AML Relapse after Allogeneic Hematopoietic Stem Cell Transplantation	ClinicalTrials.gov NCT03207191
AML 22 Phase I	Phase I Dose Escalation Study of Velcade in Combination with Lenalidomide in Patients with Relapsed Acute Myeloid Leukemia (AML) and Myelodysplastic Syndrome (MDS) after Allogeneic Stem Cell Transplantation	ClinicalTrials.gov NCT02312102
AML and MDS 55 Phase I	A Phase I Study of Nivolumab in Combination with Ipilimumab for the Treatment of Patients with High-Risk or Refractory/Relapsed Acute Myeloid Leukemia Following Allogeneic Stem Cell Transplantation	ClinicalTrials.gov NCT03600155
AML 28 Phase II	A Phase 2 Study of PF-04449913 for the Treatment of Acute Myeloid Leukemia Patients with High Risk of Post-Allogeneic Stem Cell Transplantation Relapse PF-04449913 Is a Small Molecule Inhibitor of the Hedgehog (Hh) Pathway That Inhibits the Protein Smoothened (SMO)	ClinicalTrials.gov NCT01841333
MDS, CMML, and sAML 50 Phase II	Phase-II Trial to Assess the Efficacy and Safety of Lenalidomide in Addition to 5-Azacitidine and Donor Lymphocyte Infusions (DLI) for the Treatment of Patients with MDS, CMML or AML Who Relapse after Allogeneic Stem Cell Transplantation	ClinicalTrials.gov NCT02472691
AML, JMML, ALL, and CML 24 Phase I	Phase I Study of Adoptive Immunotherapy with CD8$^+$ and CD4$^+$ Memory T Cells Transduced to Express an HA-1-Specific T Cell Receptor (TCR) for Children and Adults with Recurrent Acute Leukemia after Allogeneic Hematopoietic Stem Cell Transplantation (HCT)	ClinicalTrials.gov NCT03326921
AML and MDS 30 Phase I/II	Phase I/II Clinical Trial of Daratumumab and Donor Lymphocyte Infusion in Patients with Relapsed Acute Myeloid Leukemia Post-Allogeneic Hematopoietic Stem Cell Transplant	ClinicalTrials.gov NCT03537599
AML and MDS 350 Phase III	European Intergroup Trial on Panobinostat Maintenance after HSCT for High-Risk AML and MDS: A Randomized, Multicenter Phase III Study to Assess the Efficacy of Panobinostat Maintenance Therapy versus Standard of Care Following Allogeneic Stem Cell Transplantation in Patients with High-Risk AML or MDS	EudraCT 2017-000764-15
AML and MDS 346 Phase III	A Multi-Center, Randomized, Double-Blind, Placebo-Controlled Phase III Trial of the FLT3 Inhibitor Gilteritinib Administered as Maintenance Therapy Following Allogeneic Transplant for Patients with FLT3/ITD AML	ClinicalTrials.gov NCT02997202

B-NHL, B cell non-Hodgkin lymphoma; CML, chronic myelomonocytic leukemia; JMML, juvenile myelomonocytic leukemia.

suppress GVHD without affecting GVL a Phase I study was conducted in patients with AML relapse in which chemotherapy was followed by DLI and then azacytidine [105]. Given the lack of a typical and targetable antigen, no valid data on chimeric antigen receptor (CAR) T cell therapy for MDS and AML are yet available, but trials are ongoing.

Taken together, the available data indicate that preemptive and therapeutic DLI are effective in AML/MDS and can be recommended alone or in combination with other agents when predominant donor chimerism has been achieved, depending on the individual disease burden and GVHD risk.

Conventional Chemotherapy to Induce Remission

In cases of overt relapse, a conventional chemotherapy can be applied to reduce the tumor burden. However, chemotherapy alone generally has no curative potential in this setting. Thus, intensive chemotherapy with the goal of inducing remission is justified if a subsequent consolidation treatment, such as DLI or second allo-HCT, is feasible. Dosage adjustments may be necessary based on bone marrow function, and in general, greater toxicity should be expected. A retrospective study suggested that immunotherapy including second allo-HCT or DLI is superior to chemotherapy alone in relapsed MDS after allo-HCT; OS was 32% in the immunotherapy group, 6% in the cytoreductive chemotherapy-only group, and 2% in the palliative care-only group ($P < .001$) [129].

Taken together, the foregoing findings suggest that aggressive remission induction chemotherapy is recommended in AML/MDS relapse after allo-HCT for those patients who are likely to tolerate the toxicity and are eligible for subsequent treatment with either DLI or second allo-HCT.

Second Allo-HCT

Like DLI, second allo-HCT is an alternative strategy to facilitate a GVL effect. A retrospective registry study of 179 patients demonstrated that a second allo-HCT for relapsed leukemia including AML is feasible, yielding a 2-year OS of 25% [130]. A separate case series of patients conditioned with RIC for second allo-HCT reported a transplantation-related mortality of 31% and a 3-year OS of 18% [131]. Survival after second allo-HCT is correlated with low disease burden at the time of second allo-HCT, longer remission after first allo-HCT, and younger age [132]. The available data also show that a change of donor is neither favorable nor unfavorable [132]. In a retrospective analysis

of 418 patients with relapsed AML comparing the outcomes of second allo-HCT and DLI found no difference in OS at 2 years (26% versus 25%) or 5 years (19% versus 15%) [133]. For both approaches, OS was significantly better in patients who were in CR before the intervention and who experienced relapse more than 6 months after first allo-HCT [133]. Recent data suggest that, given the higher incidence of HLA loss in relapse after haploidentical donor allo-HCT, a new donor with a different HLA mismatch may be preferable [23].

Nonetheless, there is no prospective evidence suggesting a superior outcome with second allo-HCT compared with DLI. Given the sparse data, as well as the ongoing improvements in allo-HCT techniques, the decision regarding a second allo-HCT should be individualized for each patient.

Specific Aspects of Supportive and Palliative Care

Transfusions and antimicrobial prophylaxis should be provided based on the established principles for patients after allo-HCT. This includes the need for irradiation of blood products to prevent transfusion-associated GVHD in this era of inline-filtrated blood products, as well as prophylaxis for infections with viruses and encapsulated pathogens.

Treatment of Specific Conditions

Chloroma, or granulocytic myelosarcoma, is a solid tumor composed of AML blasts. Chloroma poses a specific challenge because it often occurs at immunoprivileged sites, such as the central nervous system, skin, and bones, which are believed to be less accessible for the GVL effect. However, ipilimumab treatment was shown to be effective in patients with chloroma [34], suggesting that immune checkpoint inhibition may help counteract the immune escape at immunoprivileged sites. Specific targeting approaches may offer new options [134]. Radiation either in palliative doses or in combination with systemic therapy may be justified to relieve organ compression.

Specific Approaches in Pediatric and Adolescent Patients

The number of patients age <18 years with AML undergoing allo-HCT is very small. Approximately 25% of these patients experience an overt relapse after allo-HCT. These low patient numbers have hindered conclusive studies, and standards have not yet been defined. A retrospective international dataset including more than 300 pediatric patients is currently being evaluated. Preliminary data suggest a low likelihood of long term survival without a second allo-HCT (personal communication, M. G. Sauer). Given their retrospective nature, these data are prone to a strong selection bias, and subgroups of patients may benefit from targeted approaches.

SUMMARY AND OUTLOOK

Relapse remains the most challenging cause of treatment failure after allo-HCT. Approaches to reduce relapse risk by increasing the intensity of the conditioning regimen or reducing immunosuppression with the aim of increasing the GVL effect carry a substantial risk of severe complications. Therefore, these interventions should be chosen individually based on relapse risk and patient-related factors, such as age and comorbidities.

Prophylaxis to reduce the risk of relapse after allo-HCT includes both pharmacologic and cellular approaches. Both strategies can reduce the risk of relapse but carry the risk of side effects in a fraction of “already cured” individuals. In contrast, preemptive interventions based on the detection of MRD or mixed chimerism as well as the treatment of overt hematologic relapse affects only patients with a very poor long-term prognosis. Besides symptomatic treatment and palliative chemotherapy, some new approaches offer long-term remission and even cure in a substantial number of patients. These treatment strategies have evolved in recent years, and most combine cytotoxic or disease-specific targeted therapy with cellular approaches to break tolerance and augment the GVL effect. Although relapse of AML and MDS after allo-HCT remains a therapeutic challenge, multiple promising therapeutic approaches have evolved over the past decade, including TKIs, cellular therapy techniques, hypomethylating agents, small molecules, and immune checkpoint antibodies. Cellular therapies based on CAR technology are intriguing, allowing leukemia rejection without eliminating healthy progenitor and stem cells. TKIs directed at FLT3, immune-modulating agents like lenalidomide, and immune checkpoint inhibitors against CTLA4 and PD1 are promising agents but carry a risk of severe GVHD. Ultimately a second or subsequent allo-HCT may be performed in eligible patients.

Future studies focusing on post-transplantation MRD monitoring and early treatment of relapse or persistent disease are needed, to facilitate treatment of patients at risk and spare patients not requiring intervention from the side effects of treatment. A better understanding of the mechanisms responsible for the persistence of LSCs in the bone marrow will lead to identification of molecular targets of treatment.

Here we have provided the treating physician with an overview of the published evidence on the use of classical therapeutic strategies and novel therapeutic targets currently being tested in clinical studies to prevent and treat relapse of AML and MDS after allo-HCT. Further improving the prognosis of patients with AML and MDS after allo-HCT remains a major challenge.

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