Harnessing the immune system after allogeneic stem cell transplant in acute myeloid leukemia

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Abstract
Allogeneic stem cell transplantation (allo-SCT) is the most successful and widely used immunotherapy for the treatment of acute myeloid leukemia (AML), as a result of its anti-leukemic properties driven by T cells and natural killer (NK) cells, leading to a graft-vs-leukemia (GVL) effect. Despite its essential role in AML treatment, relapse after allo-SCT is common and associated with a poor prognosis. There is longstanding interest in developing immunologic strategies to augment the GVL effect post-transplant to prevent relapse and improve outcomes. In addition to prophylactic maintenance strategies, the GVL effect can also be used in relapsed patients to reinduce remission. While immune checkpoint inhibitors and other novel immune-targeted agents have been successfully used in the post-transplant setting to augment the GVL effect and induce remission in small clinical trials of relapsed patients, exacerbations of graft-vs-host disease (GVHD) have limited their broader use. Here we review advances in three areas of immunotherapy that have been studied in post-transplant AML: donor lymphocyte infusion (DLI), immune checkpoint inhibitors, and other monoclonal antibodies (mAbs), including antibody-drug conjugates (ADCs) and ligand receptor antagonists. We also discuss additional therapies with proposed immunologic mechanisms, such as hypomethylating agents, histone deacetylase inhibitors, and the FLT3 inhibitor sorafenib.

1 | INTRODUCTION

Allogeneic stem cell transplantation (allo-SCT) is the most successful form of immunotherapy for acute myeloid leukemia (AML). The success of allo-SCT hinges on the GVL effect, the ability of the donor immune system to recognize and attack recipient leukemia cells. First suggested by a mouse transplantation model in 1956, this GVL effect was applied clinically in 1965 when allo-SCT was used to treat a patient with acute lymphocytic leukemia (ALL). Allo-SCT is now known to cure many patients with AML; however, post-transplant relapse remains the most frequent cause of death, with relapse rates of 37% following reduced-intensity conditioning (RIC). The GVL effect is critical for preventing relapses, but stimulating donor T lymphocytes leads to both acute and chronic GVHD that can be fatal and cause long-term morbidity. This paradox has stymied the universal adoption of treatments designed to prevent relapse by stimulating the GVL effect after allo-SCT. However, three factors will play an important role in the broader use of prophylactic post-transplant therapies to harness the immune system in the coming years: an increased ability to identify AML patients at the highest risk for post-transplant relapse, a better understanding of the mechanisms underlying those relapses, and the proliferation of novel agents and cell-based therapies with immunologic mechanisms.

A variety of pre- and post-transplant factors can identify AML patients at the highest risk for disease relapse after allo-SCT. Pre-transplant factors that confer an increased risk of relapse include measurable residual disease (MRD) by flow cytometry prior to transplant (cumulative incidence of relapse [CIR] at 3 years 63% vs 22% in MRD-negative patients), a complex karyotype at diagnosis (CIR at 2 years 46% among patients in CR1 at transplant), or a FLT3-ITD mutation (CIR at 2 years 30% vs 16% for FLT3-ITD wild type patients). One of the simplest methods for predicting relapse is the disease risk index...
(DRI), which relies on disease risk (ie, cytogenetics in AML) and disease status at the time of transplant to risk stratify patients for OS and PFS, which is driven largely by risk of relapse. Post-transplant factors that predict an increased incidence of subsequent relapse include peripheral blood T lymphocyte donor chimerism ≥85% from day +90 to +120 (CIR at 3 years 29% vs 15% for donor chimerism >85% in patients transplanted in CR1/CR2), and the presence of MRD by flow cytometry (CIR at 1 year >75% in patients with detectable MRD after allo-SCT). Post-transplant chimerism has been used to select patients for preemptive therapy, including the early withdrawal of immunosuppression, donor lymphocyte infusion (DLI), and azacitidine. However, the majority of patients who relapse after transplant have >85% peripheral blood T cell chimerism and are negative for MRD by flow cytometry. Thus, while we have made progress in identifying the patients at highest risk for AML relapse after allo-SCT, the current prophylactic targeted therapies and preemptive strategies are not applicable to the majority of patients.

AML relapse following allo-SCT occurs by a number of unique mechanisms, including genomic human leukocyte antigen (HLA) loss following mismatched transplants, downregulation of major histocompatibility complex (MHC) class II genes on leukemic blasts, and upregulation of inhibitory checkpoint molecules. Vago et al. identified genomic HLA loss as a mechanism of post-transplant AML relapse, after noting that some patients who relapsed following haploidentical transplant acquired uniparental disomy of chromosome 6p in the leukemic blasts, thereby preventing recognition by donor T cells. More recently, Christopher et al. showed that 50% of AML patients who relapsed after non-haploidentical allo-SCT had down-regulation of MHC class II. Notably, interferon-γ and other agents discussed in this review can reverse the downregulation of MHC class II, and thus potentially prevent or treat a significant percentage of AML relapses. Conversely, in post-transplant relapses with preserved expression of MHC class II, Toffalori et al. demonstrated upregulation of immune inhibitory ligands, such as PD-L1, suggesting a second, distinct mechanism of immune evasion. This finding suggests that selective immune checkpoint blockade may represent the ideal therapeutic modality to reverse the immune dysfunction that is driving relapse in these patients. Thus, recent research highlights the unique and distinct mechanisms of immune evasion underlying post-allo-SCT AML relapse, which may allow therapy to be tailored to overcome these mechanisms of relapse.

In the current review, we will outline advances in post-transplant therapy for the treatment of AML by focusing on three domains: DLIs; immune checkpoint inhibitors; and monoclonal antibodies (mAbs), including antibody drug conjugates and ligand receptor antagonists. An overview of these therapies and relevant targets, where applicable, is listed in Table 1 and shown in Figure 1. A summary of notable studies with evidence for these therapies in post-transplant AML can be found in Table 2.

We also discuss other therapies with proposed immunologic mechanisms, such as hypomethylating agents, FLT3 inhibitors, and histone deacetylase inhibitors. While our goal is to focus on prophylactic maintenance strategies to prevent relapse, we also include data on the efficacy of immunotherapies in relapsed post-transplant patients due to the relative paucity of clinical trials in the latter population. In addition to highlighting novel therapies, we seek to illustrate how our increased understanding of the risk of relapse and the mechanisms of relapse will allow us to better select patients for prophylactic post-transplant therapies, thus minimizing the risk of additional toxicities in those patients who are less likely to relapse while maximizing the benefit of the selected therapies in those patients at the highest risk of relapse.

### Table 1: Overview of immunotherapies for acute myeloid leukemia after allogeneic stem cell transplantation (allo-SCT)

<table>
<thead>
<tr>
<th>Type of therapy</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular therapy</td>
<td>DLI, NK cell infusion, *CAR T cell (CD33, CD123)</td>
</tr>
<tr>
<td>Bispecific T cell engaging techniques</td>
<td>*Bispecific antibodies (CD33 × CD3, CD123 × CD3)</td>
</tr>
<tr>
<td>Monoclonal antibodies (mAbs)</td>
<td>CSL360 (CD123), Daclizumab (CD25), BI 836858 (CD33), Daratumumab (CD38)</td>
</tr>
<tr>
<td>Antibody-drug conjugates (ADCs)</td>
<td>Gemtuzumab (CD33), 131I-BC8 (CD45), F16-IL2 (TnC-A1)</td>
</tr>
<tr>
<td>Immune checkpoint inhibitors</td>
<td>Nivolumab (Anti-PD-1), Pembrolizumab (Anti-PD-1), Ipilimumab (Anti-CTLA-4)</td>
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</table>

*Not discussed in this article.*

2 | DLI

#### 2.1 Introduction to DLI

Frequently used in the post-transplant setting, allogeneic cell infusion is a proven method for inducing GVL and treating relapsed disease. Post-transplant AML relapse is driven in part by loss of leukemic-specific T cells and loss of T cell function. DLI has been found to increase anti-leukemic T cells, but also reverses T cell exhaustion through increased IFN-γ production and reduced T cell inhibitory receptors, such as programmed cell death protein 1 (PD-1) and T cell immunoglobulin and mucin domain 3 (Tim-3). As with allo-SCT, the major obstacle with DLI is to maximize the GVL effect while minimizing GVHD. Canine studies in the 1970s illustrated the importance of delaying DLIs for at least 2 months after transplant in order to prevent fatal GVHD. This allowed for the first clinical use of allogeneic lymphocytes in a patient with relapsed, post-transplant chronic myeloid leukemia (CML), who achieved remission following DLI. Since then, DLI as the sole therapy for relapsed myeloid malignancies was shown to yield remission in ~80% of patients with relapsed chronic phase CML, but just 29% of patients with AML or MDS with very limited long-term survival even among responding patients.

2.2 DLI for relapsed AML

A number of strategies have been utilized to augment the efficacy of DLI, including pairing it with conventional AML therapy or...
lymphodepleting chemotherapy. A retrospective analysis by the EBMT of AML patients with post-transplant relapse demonstrates a 56% 2-year overall survival in patients who had a favorable karyotype or achieved remission with chemotherapy prior to DLI, whereas 2-year overall survival was just 9% in the majority of patients who were not in remission at the time of DLI.\textsuperscript{19} Thus, effective therapy

**FIGURE 1** Mechanisms of immunotherapy in AML. A, T cells and immune checkpoint inhibitors. B, NK cells, monoclonal antibodies (mAbs), and antibody-drug conjugates (ADCs); antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC)
<table>
<thead>
<tr>
<th>Therapy</th>
<th>Indication</th>
<th>Cohort size</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cellular therapy</strong></td>
<td></td>
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<tr>
<td>Prophylactic donor lymphocyte infusion + hypomethylating agent</td>
<td>High-risk AML or MDS after allo-SCT</td>
<td>Thirty patients</td>
<td>Two-y OS 66%; cumulative incidence of relapse 28% at 2; cumulative incidence of acute GVHD 32% at 2</td>
<td>Guillaume T, Yakoub-Agha I, Tabrizi R, et al. Prospective phase II study of prophylactic azacitidine and donor lymphocyte infusions following allogeneic hematopoietic stem cell transplantation for high risk acute myeloid leukemia and myelodysplastic syndrome. Blood</td>
</tr>
<tr>
<td>NK cells + IL-2 infusion</td>
<td>Relapsed or persistent AML or MDS after allo-SCT</td>
<td>Eight patients</td>
<td>Complete response in 25%; 0 GVHD; unable to detect haploidentical NK cells after infusion</td>
<td>Shaffer BC, Le Luduec JB, Forlenza C, et al. Phase II study of haploidentical natural killer cell infusion for treatment of relapsed or persistent myeloid malignancies following allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2016;22(4):705-</td>
</tr>
<tr>
<td><strong>Immune checkpoint inhibitors</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Ipilimumab</td>
<td>Relapsed hematologic malignancy after allo-SCT</td>
<td>Twenty eight patients</td>
<td>One-y OS 49%; among 22 patients receiving increased dose 10 mg/kg, complete response in 23% with dose-limiting IRAEs 14% and dose-limiting GVHD 14%</td>
<td>Ipilimumab for patients with relapse after allogeneic transplantation. N Engl J Med. 2016;375(20):2010.</td>
</tr>
<tr>
<td>Nivolumab</td>
<td>Relapsed hematologic malignancy after allo-SCT</td>
<td>Twenty eight patients</td>
<td>Dose-limiting toxicities in 2/6 patients at 1 mg/kg; early GVHD and severe IRAEs at 0.5 mg/kg resulted in two deaths and early accrual termination; partial response in</td>
<td>Davids MS, Kim HT, Costello CL, et al. A phase I/ib study of nivolumab for relapsed hematologic malignancies after allogeneic hematopoietic cell transplantation (alloHCT). Blood. 2018;132:705.</td>
</tr>
<tr>
<td>Therapy</td>
<td>Indication</td>
<td>Cohort size</td>
<td>Results</td>
<td>Reference</td>
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<tr>
<td><strong>Antibody-drug conjugates (ADCs)</strong></td>
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<tr>
<td>Gemtuzumab + azacitidine maintenance therapy</td>
<td>High-risk hematologic malignancies after allo-SCT</td>
<td>Twenty three patients</td>
<td>Compared to matched case–control group, trend toward improved OS (69% vs 48%) and cumulative relapse (33% vs 41%) at 1 y; three cases grade III acute GVHD</td>
<td>Kaito S, Najima Y, Kishida Y, et al. Post-transplant maintenance therapy with azacitidine and gemtuzumab ozogamicin for high-risk hematologic malignancies. <em>Blood</em>. 2017;130(4517).</td>
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<td><strong>FLT3 inhibitors</strong></td>
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<tr>
<td>Gilteritinib</td>
<td>Relapsed or refractory FLT3-mutated AML</td>
<td>Three hundred and seventy one patients (74 with prior allo-)</td>
<td>Median survival 9.3 months; CR 35.4% in patients with prior allo-SCT vs 17.6% in patients without prior allo-</td>
<td>Perl AE, Martinelli G, Cortes JE, et al. Gilteritinib or chemotherapy for relapsed or refractory FLT3-mutated AML. <em>N Engl J Med</em>. 2019;381(18):1728-1740.</td>
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<tr>
<td><strong>Hypomethylating agents</strong></td>
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<tr>
<td><strong>Histone deacetylase inhibitors</strong></td>
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prior to DLI can dramatically improve the durability of responses.4 Lymphodepleting therapy, such as fludarabine and cyclophosphamide, represents an alternative to disease-specific therapy that can alter the immunologic milieu to enhance the efficacy of DLI and yields a response rate of 49% in patients with relapsed, non-CML hematologic malignancies.20 However, retrospective studies have also shown an increased incidence of acute GVHD, particularly GVHD of the lower GI tract, among patients who receive lymphodepleting chemotherapy prior to DLI.21 Given the known benefit of GVHD in enhancing the GVL effect, there is a clear rationale for the use of lymphodepletion prior to DLI. Ultimately, both disease-specific chemotherapy and lymphodepletion given prior to DLI for relapsed AML may increase both response rates and/or response durability, but prospective, randomized trials are needed.

2.3 | DLI after T-cell depleted (TCD) allogeneic stem cell transplantation (allo-SCT)

There is also interest in using DLI after TCD allo-SCT. While TCD grafts can reduce GVHD and may obviate the need for GVHD prophylaxis,22 they are also associated with increased rates of graft failure and relapse.23,24 One strategy for dealing with such relapse is DLI. In a study assessing the role of DLI in 51 patients with relapsed AML or MDS after TCD allo-SCT, treatment with DLI resulted in a 5-year overall survival of 40% with a 5-year relapse rate of 69% and cumulative GVHD incidence of 45% at 5 years.25 Some have sought to enhance the GVL effect of DLI by removing CD25+ regulatory T cells. In a phase I study involving 21 patients with relapsed hematologic malignancies after transplant (15 with AML), compared to unmanipulated DLI, CD25+ depleted DLI was associated with a better response rate and improved event-free survival, although no significant differences were found in the AML population.26

2.4 | Prophylactic DLI for AML

While DLI has more limited efficacy in treating relapsed post-transplant AML, recent studies suggest DLI may successfully prevent relapse after allo-SCT. In a retrospective review comparing outcomes in 46 high-risk AML patients who received DLI, while in CR after allo-SCT, only 22% of patients relapsed (compared with 53% in the control group), and overall survival was 67% (compared with 31% in the control group) with a median follow-up of 7 years.27 More recently, a registry-based matched-pair analysis of 89 pairs (65 with AML) investigating the role of prophylactic DLI after allo-SCT found no significant difference in a standard-risk cohort, but a reduced rate of relapse (31% vs 46% in controls) and improved 5-year overall survival (70% vs 40% in controls) among patients with high-risk AML, defined as unfavorable cytogenetics or transplant not in CR. Prophylactic DLI was also associated with non-significant trend toward more chronic GVHD.28

2.5 | Prophylactic DLI with hypomethylating agents

Another method of increasing DLI efficacy is to combine it with hypomethylating agents. In a phase II study of 30 patients with high-risk AML (n = 20) and MDS (n = 10) treated with prophylactic azacitidine (AZA) followed by DLI after allo-SCT, the 2-year OS was 66% with cumulative incidence of relapse 28%, and cumulative incidence of acute GVHD 32%. While these outcomes are impressive in this high-risk, post-transplant population, approximately half of the patients who were identified as high-risk prior to transplant were unable to actually enroll in the study, primarily due to GVHD (15 patients) and early relapse (eight patients) at the time of planned enrollment. This issue confounds many post-transplant studies, as outcomes data are greatly biased by the selective inclusion of patients who survive the early post-transplant period without significant GVHD or relapse.29

2.6 | Prophylactic DLI after TCD allo-SCT

Prophylactic DLI has also been used after TCD allo-SCT in an effort to improve engraftment and immune reconstitution and thus augment the GVL effect. In a study of 62 patients with AML or MDS, 5-year overall survival was 80% with event-free survival of 65%, and cumulative incidence of GVHD 31% at 5 years.25 While the optimal timing of prophylactic DLI after TCD allo-HSCT is not established, DLI given less than 6 months after transplant may be associated with increased risk of GVHD.30

2.7 | DLI dosing

The optimal dosing and number of DLIs remain to be determined; however, initial doses >1.0 × 10⁷ cells/kg have been associated with an increased risk of GVHD without improved outcomes.31,32 Many recommend an individualized approach to DLI, such as adjusting the dose based on the type of transplant27 or adjusting the total number of DLI based on the risk of relapse.33 Perhaps the most common method is dose escalation, or increasing doses with each subsequent DLI in order to improve outcomes.34 Though a clearly defined endpoint is not always possible, some continue dose escalation until the patient develops GVHD to offer the best opportunity for clinical response.25

2.8 | Second allo-SCT for relapsed AML

An alternative to DLI is second allo-SCT. In a retrospective study of 179 patients with relapsed acute leukemia after allo-SCT (72 with AML), treatment with a second allo-HCT was associated with 25% overall survival at 2 years.36 In a retrospective study from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation involving 418 patients with post-allograft relapsed AML, outcomes were similar in patients receiving second allo-SCT (137 patients) compared to those receiving DLI.
properties. Importantly, HLA and KIR segregate independently on different chromosomes; thus, most HLA-matched donors are not KIR-KIRL matched. Of note, a second allogeneic transplant offers the opportunity to switch haplotypes, which may be particularly relevant in patients who have relapsed due to genomic haplotype loss and seems to yield improved outcomes when compared to patients undergoing a second transplant who were not transplanted with a new haplotype.38

2.9 | DLI for AML after allo-SCT: Current clinical trials

As shown in Table 3, current trials are investigating the role of post-transplant prophylactic DLI alone (NCT03597321) or with the hypomethylating agent guadecitabine (NCT03454984). Other trials are investigating the role of DLI in combination with guadecitabine (NCT02684162) and daratumumab, an anti-CD38 monoclonal antibody (NCT03537599) for relapsed AML.

3 | NK CELL-BASED THERAPIES

3.1 | Introduction to NK cell-based therapies

In addition to T cells, NK cells play a critical role in providing the anti-tumor effect of allo-SCT. This may be of particular importance in AML, where leukemic cells are more sensitive to NK-mediated cytotoxicity than solid tumors.39 The NK cells belong to innate lymphoid cells and are able to distinguish "self" from "non-self" through the interaction of NK receptors (ie, inhibitor killer immunoglobulin-like receptors [iKIRs]) with self-ligands (ie, certain MHC class I molecules, or KIR ligands [KIRLs]). This ability of NK cells to recognize tissue lacking self-MHC class I molecules, such as cancer cells, allows for their anti-tumor properties.40 Importantly, HLA and KIR segregate independently on different chromosomes; thus, most HLA-matched donors are not KIR-KIRL matched.41 Unlike T cells, which enhance both the anti-leukemic effect and GVHD in allo-SCT, alloreactive NK cells may contribute to GVL while actually protecting against GVHD. In one particularly noteworthy study involving 92 high-risk leukemia patients receiving HLA haplotype-mismatched allo-SCT, donor NK cell alloreactivity was associated with total protection against rejection, GVHD, and AML relapse. Multivariate analysis assessing conditioning regimens, number of stem cells and T cells in the graft, and disease status revealed only one independent predictor of survival: KIRL incompatibility in the GVHD direction, which was associated with 5-year event free survival in 60% of AML patients vs 5% in those without KIRL incompatibility. Interestingly, these findings were not seen in patients with ALL.42

3.2 | NK cell infusions for AML

While the use of allogeneic NK cell infusions has proven to be safe both before and after transplant,43,44 its efficacy in preventing relapse has been limited. In a phase II trial, involving 16 patients with high-risk malignancies treated with T-cell-depleted allo-SCT followed by NK cell infusions at days +30, +60, and +90 after transplant, relapse rates were similar to historical controls with four of eight AML patients relapsing. Overall, four of 16 patients (25%) developed acute GVHD grade II or higher, all of whom received a relatively high dose of CD3+ T cells (>5 × 10^7 cells/kg). The authors emphasized the need for further studies to determine optimal dosing and timing of NK cell therapy.45 One approach to increase NK cell activity is administering interleukin-2 (IL-2). In a phase II study involving eight patients (six with AML, two with MDS) with relapsed or persistent myeloid malignancy after allo-SCT, treatment with NK cells and IL-2 infusions resulted in two patients (one with AML and one with MDS) achieving complete response, with both relapsing less than 2 months later. There were no incidents of GVHD. Of note, the authors experienced difficulty detecting NK cells after transfer, indicating a lack of persistence of NK cells, which could be due to inadequate numbers of infused cells, inhibition from Tregs, or limited cytokine availability.46

3.3 | NK cells for AML after allo-SCT: Current clinical trials

As shown in Table 3, current clinical trials are further investigating the role of expanded NK cells (NCT03300492, NCT02809092), cytokine-induced memory like NK cells (CiML NK cells) (NCT02782546), and combining NK cells with fludarabine/total body irradiation (NCT00789776).

4 | IMMUNE CHECKPOINT INHIBITORS

4.1 | Introduction to immune checkpoint inhibitors

Immune checkpoint inhibitors are increasingly being used for the treatment of hematologic malignancies including the post-transplant setting.47 The theory is that by blocking immune checkpoints—such as the interaction of cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) and programmed death-1 receptors (PD-1) with their respective ligands—immune checkpoint inhibitors can prevent tumors from evading the donor immune system. There is also evidence that checkpoint blockade may enhance NK cell activity.48,49 The major concern with using immune checkpoint inhibitors after allo-SCT is the increased risk of GVHD.

4.2 | CTLA-4 blockade in AML

Ipilimumab is a monoclonal antibody against CTLA-4, a molecule that competes with CD28 for binding to B7 on antigen presenting cells (APCs) and thus downregulates T-cell activity. In a phase I/IIb study, ipilimumab was given to 28 patients with relapsed hematologic malignancy after allo-SCT (12 with AML). No clinical responses were noted.
<table>
<thead>
<tr>
<th>Identifier</th>
<th>Study title</th>
<th>Indication</th>
<th>Clinical phase</th>
<th>Intervention</th>
<th>Primary outcomes</th>
<th>Estimated enrollment</th>
<th>Sponsor</th>
<th>Country</th>
<th>Status</th>
<th>Start date</th>
<th>Completion date</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT03597321</td>
<td>Comparative phase II trial of early prophylactic donor lymphocyte infusion (DLI) after allogeneic hematopoietic stem cell transplantation for patients with acute myeloid leukemia</td>
<td>AML in complete remission after HSCT</td>
<td>Phase 2</td>
<td>Prophylactic DLI</td>
<td>Relapse free survival</td>
<td>One twenty four participants</td>
<td>Institut Paoli-Calmettes</td>
<td>France</td>
<td>Not yet recruiting</td>
<td>January 2019</td>
<td>January 2024</td>
</tr>
<tr>
<td>NCT02694162</td>
<td>A Phase II trial to assess the efficacy and toxicity of SGI-110 with DLI for the treatment of acute myelogenous leukemia (AML) or myelodysplastic syndrome (MDS) relapsing after allogeneic stem cell transplantation (HSCT)</td>
<td>AML or MDS with prior HSCT (with minimal residual disease, relapse, or in remission)</td>
<td>Phase 2</td>
<td>SGI-110, DLI</td>
<td>Response</td>
<td>Ninety participants</td>
<td>MD Anderson Cancer Center, Astex Pharmaceuticals</td>
<td>USA</td>
<td>Recruiting</td>
<td>June 2016</td>
<td>June 2021</td>
</tr>
<tr>
<td>NCT03454984</td>
<td>SGI-110 and donor lymphocyte infusions (DLI) after allogeneic stem cell transplantation</td>
<td>Very high risk MDS or AML</td>
<td>Phase 2</td>
<td>SGI-110, DLI</td>
<td>Disease free survival</td>
<td>Forty participants</td>
<td>Groupe Francophone des Myelodysplasies</td>
<td>France</td>
<td>Not yet recruiting</td>
<td>November 2018</td>
<td>March 2022</td>
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<tr>
<td>NCT03535999</td>
<td>Phase I/II clinical trial of daratumumab and DLI in patients with relapsed acute myeloid leukemia post-allogeneic hematopoietic stem cell transplant</td>
<td>AML or MDS relapse after HSCT</td>
<td>Phase 1/2</td>
<td>Daratumumab, DLI</td>
<td>Safety and feasibility</td>
<td>Thirty participants</td>
<td>Ohio State University Comprehensive Cancer Center</td>
<td>USA</td>
<td>Not yet recruiting</td>
<td>December 2018</td>
<td>September 2021</td>
</tr>
<tr>
<td>NCT03330492</td>
<td>A phase I/II single center study to assess the safety, tolerability and feasibility of pre-emptive immunotherapy with in vitro expanded natural killer (NK) cells in patients treated with Haplo-HSCT for AML/MDS</td>
<td>AML or MDS with indication for HSCT</td>
<td>Phase 1/2</td>
<td>Expanded NK-DLI after HSCT</td>
<td>Toxicity</td>
<td>Ten participants</td>
<td>University Hospital, Basel, Switzerland</td>
<td>Switzerland</td>
<td>Recruiting</td>
<td>November 2018</td>
<td>January 2023</td>
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<tr>
<td>NCT00789776</td>
<td>A phase I/II study evaluating the safety and efficacy of adding a single prophylactic DLI of NK cells early after nonmyeloablative, HLA-haploidentical hematopoietic cell</td>
<td>Various hematologic malignancies, including AML with +5% blasts</td>
<td>Phase 1/2</td>
<td>NK cells after HSCT</td>
<td>Phase 1: maximum tolerated dose; Phase 2: relapse, GVHD, and non-relapse mortality</td>
<td>Forty one participants</td>
<td>Fred Hutchinson Cancer Research Center</td>
<td>USA</td>
<td>Active, not recruiting</td>
<td>October 2008</td>
<td>May 2021</td>
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in the 6 patients who received ipilimumab at 3 mg/kg. Of the 22 patients who received ipilimumab at 10 mg/kg, five patients achieved a complete response (23%), including all three with leukemia cutis, one with myeloid sarcoma, and one with smoldering MDS developing into AML. Among these 22 patients, dose-limiting toxic effects included three cases of GVHD (two cases chronic GVHD of the liver, one case acute GVHD of the gut), as well as three cases of immune-related adverse events (IRAEs)–thrombocytopenia, colitis, and pneumonitis–including one death.50 While there is clear evidence for the efficacy of CTLA-4 blockade following allo-SCT in AML, the risks of GVHD exacerbation and IRAEs are currently too significant for prophylactic use.

4.3 | PD-1 blockade in AML

In addition to CTLA-4 inhibition, PD-1 has also been targeted in the post-transplant setting, as it has been implicated in one of the common immunologic mechanisms of relapse by Toffalori et al.14 In a phase I/Ib study of nivolumab in 28 patients with relapsed hematologic malignancy after allo-SCT (11 with AML), two of six patients treated with nivolumab at 1 mg/kg developed dose-limiting IRAEs. Subsequent dose reduction to 0.5 mg/kg was well-tolerated in an initial cohort of eight patients. However, upon enrollment of 14 more patients, accrual was terminated due to four cases of dose limiting toxicity, including two cases of grade III acute GVHD (liver and gut) resulting in two deaths. Of the 19 evaluable patients treated at nivolumab 0.5 mg/kg, the overall response rate was 16%, including partial response in one patient with AML.51 In another study, 11 patients with recurrence of hematologic malignancy after allo-SCT (eight with AML) were treated with pembrolizumab for up to 2 years. In all, seven patients experienced immune related adverse events with three dose-limiting toxicities and one treatment-limiting toxicity. Of the four AML patients evaluable for response, two had stable disease and two experienced disease progression. This study is ongoing.52

4.4 | Safety concerns and future role of immune checkpoint inhibition after allo-SCT

Despite the efficacy of checkpoint inhibitors in the post-transplant setting, immune related adverse events and GVHD remain significant concerns. In a review of 24 articles describing the use of checkpoint inhibitors for hematologic malignancies including 176 patients receiving a checkpoint inhibitor after allo-SCT, 14% developed acute GVHD and 9% developed chronic GVHD. The risk of GVHD in this patient population varies based on history of prior GVHD, allograft source, and post-transplant GVHD prophylaxis.53 In the same study, there were 40 reported deaths, of which 28% were GVHD related. This represents a remarkably high rate of death from GVHD (7%) among patients receiving checkpoint inhibitors after allo-SCT. However, the overall response rate to post-transplant checkpoint inhibition for relapsed disease was 54%, with complete response in 33% of patients, though the majority of patients were being treated for Hodgkin
lymphoma (89 of 176). Thus, while the safety of post-transplant checkpoint inhibition remains a major concern, this should be interpreted in the context of promising response rates for a patient population with very limited treatment options. Further studies are needed to understand the durability of these responses, whether dosing can be adjusted to improve the safety profile while retaining efficacy, and how patients’ prior GVHD prophylaxis might impact their risk of GVHD. In AML patients, Noviello et al. have demonstrated that the detection of severely exhausted (PD-1+Eomes+T-bet+) T cells in the bone marrow shortly after transplant predicts relapse. This finding suggests the possibility that intervention with immune checkpoint inhibitors could be used to prevent relapse in patients with early evidence of T cell exhaustion following transplant, but the aforementioned safety concerns currently limit the appeal of such an approach. Thus, while there is not yet a role for the prophylactic or preemptive use of immune checkpoint inhibitors to prevent post-transplant relapse in AML, further studies may allow for the identification of patient populations that can safely be given immune checkpoint inhibitors in the post-transplant setting with appropriate dosing.

### 4.5 Immune checkpoint inhibition for AML after allo-SCT: Current clinical trials

Current clinical trials continue to explore the role of immune checkpoint inhibitors for relapsed AML after allo-SCT using pembrolizumab (NCT03286114, NCT02981914), ipilimumab or nivolumab (NCT01822509), and ipilimumab + nivolumab (NCT03600155, NCT02846376). Other trials are studying the role of these therapies in relapsed/refractory AML allowing for prior allo-SCT: pembrolizumab + azacitidine (NCT02845297), ipilimumab + decitabine (NCT02890329), and nivolumab + cyclophosphamide (NCT03417154) (Table 4).

### 5 ANTIBODY-DRUG CONJUGATES (ADCs) AND LIGAND-RECEPTOR ANTAGONISTS

#### 5.1 Introduction to ADCs and ligand-receptor antagonists

Monoclonal antibodies (mAbs) comprise a variety of immunotherapeutic modalities. For the purpose of this review, we will focus on ADCs and ligand-receptor antagonists. ADCs are mAbs covalently bound to a drug or radioisotope, allowing for targeted delivery and cytotoxicity based on tumor-specific cell surface antigens. Ligand-receptor antagonists are mAbs that either bind to and block certain pathologic or immunologic pathways or stimulate antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), or complement-dependent cytotoxicity (CDC). While a variety of antibody-based approaches have proven successful in other hematologic malignancies—including acute lymphoblastic leukemia (ALL), B cell non-Hodgkin lymphoma (NHL), Hodgkin lymphoma, and multiple myeloma—the antigens commonly expressed on AML cells have substantial overlap with those expressed on normal hematopoietic cells; thus, targeting these antigens could lead to significant myelosuppression.

#### 5.2 ADCs and ligand-receptor antagonists in AML

The most frequently targeted antigen in AML thus far is CD33, an antigen that is variably expressed in leukemic blasts in 85-90% of patients with AML. The most notable drug to target CD33 is gemtuzumab ozogamicin (GO), a humanized anti-CD33 IgG4 antibody conjugated to calicheamicin. Though its role in the post-transplant setting is still being determined, there at least two case reports of GO successfully treating isolated extramedullary AML relapse after allo-SCT. Of note, hepatotoxicity—notably, venoocclusive disease (VOD)—has been a particularly concerning side effect that may be more common in patients who undergo allo-SCT. GO has also been used in combination with AZA as maintenance therapy after allo-SCT. In a study of 10 patients with high-risk AML (eight not in CR at the time of transplant), AZA and GO after allo-SCT resulted in 40% cumulative incidence of relapse with a median follow-up of 474 days. Though all patients had already developed grade I/II acute GVHD before receiving AZA-GO, only one developed a GVHD exacerbation during treatment, and no patients developed severe chronic GVHD.

A follow-up study was then performed using 23 patients with high-risk hematologic malignancies (21 with AML) possessing CD33+ leukemic blasts. Compared to matched case-control group, patients receiving AZA-GO maintenance had no significant difference in outcomes, though with a trend toward improved overall survival (69% vs 48%) and cumulative incidence of relapse (33% vs 41%) at 1 year.

Note, CD123, the interleukin-3 receptor alpha chain (IL-3Ra), is another popular target in AML given its high levels of expression in AML blasts, which could be effectively targeted even in the minority of patients who do not express CD33. Despite high levels of expression in CD34+/CD38- leukemic stem cells from which malignant cells in AML are thought to arise, studies have shown very little detectable expression of CD123 in CD34+/CD38- derived cells from normal bone marrow, making it an especially exciting target. For CSL360, it is a recombinant, chimeric IgG1, anti-CD123 monoclonal antibody that neutralizes IL-3. In a phase I study involving 40 patients with advanced AML (seven who had undergone allo-SCT), five dose levels of CSL360 were administered at doses of 0.1-10.0 mg/kg. Despite complete saturation and downregulation of CD123, indicating successful IL-3 signal blockade, only two patients responded. However, it is worth noting both responders had undergone prior allo-SCT.

The antibody drug conjugate F16-IL2 targets certain alternatively spliced extradoms of fibronectin (EDF-Fn and EDB-Fn) and tenascin-C (TenC-A1), that are strongly expressed at sites of angiogenesis, including the bone marrow of patients with AML. While highly expressed in tumor-associated vasculature and stroma, these markers are notably absent in normal organs. Thus, F6-IL2 provides targeted delivery of the...
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<tr>
<th>Identifier</th>
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<th>Intervention</th>
<th>Clinical phase</th>
<th>Primary outcomes</th>
<th>Estimated enrollment</th>
<th>Sponsor</th>
<th>Country</th>
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<td>NCT03286114</td>
<td>Augmentation of the graft vs leukemia effect via checkpoint blockade with pembrolizumab for relapse of primary malignancy after allogeneic hematopoietic stem cell transplant: a feasibility study</td>
<td>Relapsed myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), or acute lymphocytic leukemia (ALL) after allogeneic stem cell transplantation (HSCT)</td>
<td>Pembrolizumab</td>
<td>Phase 1</td>
<td>Clinical benefit, response, and graft versus host disease (GVHD)</td>
<td>Twenty patients</td>
<td>University of Michigan Cancer Center</td>
<td>USA</td>
<td>Recruiting</td>
<td>June 2016</td>
<td>October 2021</td>
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<td>NCT02981914</td>
<td>Pilot study of pembrolizumab treatment for disease relapse after allogeneic stem cell transplantation</td>
<td>Hodgkin lymphoma, B-cell non-Hodgkin lymphoma, AML, MDS</td>
<td>Pembrolizumab</td>
<td>Early phase 1</td>
<td>Tolerability, adverse events</td>
<td>Twenty six patients</td>
<td>University of Chicago</td>
<td>USA</td>
<td>Recruiting</td>
<td>March 2017</td>
<td>February 2020</td>
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<tr>
<td>NCT01822509</td>
<td>A Phase I/II study of ipilimumab or nivolumab in patients with relapsed hematologic malignancies after allogeneic hematopoietic cell</td>
<td>Various hematologic malignancies, including AML, with relapse after</td>
<td>Ipilimumab or nivolumab</td>
<td>Phase 1/1b</td>
<td>Maximum-tolerated dose</td>
<td>One hundred and thirteen participants</td>
<td>National Cancer Institute</td>
<td>USA</td>
<td>Active, not recruiting</td>
<td>April 2013</td>
<td>April 2022</td>
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<td>NCT03600155</td>
<td>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</td>
<td>Relapsed or refractory AML after HSCT</td>
<td>Ipilimumab and nivolumab, alone and in combination</td>
<td>Phase 1</td>
<td>Optimal dosing</td>
<td>Fifty five participants</td>
<td>MD Anderson Cancer Center</td>
<td>USA</td>
<td>Recruiting</td>
<td>October 2018</td>
<td>January 2020</td>
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<tr>
<td>NCT02846376</td>
<td>Phase I study of single-agent and combined checkpoint inhibition after allogeneic hematopoietic stem cell transplantation in patients at high risk for post-transplant</td>
<td>High risk AML, eligible for HSCT</td>
<td>Ipilimumab and nivolumab, alone and in combination, after HSCT</td>
<td>Phase 1</td>
<td>Toxicity</td>
<td>Twenty one participants</td>
<td>Hackensack Meridian Health</td>
<td>USA</td>
<td>Recruiting</td>
<td>October 2018</td>
<td>January 2023</td>
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<td>NCT02845297</td>
<td>Phase 2 study of azacytidine in combination with pembrolizumab in relapsed/refractory acute myeloid leukemia (AML) patients and in newly diagnosed older (≥65 y) AML</td>
<td>Relapsed or refractory AML (not excluding those with prior HSCT)</td>
<td>Pembrolizumab, azacytidine</td>
<td>Phase 2</td>
<td>Maximum tolerated dose</td>
<td>Forty participants</td>
<td>Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins</td>
<td>USA</td>
<td>Recruiting</td>
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pro-inflammatory cytokine IL-2 to the bone marrow and extramedullary AML sites, leading to both NK cell and CD8+ T cell expansion with associated tumor eradication.66 In a small study involving four patients with relapsed AML after allo-SCT, treatment with F16-IL2 combined with low-dose cytarabine resulted in one patient with disseminated extramedullary AML achieving a complete metabolic response lasting for 3 months, and two patients achieving blast reduction with transient molecular negativity. Significant toxicity in the form of cytokine release syndrome developed in two of the four patients, though this was effectively managed with high-dose glucocorticoids. Ultimately both patients tolerated re-exposure to lower doses of F16-IL2.67

Daclizumab is a mAb that binds CD25, the interleukin-2 receptor alpha chain (IL-2Ra) on T cells. Prior to the discovery of CD25+FOXP3+ regulatory T cells, there was some thought that daclizumab may prevent acute GVHD by blocking T cell activation. During this time, a double-blind clinical trial involving 210 patients (30 with AML) found that, while daclizumab did not reduce acute GVHD, there were non-significant trends toward reduced relapse, which was ultimately attributed to the anti-CD25 inhibitory effects on regulatory T cell expansion/activity and associated enhancement in GVL response. However, subgroup analysis showed reduced relapse in CML only, not the combined group of AML/ALL/MDS.68

5.3 ADCs and ligand-receptor agonists/antagonists for AML after allo-SCT: current clinical trials

Current clinical trials investigating the role of ADCs for relapsed AML with prior allo-SCT include the study of F16-IL2 with cytarabine (NCT02957032) and F16-IL2 with the anti-CD33 Ab BI 836858 (NCT03207191). ALT-803 is an IL-15 superagonist, which may enhance ADCC by modulating NK cell activation and cytotoxicity,69 and is currently being studied for the treatment of post-transplant relapse of hematologic malignancies including AML (NCT01885897). Other trials studying relapsed/refractory AML, not excluding post-allo-SCT are investigating gemtuzumab (NCT03737955) and daratumumab, an anti-CD38 mAb expressed in only a subset of AML patients but upregulated by all-trans retinoic acid70 (NCT03067571) (See Table 5). Of note, various studies are also assessing the role of mAbs prior to transplant either for preventing relapse or GVHD, including the use of I-BC8, an ADC targeting the tyrosine phosphatase CD45, which is highly expressed on most AML blasts71 (NCT00119366, NCT00002554).

6 OTHER THERAPIES WITH IMMUNOMODULATORY ACTIVITY

6.1 Introduction to other therapies with immunomodulatory activity in AML

Additional therapies used in AML are thought to depend partly on immunomodulatory effects, including hypomethylating agents, the
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<th>Identifier</th>
<th>Study title</th>
<th>Indication</th>
<th>Intervention</th>
<th>Clinical phase</th>
<th>Primary outcomes</th>
<th>Estimated enrollment</th>
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<tr>
<td>NCT02957032</td>
<td>A dose-finding phase I study of the tumor-targeting human F16IL2 monoclonal antibody-cytokine fusion protein in combination with very low-dose cytarabine in patients with acute myeloid leukemia (AML) relapse after allogeneic hematopoietic stem cell transplantation (HSCT)</td>
<td>Relapsed AML after HSCT</td>
<td>F16IL2, cytarabine</td>
<td>Phase 1</td>
<td>Dose limiting toxicity, maximum tolerated dose, and recommended dose</td>
<td>Thirty patients</td>
<td>Philogen S.p.A</td>
<td>Germany</td>
<td>Active, not recruiting</td>
<td>April 2016</td>
<td>December 2019</td>
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<tr>
<td>NCT03207191</td>
<td>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 (HSCT)</td>
<td>Relapsed AML after HSCT</td>
<td>F16IL2, BI 836858</td>
<td>Phase 1</td>
<td>Dose limiting toxicity, maximum tolerated dose, and recommended dose for</td>
<td>Fifty two patients</td>
<td>Philogen S.p.A</td>
<td>Germany</td>
<td>Recruiting</td>
<td>November 2016</td>
<td>December 2019</td>
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<td>NCT03737955</td>
<td>Fractionated gemtuzumab ozogamicin to eradicate measurable residual disease (MRD) in acute myeloid leukemia (AML) patients (GO for MRD)</td>
<td>AML with minimal residual disease (not excluding prior HSCT)</td>
<td>Gemtuzumab ozogamicin</td>
<td>Phase 2</td>
<td>Clinical response rate</td>
<td>Thirty six patients</td>
<td>University of Washington</td>
<td>USA</td>
<td>Not yet recruiting</td>
<td>December 2018</td>
<td>August 2020</td>
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FLT3 inhibitor sorafenib, and histone deacetylase inhibitors. The transmembrane tyrosine kinase FLT3 is expressed in 70%-100% of leukemic cells that becomes mutationally activated in 30% of patients with AML, most often as a result of internal tandem duplication (ITD). The use of FLT3 inhibitors has been particularly useful in patients with the FLT3-ITD mutation, which carries an unfavorable prognosis. However, these therapies inhibit multiple off-target receptor tyrosine kinases, which may contribute to their anti-leukemic effects and potential toxicities. This is particularly true of the first-generation FLT3 inhibitors, such as sorafenib and midostaurin.

6.2 | FLT3 inhibitors in AML

Sorafenib has been found to increase IL-15 production in mutant FLT3-ITD+ leukemia cells, which enhances the GVL effect by augmenting CD8⁺CD107a⁺IFN-γ⁺ T cells with features of longevity—that is, high levels of Bcl-2 and low levels of PD-1. Sorafenib also increases IFN-γ production, particularly in responders, which may reverse the down-regulation of MHC Class II that has been implicated in AML relapse following non-haploidentical transplant. In a study involving 53 patients with FLT3-ITD AML (51 with relapsed or refractory disease with 29 having undergone prior allo-SCT) who were treated with sorafenib monotherapy, 15 patients (23%) achieved complete remission, including seven patients (24%) with prior allo-SCT. The study also noted less sorafenib resistance and a higher rate of complete molecular response in the post-transplant group compared to the conventional therapy group (24% vs 8%, respectively). A phase I trial of maintenance sorafenib after allo-SCT for 22 patients with FLT3-ITD AML resulted in 1-year progression free survival of 85% and 1-year overall survival of 95%, with only one case of grade II acute GVHD.

While on-target FLT3 inhibition is largely responsible for the efficacy of sorafenib, it does not explain the response disparity between patients with or without prior allo-SCT, suggesting enhanced GVL activity may also be playing a role. In a recent phase III study involving 371 patients with relapsed or refractory FLT3-mutated AML (74 with relapsed or refractory disease with 29 having undergone prior allo-SCT) who were treated with sorafenib monotherapy, 15 patients (23%) achieved complete remission, including seven patients (24%) with prior allo-SCT. The study also noted less sorafenib resistance and a higher rate of complete molecular response in the post-transplant group compared to the conventional therapy group (24% vs 8%, respectively). A phase I trial of maintenance sorafenib after allo-SCT for 22 patients with FLT3-ITD AML resulted in 1-year progression free survival of 85% and 1-year overall survival of 95%, with only one case of grade II acute GVHD.
As demonstrated above, hypomethylating agents such as AZA and DAC are frequently used in combination with other immunotherapies—not only for their direct cytotoxic effects but also their immunomodulatory properties. Anti-tumor effects of HMAs involve increased tumor recognition by upregulation of tumor cell antigens—notably, cancer testis antigens (CTAs)\textsuperscript{80,81}—as well as enhanced T cell reactivity by increased expression of HLA class I antigen and co-stimulatory molecules.\textsuperscript{82,83} Both AZA and DAC have also been shown to demethylate the FOXP3 promoter, increasing FOXP3 expression and expansion of regulatory T cells.\textsuperscript{84,85} Animal models suggest Tregs may reduce GVHD by suppressing early expansion of alloreactive donor T cells without suppressing T cell activation, thus preserving the GVL effect.\textsuperscript{86} Of note, there is also evidence to suggest differential use of T cell-mediated cytolytic pathways: while GVHD is mediated primarily by the FasL effector pathway, GVL responses rely largely on the perforin pathway.\textsuperscript{87}

As previously discussed, low levels of donor chimerism have been associated with increased risk of relapse.\textsuperscript{8} The RELAZA study investigated the use of standard doses of azacitidine (75 mg/m\textsuperscript{2} over 7 days) in AML/MDS patients judged to be at risk of imminent relapse based on declining post-transplant CD34 chimerism, and showed that this strategy restored donor chimerism in the majority of patients. Although most patients eventually suffered a hematologic relapse, this may have been delayed by therapy.\textsuperscript{10} More recently, a randomized controlled trial sought to determine the role of AZA maintenance monotherapy in 187 patients with AML or MDS after allo-SCT; however, the study was closed early due to slow accrual over 8 years. While relapse free survival was comparable between those who received AZA and those who did not, stratification by number of AZA cycles received showed a trend toward improved relapse free survival in patients receiving more cycles of AZA therapy.\textsuperscript{88} In addition to injectable azacitidine, oral azacitidine (CC-486) has also been used for post-transplant maintenance therapy. In a phase I/II dose-finding study involving 30 patients with AML or MDS (26 with AML) in CR after allo-SCT, maintenance therapy with CC-486 was associated with relatively low 1-year rate of relapse or disease progression at 21% of evaluable patients (6/28), as well as 10% grade III acute GVHD.\textsuperscript{89}

Given the potential reduction in GVHD provided by AZA-mediated expansion of Tregs, AZA has been used in combination with the immunomodulatory agent lenalidomide, which was found to be a well-tolerated salvage therapy in post-transplant relapsed AML.\textsuperscript{90} Others have suggested combining hypomethylating agents with BCL-2 inhibitors, such as venetoclax, which may sensitize AML cells to the effects of hypomethylating agents.\textsuperscript{91} This combination will be investigated as a maintenance therapy for high-risk, post-transplant AML in an upcoming phase II trial (NCT04128501). Finally, azacitidine and cytarabine have been found to induce expression of the MHC class II antigen HLA-DR in leukemic cells of patients with AML,\textsuperscript{92} possibly overcoming a significant immunologic mechanism of post-transplant AML relapse following non-haploidentical transplantation.

### 6.4 Histone deacetylase inhibitors in AML

Histone deacetylase inhibitors (HDACis), such as panobinostat, have also been used to treat AML after allo-SCT. In addition to inducing apoptosis and differentiation, HDACis are thought to possess important immunomodulatory effects, including increased expression of tumor antigens, MHC class I and II molecules, costimulatory molecules, and NK cell-activating ligands.\textsuperscript{93-96} In an open-label, multicenter phase I/II trial, prophylactic panobinostat after allo-SCT was given to 42 patients with high-risk AML or MDS (37 with AML, 67% with active disease at the time of transplant) to determine the maximum tolerated dose. At 2 years, only 20% of patients had relapsed and 7% developed grade III acute GVHD. Of note, 18 patients (43%) received a median of two DLIs, as DLIs were allowed throughout the study at the discretion of the treating physician.\textsuperscript{97}

### 7 DISCUSSION

Despite the ability of allo-SCT to successfully treat AML, post-transplant relapse remains a significant concern. Various immunotherapies are now being used for the treatment of relapsed disease after allo-SCT, but these same approaches may also prevent relapse. Given the high risk of relapse in a select subset of AML patients, the prophylactic use of certain therapies after allo-SCT may be especially beneficial in these patients. With the exception of those patients with a history of severe acute GVHD, severe infection, or need for immunosuppression aside from GVHD prophylaxis (eg, active autoimmune disease requiring treatment), there are no specific contraindications. As with any therapy, the increased risk of complications among older patients, and those with comorbidities, must be balanced against the likelihood of post-transplant relapse in determining the risk-benefit of maintenance therapies. Since the median time to relapse after transplant in AML is 7.5 months,\textsuperscript{98} therapies aiming to reduce the risk of relapse should be started relatively soon after transplant.

As both our understanding of the mechanisms underlying AML relapse following allo-SCT and our arsenal of therapeutic options continue to expand, our ability to harness the immune system after allo-SCT should become increasingly nuanced. A number of therapies highlighted in this review—including DLI, immune checkpoint inhibitors, FLT3 inhibitors, and hypomethylating agents—possess the theoretical ability to reverse or prevent recently characterized immunologic mechanisms of AML relapse following allo-SCT. Historically, the challenge has been to enhance the powerful graft vs leukemia effect without augmenting graft vs host disease. The post-transplant use of DLI and immune checkpoint inhibitors, as highlighted in this review, illustrate this difficult compromise. However, with our improved understanding of the immunologic mechanisms underlying relapse, it may now be possible to select prophylactic maintenance therapies that overcome these mechanisms without exacerbating GVHD.

An additional limitation in AML has been the lack of ideal target antigens, which hinders the use of ADCC, T cell engaging techniques, and CAR (chimeric antigen receptor) T-cells. Nonetheless, there are
numerous ongoing trials investigating the role of CAR T-cells for AML with major targets including CD33, CD123, and FLT3. Similarly, T-cell engaging techniques are now being used in AML, mostly targeting CD33 and CD123. The role of these therapies in the post-transplant setting remains to be determined. Ultimately, our enhanced understanding of the mechanisms underlying post-transplant relapse could help us to identify novel antigens and treatment strategies needed to overcome these mechanisms, prevent relapse, and treat relapsed disease.

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