Relapse of hematologic malignancy is the major reason for treatment failure after allogeneic stem cell transplantation. Graft-versus-leukemia (GVL) responses are critical to prevent relapse but may fail as a result of tumor immune evasion, for example through antigen loss or T cell dysfunction. In silico pipelines for the discovery of tumor-expressed antigens and novel therapies that target immune evasion mechanisms (small molecule, biological, immune cell transfer) are potential strategies to sustain or reimpose GVL. A key challenge will be how experimental therapies to enhance GVL can progress from first-in-human trials to prospective, controlled trials that evaluate efficacy and lead to regulatory approval.

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INTRODUCTION

Allogeneic hematopoietic stem cell transplant (allo-HSCT) remains a major treatment approach for patients with blood cancers. Although increasing use of small molecular inhibitors, biologics, and chimeric antigen receptor-transduced T (CAR-T) cells has modified the indications or timing, the number of transplants performed continues to rise worldwide. Improvements in initial or salvage treatments, the use of reduced intensity conditioning regimens, and the increasing use of haploidentical related donors have all contributed to widening eligibility. Allo-HSCT therefore still represents the most common cellular immunotherapy for cancer, in which graft-derived immune cells reject residual malignant cells in the patient. This graft-versus-leukemia (GVL) or graft-versus-tumor response is often very powerful and enduring, reflecting a response against multiple tumor-expressed antigens and incorporating multiple immune cell types. However, the breadth and magnitude of immunity initiated by allo-HSCT is also a disadvantage, leading to peripheral tissue injury from graft-versus-host disease (GVHD). Even with new treatments such as JAK 1/2 inhibitors, approximately one in 10 patients will still die as a result of GVHD and one in three patients are left with chronic disability.

Crucially, deficiencies in initiating or sustaining GVL responses continue to represent the major cause of treatment failure across all settings. Composite measures of outcome such as GVHD and relapse-free survival (GRFS) may provide a better overall assessment of treatment success; recent studies have shown wide variation in 1-year GRFS in the range, 8%-58% dependent on age, HLA matching, stem cell source, and GVL prophylaxis. Thus, there is an urgent need to intensify GVL without incurring GVHD. The purpose of this review is to provide the reader with a summary of recent advances in our understanding of GVL mechanisms, together with an appraisal of current and future approaches to increasing its efficacy. We will not summarize the wealth of new information in relation to GVHD or its prevention and treatment, which have been covered recently in several excellent reviews.

MECHANISMS OF GVL

Mechanisms of GVL are difficult to recapitulate in model systems because of important differences between humans and mice (eg, the lack of conservation of natural killer [NK] receptors) and the use of transplantable tumors which are unrepresentative of the clinical situation. Furthermore, reductionist models have mostly focused on possible strategies to prevent GVHD while sparing GVL; here, the findings reflect the higher threshold for inducing GVHD and often fail to give additional insight into mechanisms of antitumor immunity. The precise repertoire of immune effectors and the recognition mechanisms they employ vary according to tumor type, donor-recipient matching for the HLA system, donor source, and strategy to prevent GVHD. Ideally, precise targeting would involve targeting of molecules selectively expressed by leukemic cells that are also required for their survival (to prevent immune escape) and have no expression on normal tissues (to prevent GVHD or other on-target toxicities). The reality is that this is
difficult to achieve in current practice and the approach is usually agnostic to the target(s) recognized by immune cells. Although mismatching at one or more HLA loci or non-HLA antigen recognition systems have the potential to affect GVL, this review will focus on graft T cell reactivity to multiple mismatched minor histocompatibility antigens (miHAs) following HLA-identical transplantation. For more information on the former mechanisms, the reader is referred to the Appendix (online only) and to an accompanying paper in this series, “Optimizing Donor Choice and GVHD Prophylaxis in Allogeneic Hematopoietic Cell Transplantation.”

miHAs are a diverse collection of HLA-bound peptides that can act as alloantigens; they are a consequence of allelic polymorphism that translates into disparity in major histocompatibility complex-peptide epitopes between the donor and the recipient. Although the donor and the recipient are mismatched for several thousand non-synonymous coding region single nucleotide variants (SNVs), only a small fraction will be predicted to generate immunogenic miHAs capable of eliciting a T cell response: variations in intracellular processing of variant peptides, binding affinity of peptides to HLA, and tumor expression of HLA all influence immunogenicity. Following female into male allo-HSCT, immunity may be directed against multiple polymorphic genes encoded by the Y-chromosome (eg, UTY, SMCY) that are lacking in the female donor. Autosomal miHAs usually arise from SNVs in coding exons, leading to amino acid substitutions in proteins that are translated from primary gene transcripts in the normal or alternative reading frames (eg, HA-1, C19orf48). Other mechanisms for generation of autosomal miHAs include the creation of novel peptide sequences by SNVs that disrupt stop codons, or frameshift indels, or exon/intron SNVs that alter splicing. Preclinical data and individual patient responses have implicated miHA-directed responses in induction of GVL, and this mechanism is consistent with the reduced relapse of chronic myeloid leukemia (CML) observed where patients and donors are mismatched for the HLA-A*0201-restricted hemopoietic miHA, HA-1. Attempts to measure the impact of other hematopoietic-restricted miHA in larger patient cohorts are hindered by confounding interactions with GVHD, or biases introduced by inclusion of the small number of miHAs for which typing is available, but broadly support their role in GVL. The Falkenburg group has recently demonstrated that miHAs inducing GVL or GVHD overlap considerably although selective GVL was linked to lower magnitude responses toward a narrower repertoire of antigens. In the near future, high-throughput pipelines for identification of novel miHAs using whole exome sequencing and variant calling for donor-recipient coding SNVs, RNA sequencing of tumor cells versus normal tissues, and prediction of peptide binding to HLA in silico combined with the validation of HLA-peptide complex immunogenicity, will generate much larger numbers of candidate miHAs; these data will provide a more unbiased approach to evaluate the overall impact of multiple miHAs on GVL. Studies are urgently required to demonstrate that these in silico approaches can discover clinically relevant miHAs, that is, identifying antigens presented endogenously by tumor cells that are also capable of eliciting antitumor immunity. These pipelines will need to incorporate rigorous procedures to exclude miHA presentation by nonhematopoietic cells, for example, under inflammatory conditions. If proof of concept of these strategies can be shown and be scaled to the clinic, they offer the opportunity for designing novel therapeutics based on peptide vaccination or adoptive transfer of antigen-specific T cells. An important goal will be to identify public miHAs which have a high degree of polymorphism in the human population as these will have broader applicability.

Discovery pipelines based on deep sequencing of non-synonymous SNVs arising in solid tumors versus germline have also been used to identify candidate neoantigens, especially for cancers arising in the context of mutagen
exposure (eg, melanoma).25 The burden of somatic mutations is much lower in hematological cancers than mutagen-induced cancers, and for any particular donor-recipient pair, the number of candidate neoantigens is likely to be far less than the number of miHAs.26 Nevertheless, recent studies have suggested that T cell responses can be elicited to variant peptides derived from driver mutations (eg, CALR27 and JAK228 in myeloproliferative neoplasms [MPN]) and NPM129 in acute myeloid leukemia (AML); it will therefore be interesting to discover whether responses to these public neoantigens are also observed in patients following allo-HSCT. Alternatively, tumor-specific antigens (TSAs) could arise from frameshift mutations, gene fusions, or endogenous retroelements.30 Of particular interest is the possible generation of new antigens via splice variant mRNAs as a consequence of spliceosome defects that are common in hematological malignancies (eg, AML and MPN). For example, Schischlik et al31 have recently reported that MPN patients with mutations in the spliceosome gene SF3B1 show a high frequency of 3’ splicing variants detectable by RNA sequencing, many with the potential to generate neoantigens if translated into proteins. A key question is whether these splicing variants are unique to the neoplastic cells or also exist to some degree in normal tissues.30 Overexpressed tumor-associated antigens (TAAEs) (eg, cancer testis antigens, WT1, and proteinase-3; reviewed in ref. 32) could also potentially contribute to GVL although donor T cells with high affinity for these self-antigens will be reduced through mechanisms of thymic tolerance.33 Additional strategies including vaccination or gene transfer of antigen-specific T cell receptor (TCR) into nontolerant T cells may be required to target these antigens.

MECHANISMS OF RELAPSE

The aim of transplant is to impose a GVL response of sufficient breadth and depth to eliminate all remaining cancerous cells. Evidence in support of immune-mediated elimination is provided by patients converting from minimal residual disease (MRD) positive to permanent negative status following the withdrawal of immune suppression or after donor lymphocyte infusion (DLI), although this interpretation is limited by the sensitivity of molecular testing.34,35 At the other end of the scale, early progression or relapse may occur because GVL responses are non-existent or insufficient to arrest rapid growth kinetics of tumor cells; this might be a consequence of T cell depletion as GVHD prophylaxis36 or intrinsic defects in T or NK cell function/survival (eg, through exhaustion or apoptosis37,38). In between these two extremes, it is likely that donor immune cells exert a degree of immune pressure sufficient to suppress tumor growth but insufficient to eliminate all cells. This scenario is likely to lead to an unstable equilibrium, which is eventually superseded by immune escape from minority populations that have adapted to avoid immune surveillance (Fig 1).39 This process of immune editing can involve antigen loss or the emergence of immune suppressive microenvironments, as described below.

(i) Antigen loss: Evidence of this model of immune editing was first shown in AML by the copy neutral loss of heterozygosity, eliminating the mismatched HLA haplotype following haploidentical stem cell transplantation,40 although it is noteworthy that this mechanism has not been reported so far following cord transplantation where mismatched HLA alleles are frequently distributed across haplotypes. Reduced transcription of HLA class II genes and their regulators (eg, class II major histocompatibility complex regulator, CIITA) has also recently been reported following HLA-identical transplantation41,42; together genomic loss or altered transcription of HLA is evident in approximately 45% of patients (Fig 1A).42 Thus, the escape variants can evade continued surveillance by T cells that respond to mismatched HLA molecules or HLA class II–restricted peptides. Whether miHA or tumor-specific neoantigens are also edited by mutation or epigenetic change is not known but will be an important focus for future studies.

(ii) Immune suppression: In a mutually exclusive group of patients with relapsed AML (approximately 20% of patients), cancer cells upregulate inhibitory ligands, such as PD-L1 or B7-H3 with concomitant changes in the expression of inhibitory receptors that are associated with T cell dysfunction42,43 (Fig 1A). In the remaining patients who relapse, the mechanisms are not always clear although several potential mechanisms have been described (Fig 1B). For example, tumor adaptations may include a shift away from pro-inflammatory cytokine generation toward more immune suppressive cytokines (eg, transforming growth factor beta [TGF-β]44 or the active recruitment of regulatory populations (eg, myeloid-derived suppressor cells [MDSC])45 and Treg46 (Fig 1B). Tumor cells may also upregulate enzymes whose products directly inhibit T cell and antigen-presenting cell function including indoleamine 2,3-dioxygenase-1,47 arginase,48 or ectonucleotidases (CD73 and CD39).49,50 While emerging evidence supports these or other mechanisms underpinning relapse, there is an important need to apply more systematic, unbiased analyses for the delineation of the tumor immune landscape after transplant; for example, a recent immunogenomic approach across multiple cancer types identified several distinct immune types, common across multiple tumors (wound healing, interferon-γ dominant, inflammatory, lymphocyte depleted, immunologically quiet, and TGF-β dominant), which were closely related to prognosis.51

Although investigators are documenting an increasing array of mechanisms that permit immune evasion and relapse,
FIG 1. Mechanisms of leukemia relapse. (A) Genomic loss or altered transcription of HLA or T cell dysfunction is thought to account for the majority of AML relapses following allo-SCT, with other mechanisms thought to contribute to the remaining one-third of cases, as shown in (B). AML, acute myeloid leukemia; CIITA, class II major histocompatibility complex transactivator; IDO1, indoleamine 2,3-dioxygenase 1; iNOS, inducible nitric oxide synthase; MDSC, myeloid-derived suppressor cell; PD-L1, programmed cell death ligand 1; TAA, tumor-associated antigen; TSA, tumor-specific antigen. Diagrams created with BioRender.112

most of these have been described at the point of relapse. Currently, there are no immune biomarkers that reliably predict relapse and which can be used to design risk stratification or earlier intervention; this unmet need will require serial sampling of bone marrow or blood to track immune and cancer cell dynamics.

CURRENT APPROACHES TO PREVENT OR TREAT RELAPSE

Managing failure of GVL is challenging, and with a few exceptions, is associated with poor outcomes; following relapse, 3-year survival was < 20% in one large study.52 Outside the setting of clinical trials, post-transplant protocols have been adapted to bolster early GVL immunity through prophylactic or pre-emptive transfer of nontolerant donor T cells in the form DLI. Pre-emptive transfer of T cells is often performed according to the detection of mixed chimerism.53,54 Based on the rationale that this state is a surrogate for immune tolerance. While the use of pre-emptive DLI to correct mixed chimerism is widely used, definitive evidence for this approach or information regarding the best strategy to employ (timing, dose, and frequency of DLI) is lacking. Where an MRD marker is available, early withdrawal of immune suppression with or without subsequent DLI may re-establish molecular remission.54,55 DLI can reinduce remissions in patients with CML or other MPNs as well as in more indolent lymphomas but has much lower efficacy in tumors with rapid growth kinetics such as acute leukemias or high-grade lymphomas.56 The need to apply other measures (eg, chemotherapy or antibodies) in addition to DLI to control rapidly progressing tumors is likely to obscure true efficacy. Furthermore, while gradual dose escalation may mitigate against the risk, the frequency of severe GVHD is high following unrelated donor DLI and is associated with significant mortality.57 In selected patients with good performance status who relapse late after allo-HSCT and in whom disease control can be re-established, a second allogeneic transplant may induce durable remission.58 Although some centers routinely switch to an alternative donor, the data to support this strategy are lacking;59 however, where AML relapse after haploidentical transplant involves genomic loss of the mismatched HLA haplotype, it would be logical to use a donor who is mismatched for the remaining haplotype.60 Thus, prevention and management of relapse after transplant remains a continuing area of unmet need, and enrollment of at-risk or relapsed patients into prospective clinical trials, ultimately involving randomized comparison to controls is essential to advance the field.

FUTURE PERSPECTIVES

ClinicalTrials.gov (as of June 8, 2020) currently lists more than 100 phase I-III intervention trials focusing on the
<table>
<thead>
<tr>
<th>Name of Intervention</th>
<th>Start Date</th>
<th>Target Enrollment</th>
<th>Phase</th>
<th>Sponsoring Institution</th>
<th>NCT Reference</th>
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<tr>
<td>A trial of the FLT3 inhibitor gilteritinib administered as maintenance therapy</td>
<td>July 2017</td>
<td>346 (actual)</td>
<td>3 (RCT)</td>
<td>Astellas Pharma</td>
<td>NCT02997202</td>
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<td>following allogeneic transplant for patients with FLT3/ITD AML (MORPHO)</td>
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<td>Randomized study of oral azacitidine vs placebo maintenance in AML or MDS patients</td>
<td>June 2019</td>
<td>324</td>
<td>3 (RCT)</td>
<td>University of Birmingham</td>
<td>NCT04173533</td>
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<td>after allo-SCT (AMADEUS)</td>
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<td>Panobinostat maintenance after HSCT for high-risk AML and MDS (ETAL-4/HOVON-145)</td>
<td>July 2018</td>
<td>350</td>
<td>3 (RCT)</td>
<td>Goethe University</td>
<td>NCT04326764</td>
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<td>(panobinostat arm incorporates DLI vs control arm of DLI alone)</td>
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<td>A study evaluating safety and efficacy of venetoclax in combination with azacitidine</td>
<td>February 2020</td>
<td>424</td>
<td>3 (RCT)</td>
<td>AbbVie</td>
<td>NCT04161885</td>
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<td>and standard of care after allogeneic stem cell transplantation in participants with</td>
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<td>AML (VIALE-T)</td>
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<td>Vaccination/Biologics</td>
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<td>Dendritic cell/AML fusion cell vaccine following allogeneic transplantation in AML</td>
<td>October 2018</td>
<td>45</td>
<td>1</td>
<td>Dana-Farber</td>
<td>NCT03679650</td>
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<td>patients (incorporates 2 arms, one in which vaccine is administered alone and the</td>
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<td>other in which decitabine is also incorporated)</td>
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<td>Nivolumab and ipilimumab after donor stem cell transplant in treating patients with</td>
<td>October 2018</td>
<td>55</td>
<td>1b</td>
<td>MD Anderson</td>
<td>NCT03600155</td>
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<td>high-risk refractory or relapsed AML or MDS</td>
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<td>Augmentation of the graft vs leukemia effect via checkpoint blockade with pembrolizumab</td>
<td>December 2017</td>
<td>20</td>
<td>1b</td>
<td>University of Michigan</td>
<td>NCT03286114</td>
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<td>(patients with MDS, AML, and ALL relapsing post allo-HSCT)</td>
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<td>Inotuzumab ozogamicin post-transplant for ALL</td>
<td>March 2017</td>
<td>44</td>
<td>1/2</td>
<td>Case Comprehensive Cancer Center</td>
<td>NCT03104491</td>
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<td>T and NK cell transfer</td>
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<td>Prophylactic DLI for the prevention of relapse post HSCT in patients with high-risk</td>
<td>December 2016</td>
<td>124</td>
<td>2</td>
<td>King’s College Hospital</td>
<td>NCT02856464</td>
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<td>myeloid malignancy (PRO-DLI)</td>
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<td>TCR αβ–depleted progenitor cell graft with additional memory T-cell DLI, plus selected</td>
<td>January 2019</td>
<td>140</td>
<td>2</td>
<td>St. Jude</td>
<td>NCT03849651</td>
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<td>use of blinatumomab, in naive T cell–depleted haploidentical donor hematopoietic cell</td>
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<td>transplantation for hematologic malignancies</td>
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<tr>
<td>HA-1 TCR T cell immunotherapy for the treatment of patients with relapsed or refractory</td>
<td>February 2018</td>
<td>24</td>
<td>1</td>
<td>Fred Hutchinson</td>
<td>NCT03326921</td>
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<td>acute leukemia after donor stem cell transplant</td>
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(continued on following page)
TABLE 1. Examples of Current Clinical Trials of Interventions Following Allo-HSCT (continued)

<table>
<thead>
<tr>
<th>Name of Intervention</th>
<th>Start Date</th>
<th>Target Enrollment</th>
<th>Phase</th>
<th>Sponsoring Institution</th>
<th>NCT Reference</th>
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<tr>
<td>Antigen-specific T cell therapy for AML or MDS patients with relapsed disease after allo-HCT (donor-derived CTLs targeting WT1, PRAME, and Cyclin A1)</td>
<td>February 2020</td>
<td>22</td>
<td>1/2</td>
<td>NexImmune</td>
<td>NCT04284228</td>
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<td>Haploidentical bone marrow transplant with or without NK cell infusion in AML and MDS (Bigeminy)</td>
<td>January 2020</td>
<td>116</td>
<td>2 (RCT)</td>
<td>Fondazione Policlinico Univ. Agostino Gemelli</td>
<td>NCT04166929</td>
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<tr>
<td>Cytokine-induced memory-like NK cell adoptive therapy after haploidentical donor hematopoietic cell transplantation (for high-risk AML, with IL-15 superagonist)</td>
<td>January 2017</td>
<td>60</td>
<td>2</td>
<td>Washington University</td>
<td>NCT02782546</td>
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<tr>
<td>CD19.CAR/multivirus-specific CTLs for patients with CD19+ B-ALL or NHL undergoing related allogeneic HSCT (CARMA)</td>
<td>Not yet recruiting</td>
<td>34</td>
<td>1</td>
<td>Baylor</td>
<td>NCT03768310</td>
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</table>

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CAR, chimeric antigen receptor-transduced; CTL, cytotoxic T-lymphocyte; DLI, donor lymphocyte infusion; HSCT, hematopoietic stem cell transplant; MDS, myelodysplastic syndrome; NHL, non-hodgkin lymphoma; NK cell, natural killer cell; RCT, randomized controlled clinical trial; TCR, T cell receptor.

Prevention or treatment of relapse following allo-HSCT, indicating that addressing this issue represents a major priority within the field (selected studies are listed in Table 1, enrollment status per ClinicalTrials.gov on June 8, 2020). While some strategies focus on the enhancement of GVL, others aim to suppress early tumor progression before GVL is established (eg, some maintenance treatments) or are designed to promote antitumor immunity in ways that could also be exploited in an autologous setting (Fig 2). Below, we have highlighted novel approaches that may have an impact in the future, particularly those being evaluated in ongoing current phase III trials.

Small Molecular Agents

**Tyrosine kinase inhibitors.** Tyrosine kinase inhibitors (TKI) targeting breakpoint cluster region protein-ABL1 (BCR-ABL) in CML can re-establish molecular remissions after allo-HSCT even in the absence of DLI. In addition to targeting BCR-ABL, TKI may modulate GVL through direct inhibitory effects on MDSC or expansion of NK and T cells. While maintenance TKIs after allo-HSCT in CML and Ph+ acute lymphoblastic leukemia (ALL) are reported to reduce the risk of early relapse, this is not a consistent finding and can lead to depression of graft function. An alternative approach involves the pre-emptive use of TKI on the basis of MRD detection. In one randomized trial in ALL, there were no differences in overall survival depending on whether a pre-emptive or maintenance strategy was used. For poor-risk AML with the FLT3-ITD mutation relapsing after allo-HSCT, sorafenib induces durable remissions in 17% of patients. Sorafenib has direct effects on FLT3-ITD AML blasts inducing an interferon regulatory factor 7-dependent increase in IL-15 that in turn promotes the expansion of cytotoxic donor CD8+ T cells. Other early phase prospective trials have shown the feasibility of maintenance with sorafenib and other, more specific, TKI, often with encouraging results; these findings prompted randomized phase II/III trials of maintenance TKI in FLT3-ITD AML (Table 1). Most recently, the SORMAIN trial, a randomized, double-blind phase II trial comparing sorafenib maintenance with placebo after allo-HSCT for FLT3-ITD AML in 83 patients demonstrated a significant improvement in relapse-free survival with sorafenib (85% v 53% at 2 years). A phase III study comparing maintenance gilteritinib and placebo (MORPHO, ClinicalTrials.gov identifier: NCT02997202) has recently completed recruitment, and the results are eagerly anticipated.

**Hypomethylating agents.** Azacitidine given post-transplant enhances the expression of epigenetically silenced TAA and increases antigen-specific CD8+ T cell reactivity. It is postulated that the potential risk of GVHD is mitigated by the capacity of azacitidine to enhance the expansion of Treg. While several reports have shown that a significant minority of patients with AML or myelodysplastic syndrome (MDS) relapsing after allo-HSCT will respond to hypomethylating agents, (either alone or in combination with DLI or with immune-modifying drugs eg, lenalidomide), there is a need for prospective randomized trials to determine efficacy. The orally available hypomethylating drug CC-486 is well tolerated after allo-HSCT and is currently being evaluated in a phase III trial as a post-transplant maintenance therapy in patients with AML or MDS (Table 1, AMADEUS trial).

**Biologics**

**Vaccination.** A variety of approaches including peptide- or dendritic cell (DC)-based vaccines have long been posited as maneuvers to enhance immunity against hematological
malignancies and mostly tested in the autologous setting.\textsuperscript{32} Vaccine targets have mostly involved leukemia-associated antigens (eg, WT-1 and PR-1)\textsuperscript{30,31} or lineage-restricted miHA\textsuperscript{32} although tumor-based vaccines (eg, AML-DC fusions and GVAX\textsuperscript{33,34}) have also been assessed. Although these approaches can elicit T cell responses detectable ex vivo, clinical responses have so far been modest.\textsuperscript{35} New analytical pipelines designed to discover novel leukemia-expressed antigens and possible combinations with other therapies, for example, gene-modified T cells, merit further investigation.

**Checkpoint inhibitor drugs.** Based on their encouraging efficacy in nontransplant settings, several groups have explored whether checkpoint inhibitor (CPI) drugs can re-establish GVL after allo-HSCT.\textsuperscript{36} At higher doses, seven of the 22 patients relapsing after allo-HSCT showed objective responses to ipilimumab.\textsuperscript{37} GVHD occurred in four patients (all resolving with corticosteroids), and six patients developed immune-related adverse events (irAE), one of which was fatal.\textsuperscript{38} The therapeutic index for anti-PD mono-clonal antibodies (mAbs) is narrower than that for ipilimumab. In a recent phase I trial of nivolumab for relapsed disease, the

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**FIG 2.** Strategies for boosting GVL following allo-HSCT. TKI such as imatinib targeting the BCR-ABL fusion protein in ALL and CML, sorafenib or gilteritinib targeting FLT3-ITD in AML, and ibritinib targeting BTK in CLL have been investigated in both the maintenance and relapse settings. Hypomethylating agents (eg, azacitidine) act through inhibition of DNA methyltransferases in AML and MDS, which can be associated with hypermethylated DNA and silencing of tumor suppressor genes. A variety of vaccination approaches have been explored including DCs pulsed with tumor lysate, DC/AML hybrids, and DCs modified to reduce the expression of inhibitory ligands. Use of checkpoint inhibitors post allo-HSCT can be associated with exacerbation of GVHD as well as other immune-related adverse events; more targeted treatments may be necessary. BITEs such as blinatumomab and antibody-drug conjugates such as inotuzumab ozogamicin are employed frequently in relapsed disease and are now being investigated specifically in the post-transplant setting. Cellular therapies include T and NK cells engineered to express CARs or TAA/TSA-specific TCR or allogeneic NK cells. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BCR-ABL, breakpoint cluster region protein-ABL1; BiTE, bispecific T cell engager; BTK, Bruton’s tyrosine kinase; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DC, dendritic cell; FLT3-ITD, FMS-like tyrosine kinase-3 internal tandem duplication; GVHD, graft-versus-host disease; GVL, graft versus leukemia; HSCT, hematopoietic stem cell transplant; mAb, monoclonal antibody; MDS, myelodysplastic syndrome; NK cell, natural killer cell; PD-1/PD-L1, programmed cell death ligand 1; TKI, tyrosine kinase inhibitor; TAA, tumor-associated antigen; TCR, T cell receptor; Tm, memory T cell; TSA, tumor-specific antigen. Diagrams created with BioRender.\textsuperscript{112}
low dose initially given (1 mg/kg) had to be de-escalated further because of toxicity, yet the lower doses (0.5 mg/kg) were still complicated by irAE and worsening GVHD.  

Although the overall response rate was 32%, only one of the 25 patients attained a complete response. Furthermore, a phase I trial to evaluate low-dose maintenance nivolumab in patients with high-risk disease was halted after enrollment of only four patients because of an unexpectedly high rate of irAE. Together, the available data indicate that more targeted CPI treatments will be necessary to rescue GVL; these could include CPI that target primarily NK cells (eg, anti-NKG2A) or the use of CPI in combination with anti-inflammatory drugs (eg, anti-IL-6 receptor mAb; ClinicalTrials.gov identifier: NCT03588936).

**mAbs and bispecific T cell engagers.** Anti-CD20 and brentuximab vedotin mAbs are employed frequently for relapsed B-cell or Hodgkin’s lymphoma after allo-HSCT, often in combination with DLI. Although it is possible that antibody-dependent cellular cytotoxicity will increase the cross-presentation of lymphoma antigens, the available data do not show a clear interaction with GVL. More direct induction of donor immunity could be triggered by the use of bispecific T cell engagers (BiTEs), and several trials are currently evaluating the prophylactic use in the post-transplant setting (Table 1). Because these reagents usually have a short half-life, a key question will be whether discontinuation of treatment will prevent the progression of GVHD should it occur.

**IL-15 superagonists.** A recent phase I trial demonstrated the safety of ALT-803, a complex of recombinant IL-15-IgG1 Fc that increases IL-15 bioavailability by mimicking its trans-presentation on the surface of IL-15Ra–expressing DCs. The agonist complex had a longer half-life than the native cytokine, was well tolerated when given subcutaneously without an increased risk of GVHD, and induced rapid expansion of NK and memory T cell populations. Although clinical responses were only observed in a minority of patients, future trials will evaluate ALT-803 in combination with other approaches including BiTEs and T or NK cells.

**T and NK Therapies**

**T cell subsets.** To improve the therapeutic index of DLI, infusions enriched for CD4+ T cells are designed to direct the GVL response to the hematopoietic compartment because HLA class II–restricted antigen presentation by peripheral tissues is relatively low in the steady state. CD4+-enriched T cell infusions can induce antitumor responses and reverse dysfunction of bone marrow T cells without excessive GVHD; randomized trials of prophylactic CD4+ T cells (using high stringency immunomagnetic selection) versus no DLI have recently completed recruitment (ClinicalTrials.gov identifier: NCT01240525; ISRCTN51398568). An alternative approach, based on the reduced alloreactive potential of memory T cells, is the use of naïve T cell–depleted DLI, and preliminary phase I data have confirmed safety and some treatment responses in patients with active disease. More complex approaches including the selection and expansion of T cell lines with specificity against TAA or TSA have also been reported; these approaches may become more widely adopted with the identification of new mHA or TAA. Expansion and infusion of bone marrow-infiltrating T cells, which are potentially enriched for tumor-reactive T cells, is also being tested.

**NK cells.** NK infusions can induce transient clinical responses and are generally well tolerated, although preactivation of cells with IL-15 was associated with GVHD in one clinical trial. There is now a major focus on developing methods to enhance the survival of infused NK cells, for example, through selective expansion of NKG2C+ memory-like NK cells. The potential of NK infusions to augment GVL has recently been comprehensively reviewed elsewhere and will not be reproduced here.

**TCR-transduced T cells.** Transfers of large numbers of TCR-transduced T cells targeting leukemia-expressed intracellular antigens are likely to become increasingly relevant. In one recent phase I trial, T cells transduced with a high-avidity TCR reactive with an HLA-A*2-01–restricted WT-1 peptide were infused prophylactically into 12 patients with high-risk AML undergoing allo-HSCT; no excess GVHD was observed, and no patient relapsed despite the prolonged follow-up. Future iterations of this approach are likely to include editing strategies to knockdown the endogenous TCR (to prevent TCR chain mispairing and the risk of GVHD) or the use of banks of allogenic cells with some degree of HLA matching with the patient.

**CAR-T and CAR-NK.** Infusion of donor-derived CD19 CAR-T cells following allo-HSCT has been applied for a variety of lymphoid malignancies; in contrast to the autologous setting, lymphodepletion is omitted. Remission is attained at rates similar to those for the autologous setting, and in most studies, the rates of GVHD are low (0%-10%) despite the use of nontolerant T cells from the original donor. Preclinical models have suggested that CAR-T cells with the potential for alloreactivity undergo rapid deletion via activation-induced cell death, although this may depend on the CAR intracellular domains used. As for TCR-transduced T cells, the CAR field is focused on the development of universal off-the-self reagents; these could include non–HLA-matched CAR-NK cells derived from cord blood. The new era of synthetic biology offers huge opportunities for further revision and development of CAR-T–based therapies, including synthetic Notch receptors, combinatorial target antigen recognition, and logic gate technologies.

The plethora of early phase clinical trials heralds an exciting new era for GVL research. In the future, the concept of GVL may become uncoupled from allo-HSCT and instead be
used to refer to the use of any donor or third-party cell designed to target leukemia selectively; these therapies will need to provide the breadth and depth of classical GVHD while avoiding on- or off-target toxicities. The challenge will be how such therapies can progress from first-in-human trials to prospective, controlled trials that evaluate efficacy and lead to regulatory approval. The complexity and cost of newer therapies, coupled with a high attrition rate when tested at the phase I/II level, mean that most experimental therapies are unlikely to be incorporated into large randomized prospective trials; this could act as a powerful disincentive to the biopharmaceutical industry investment. There is therefore an important need to create collaborative groups including industry that seek to evaluate multiple therapies and to use adaptive trial designs to select winning strategies.

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REFERENCES
2. Blazar BR, Hill GR, Murphy WJ: Dissecting the biology of allogeneic HSCT to enhance the GvT effect whilst minimizing GvHD. Nat Rev Clin Oncol 17:475-492, 2020

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85. Soffer RJ, Davids MS, Chen YB: Tyrosine kinase inhibitors and immune checkpoint blockade in allogeneic hematopoietic cell transplantation. Blood 131: 1073-1080, 2018
89. Andrè P, Denis C, Sourlas C, et al: Anti-NKG2A mAb is a checkpoint inhibitor that promotes anti-tumor immunity by unleashing both T and NK cells. Cell 175: 1731-1743, 2018
95. van Balen P, van Bergen CAM, van Luxemburg-Heijs SAP, et al: CD4 donor lymphocyte infusion can cause conversion of chimerism without GVHD by inducing immune responses targeting minor histocompatibility antigens in HLA class II. Front Immunol 9:3016, 2018
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Graft Versus Leukemia: Current Status and Future Perspectives

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Mismatches of HLA loci between donor and recipient have been shown to influence the risk of GVHD and GVL responses. Several studies have reported that HLA-DPB1 mismatches are associated with an increased risk of relapse after allogeneic hematopoietic stem cell transplantation (allo-HSCT) (reviewed in Cooley EW, et al: Blood 2015;110:4560-4566). Furthermore, HLA-DQB1 mismatches have been linked to a lower risk of relapse (et al: Cancer Immunol Res 3:425-435, 2015) or γδT cell activation by a diverse array of ligands (eg, stress proteins, small peptides, phospholipids, and prenyl pyrophosphates) that are bound to or expressed on the target cell surface (Hayday AC: J Immunol 203:311-320, 2019). While it is possible that these elements also trigger antitumor responses that synergize with classical T- or NK-mediated GVL, their current role in GVHD biology is not fully understood.

**Appendix**

**Mismatching at one or more HLA loci provokes direct T cell alloreactivity with the potential to affect both GVHD and GVL.**

**OTHER POTENTIAL MECHANISMS OF GVL**

Mismatches of HLA loci between donor and recipient have been shown to influence the risk of GVHD and GVL responses. Several studies have reported that HLA-DPB1 mismatches are associated with an increased risk of relapse after allogeneic hematopoietic stem cell transplantation (allo-HSCT) (reviewed in Cooley EW, et al: Blood 2015;110:4560-4566). Furthermore, HLA-DQB1 mismatches have been linked to a lower risk of relapse (et al: Cancer Immunol Res 3:425-435, 2015) or γδT cell activation by a diverse array of ligands (eg, stress proteins, small peptides, phospholipids, and prenyl pyrophosphates) that are bound to or expressed on the target cell surface (Hayday AC: J Immunol 203:311-320, 2019). While it is possible that these elements also trigger antitumor responses that synergize with classical T- or NK-mediated GVL, their current role in GVHD biology is not fully understood.

**Prospective validation in clinical trials before it can be applied generally.** Mismatches for class I HLA may also provoke NK reactivity, and this subject has recently been reviewed in refs. 2 and 101. The most striking evidence in support of NK-mediated GVL occurs following haploidentical allo-HSCT for AML using stringent T cell depletion, where grafts containing alloreactive NK cells with inhibitory receptors to HLA class I ligands present in the donor but not in the recipient are associated with a lower risk of relapse (Ruggeri L, et al: Science 295:2097-2100, 2002). Following HLA-matched or partially matched transplantation, selection of donors with higher numbers of activating NK receptors (eg, with 1 or 2 KIR B haplotypes) may also be associated with lower risks of relapse (Cooley S, et al: Blood 113:726-732, 2009; Venstrom JM, et al: Blood 115:3162-3165, 2010; Venstrom JM, et al: N Engl J Med 367:805-816, 2012). However, these observations in support of NK-mediated GVL are not consistently observed for non-AML blood cancers or for all transplant settings (eg, using alternative methods of GVHD prophylaxis).

Outside the setting of transplant, tumor immunosurveillance may be mediated by other recognition systems that are independent of HLA, for example, those mediated by NKT recognition of CD1d-restricted glycolipids (McEwen-Smith et al: Cancer Immunol Res 3:425-435, 2015) or γδT cell activation by a diverse array of ligands (eg, stress proteins, small peptides, phospholipids, and prenyl pyrophosphates) that are bound to or expressed on the target cell surface (Hayday AC: J Immunol 203:311-320, 2019). While it is possible that these elements also trigger antitumor responses that synergize with classical T- or NK-mediated GVL, they are currently very difficult to measure directly (Godder KT, et al: Bone Marrow Transplant 39:751-757, 2007; Malard F, et al: Blood 127:1828-1835, 2016); determination of the capacity of each population to induce GVL will depend on isolation and transfer of individual populations.