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Review

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REVIEW

T cell optimization for graft-versus-leukemia responses

Melinda A. Biernacki,[1,2] Vipul S. Sheth,[1] and Marie Bleakley[1,3]

[1]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA. [2]Department of Medicine, and [3]Department of Pediatrics, University of Washington, Seattle, Washington, USA.

Protection from relapse after allogeneic hematopoietic cell transplantation (HCT) is partly due to donor T cell–mediated graft-versus-leukemia (GVL) immune responses. Relapse remains common in HCT recipients, but strategies to augment GVL could significantly improve outcomes after HCT. Donor T cells with αβ T cell receptors (TCRs) mediate GVL through recognition of minor histocompatibility antigens and alloantigens in HLA-matched and -mismatched HCT, respectively. αβ T cells specific for other leukemia-associated antigens, including nonpolymorphic antigens and neoantigens, may also deliver an antileukemic effect. γδ T cells may contribute to GVL, although their biology and specificity are less well understood. Vaccination or adoptive transfer of donor-derived T cells with natural or transgenic receptors are strategies with potential to selectively enhance αβ and γδ T cell GVL effects.

Introduction

HCT

Allogeneic hematopoietic stem cell transplantation (HCT) is standard consolidation therapy for high-risk leukemia in children and adults (1, 2) and is associated with a lower relapse risk than chemotherapy alone (3, 4). HCT is indicated for a given patient when the risk of death due to relapse or nonrelapse mortality (NRM) with chemotherapy alone exceeds the probability of death with HCT. This decision is informed by known risk factors for leukemic relapse, including cytogenetic and/or molecular characteristics of the leukemia and its chemotherapy response, as reflected by measurable residual disease (MRD) at the end of induction and consolidation (1, 2, 5). The decision to perform HCT also considers NRM risk, which depends on age and patient comorbidities. NRM rates are higher following HCT than after chemotherapy alone, although the magnitude of this difference has declined over time. In a large cohort of patients transplanted in the current era for hematological neoplasms (n = 47,591), including acute leukemia (57.8%), the probability of 3-year disease-free survival (DFS) following HCT was 50.5%, with a 3-year incidence of relapse and NRM of 34.1% and 23.5%, respectively (6).

GVL. Two main elements of HCT account for protection from relapse: the pre-HCT preparative regimen (conditioning, involving chemotherapy and/or radiotherapy) and the presence of donor T cells in the hematopoietic cell graft. Conditioning primarily mediates relapse protection early after HCT (0–12 months), while the effect of donor T cells, the graft-versus-leukemia (GVL) effect, occurs later (≥12 months) (7, 8) (Figure 1). Conditioning intensity varies, and the GVL effect is particularly critical in minimally intensive nonmyeloablative and reduced-intensity HCT, whereas conditioning and the GVL effect both contribute to relapse protection in intensive myeloablative HCT. The importance of donor T cells in mediating GVL was originally inferred from clinical data demonstrating increased relapse risk with extensive ex vivo T cell depletion from donor grafts before infusion into patients (9, 10). Clinical studies also demonstrated a lower risk of relapse in recipients of allogeneic, as compared with syngeneic, HCT grafts, indicating that polymorphic antigens are major molecular targets of donor T cell–mediated GVL (9, 11, 12).

T cells as mediators of GVL

Donor T cells respond to non-donor self-antigens on recipient cells encoded by recipient genomic polymorphisms, including (a) complexes of allelic variants of human leukocyte antigen/major histocompatibility antigen (HLA/MHC) molecules presenting self- or other peptides in HLA-mismatched HCT (13);

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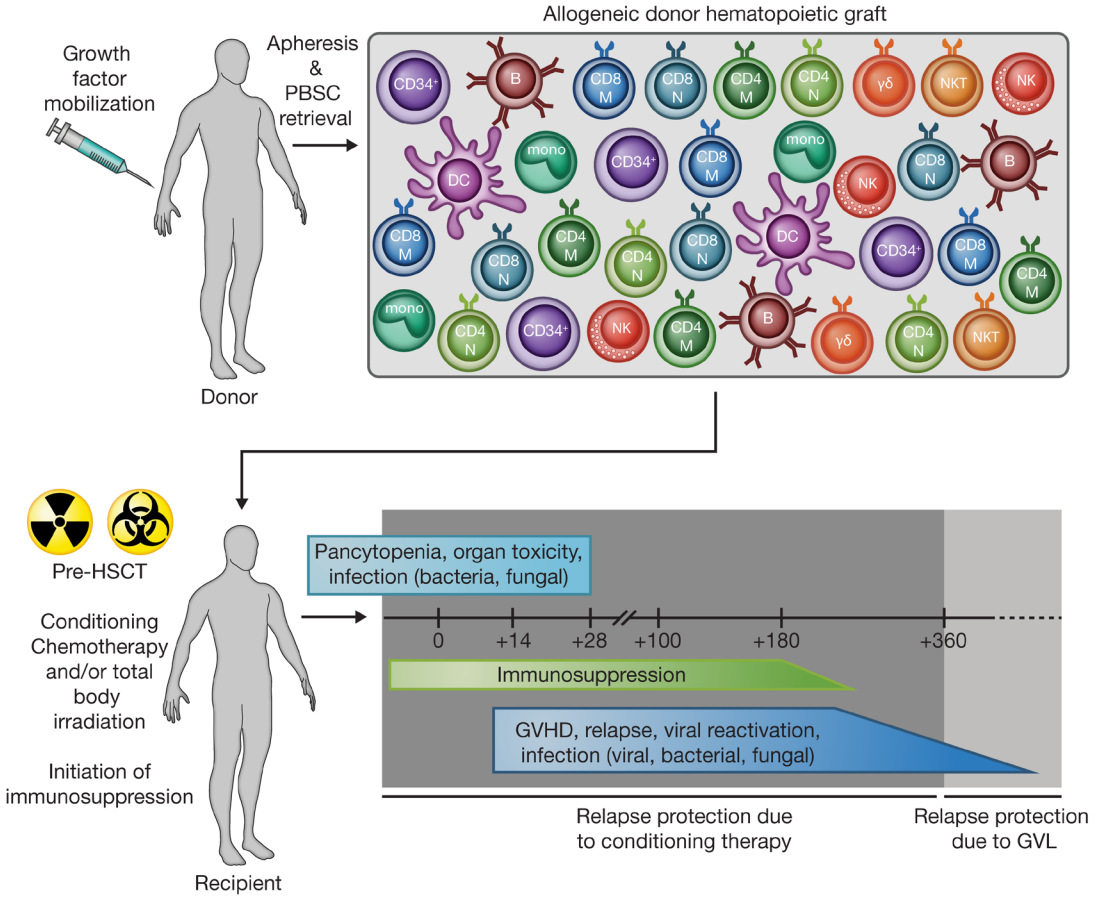

Figure 1. Overview of allogeneic hematopoietic cell transplantation, including cellular components of an unmanipulated T cell–replete peripheral blood stem cell (PBSC) graft. Key cellular components of the hematopoietic graft are indicated by pictograms, including αβ T cells ($CD4^+CD3^+$, green; $CD8^+CD3^+$, blue; Tn are indicated in lighter colors and Tm darker) and γδ T cells (gray with TCR). The green bar indicates the approximate time frame in which patients receive immunosuppressive medications for prevention and/or treatment of GVHD. Blue bars indicate usual periods of risk for post-HCT complications: light blue indicates early post-HCT risks primarily related to conditioning, darker blue indicates later post-HCT risks related primarily to immunosuppression and GVHD. Gray shading indicates the primary origin of relapse protection at different times after HCT: in the first 12 months due to conditioning therapy (dark gray), and after 12 months due to donor-derived GVL responses (lighter gray). Illustrated by Rachel Davidowitz.

(b) peptide epitopes derived from mismatched, allogeneic HLA molecules that are presented by shared HLA molecules (14); and (c) minor histocompatibility (H) antigens. Minor H antigens are HLA-presented polymorphic peptides derived from normal self-proteins that differ in amino acid sequence between donor and recipient due to genetic polymorphisms outside of the HLA loci on chromosome 6 (12). The dominant role of alloantigen- and minor H antigen–specific T cells in GVL does not negate the possibility that donor T cells specific for nonpolymorphic leukemia–associated antigens (LAAs) or neoantigens also contribute to relapse protection after HCT. Alloantigen- and minor H antigen–specific T cells are also involved in the pathogenesis of graft-versus-host disease (GVHD) when their cognate antigens are presented on healthy nonhematopoietic tissues.

Relapse after HCT

Although HCT reduces the risk, relapse remains the major cause of death after HCT for leukemia (6). Reported post-HCT relapse rates are variable: 10%–30% for patients transplanted with leukemia in MRD-negative remission, 20%–70% for those in remission but with MRD, and 50%–90% for those in relapse (15, 16). Long-term survival after post-HCT relapse is infrequent. Reported 2-year overall survival (OS) in patients relapsing at less than 3 months, 3–6 months, and greater than 6 months is 3%, 9%, and 19%, respectively, while average survival after post-HCT relapse is 4 months (17–20). More options now exist to treat post-HCT relapse, including CD19 chimeric antigen receptor (CAR) T cells for patients with B lineage acute lymphoblastic leukemia (ALL) and hypomethylating agents for patients with acute myeloid

leukemia (AML), among other approaches. However, most patients with post-HCT relapse ultimately succumb to their disease or treatment complications (21), highlighting an urgent need to develop new HCT strategies that include administration of T cells optimized to deliver potent GVL effects.

Graft engineering

The optimal composition of donor cells infused during HCT for patients with leukemia has not been determined, but certain functions are prerequisite. At a minimum, the donor product must include hematopoietic stem cells (HSCs) capable of regenerating hematopoiesis in a recipient who has received myeloablative or nonmyeloablative conditioning. The need for T cells to facilitate HSC engraftment and achieve stable donor hematopoietic chimerism depends on the number of HSCs, the HLA match between recipient and donor, the presence of recipient anti–donor HLA antibodies, and the intensity of pre-HCT conditioning myeloablation and lymphoablation. During the early post-HCT period, before new HSC-derived lymphocytes are generated, donor lymphocytes transferred with the graft provide important protection against opportunistic pathogens. For patients with leukemia, administration of lymphocytes with antileukemic activity is also highly desirable. Donor lymphocytes with reported antileukemic activity include natural killer (NK) cells, invariant NK T (iNKT) cells, and T cells with either an αβ or γδ T cell receptor (TCR) (22–24). Some subsets of αβ T cells mediate detrimental GVHD as well as GVL (25). Conversely, NK, iNKT, and γδ T cells do not appear to be major mediators of GVHD, and iNKT may be protective against the condition (23). It is likely that a combination of NK, iNKT, γδ T cells, and selected αβ T cells will prove to provide safe and effective protection against relapse. The antileukemic activity of NK and iNKT cells has been described previously (22, 23). This review will focus on optimizing the donor graft for αβ or γδ T cells with antileukemic activity. αβ and γδ T cells are naturally present in unmanipulated T cell–replete HCT (Figure 1) but may be partially or completely depleted in various graft engineering strategies that aim to minimize GVHD, such as CD34 selection to produce a pan-T cell–depleted (pan-TCD) graft (Figure 2A), selective CD45RA depletion to remove naive T (Tn) cells (Figure 2B), or selective αβ TCR depletion to remove αβ T cells (αβ-TCD) (Figure 2C). Therefore, in engineered grafts an important goal is to preserve and enrich for selected αβ and γδ T cell subsets that can mediate GVL with minimal or no GVHD activity.

αβ T cells

T cells with αβ TCRs represent the majority of mature postthymic human $CD3^+$ T cells, recognize peptide antigens presented in association with HLA class I or II molecules, and serve a number of functions, including production of cytokines and cytolytic granules in response to virus-infected and malignant cells. αβ T cells can be divided into $CD8^+CD3^+$ and $CD4^+CD3^+$ subsets, each of which is composed of phenotypically, functionally, epigenetically, and metabolically diverse T cell pools (Table 1), including antigen-inexperienced Tn cells, antigen-experienced memory T cells (Tm) (26), and others such as Tregs, T follicular helper cells, and tissue-resident memory T cells (27–29). Because minor H antigens are the molecular target of GVHD-initiating donor T cells in MHC-matched HCT, Tn cells should have greater potential to cause GVHD than Tm, a hypothesis supported by studies performed in murine GVHD models by the Shlomchik group and others (25, 30–36). In vitro studies of human cells confirmed a higher frequency of minor H antigen–specific T cells among Tn than Tm (37). Moreover, single-arm clinical trials of Tn-depleted peripheral blood stem cell HLA-matched HCT demonstrated substantially lower rates of chronic GVHD than concurrent controls (9% versus 50%) and a trend toward lower rates of severe acute GVHD (38).

Targets of αβ T cells with GVL

Minor H antigens and GVL

Minor H antigen–specific T cells within the Tn population have potent GVL activity. Most minor H antigens are ubiquitously expressed, including on epithelial tissues, and thereby also trigger GVHD. However, some minor H antigens are expressed predominantly or exclusively on hematopoietic cells. As the hematopoietic system in an HCT recipient is primarily of donor origin and donor hematopoietic cells do not present recipient minor H antigens, donor T cells specific for hematopoietically restricted recipient minor H antigens can mediate GVL without GVHD or damage to the normal donor–derived hematopoietic system after HCT (39). Conventional unmanipulated HCT involves the transfer of donor T cells that target both hematopoietically restricted minor H antigens (selective GVL) and ubiquitously expressed minor H anti-

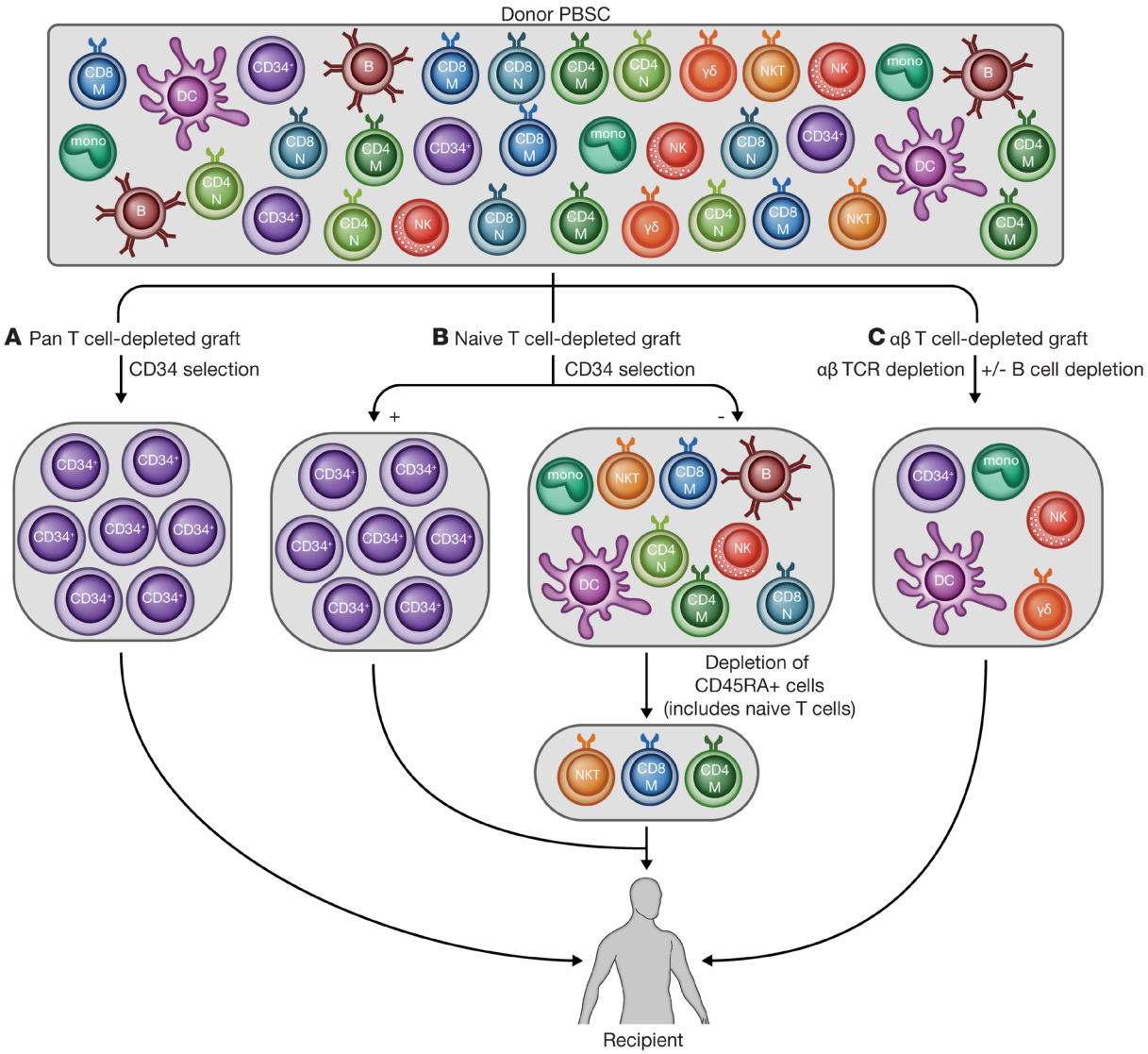

Figure 2. Illustration of 3 PBSC graft engineering strategies to reduce GVHD. (**A**) In pan-T cell–depleted (pan-TCD) grafts, only CD34$^+$ HSCs (purple) that have been positively selected from donor PBSCs are infused into the recipient. (**B**) In naive T cell–depleted grafts, CD34$^+$ HSCs are first isolated from PBSCs by positive selection as in **A**. The CD34$^-$ fraction is then depleted of CD45RA$^+$ cells, which removes CD45RA$^+$ naive T cells. The CD34$^+$ HSC and CD45RA$^-$ fractions (CD4$^+$CD3$^+$ Tm, dark green; CD8$^+$CD3$^+$ Tm, dark blue; iNKT, yellow) are then infused into the recipient. (**C**) In αβ T cell–depleted (αβ-TCD) grafts, donor PBSCs are depleted of αβ TCR$^+$ cells and often CD19$^+$ cells, which removes αβ T cells and iNKT cells, and B cells, respectively. The αβ TCR$^-$CD19$^-$ fraction, including NK (red with granules) and γδ (orange with TCR) T cells, is infused into the recipient. Illustrated by Rachel Davidowitz.

gens (GVL and GVHD). In Tn-depleted HCT, the transfer of donor minor H antigen–targeting T cells is substantially reduced. However, a recent study in murine HCT models demonstrated that newly generated CD8$^+$ T cells specific for hematopoietically restricted minor H antigens can evade full thymic deletion, and deletion-escapee T cells can mediate GVL in recipients of hematopoietically restricted minor H antigen–mismatched HCT (40). This finding may explain why relapse does not appear to be increased and GVL can be separated from serious GVHD in Tn-depleted HCT.

We and others are investigating whether administration of additional hematopoietically restricted minor H antigen–specific T cells with or after HCT can further augment GVL (41–43). We developed T cell immunotherapy employing donor Tm transduced with a lentiviral vector encoding a TCR specific for the hematopoietically restricted minor H antigen HA-1 (41) (Figure 3A), and are currently evaluating this approach in a phase I clinical trial for the treatment of post-HCT MRD or relapse (NCT03326921).

Table 1. Key T cell subsets in GVL and GVHD and their characteristics

Subset	Phenotype
Naive T cells (Tn)	$CD45RA^+CD45RO^-CD62L^+CD95^-$
T memory stem cells (Tscm)	$CD45RA^+CD45RO^-CD62L^+CD95^+$
Central memory T cells (Tcm)	$CD45RO^+CD62L^+$
Effector memory T cells (Tem)	$CD45RO^+CD62L^-$
$CD45RA^+CD62L^-$ effector T cells (Temra)	$CD45RA^+CD62L^-$
T regulatory cells (Treg)	$CD4^+CD25^+CD127^{lo}FOXP3^+$
T follicular helper cells (Tfh)	$CD4^+CXCR5^+$
$CD8^+$ tissue-resident memory T cells ($CD8^+$ Trm)	$CD8^+CD69^+CD103^+$
$CD4^+$ tissue-resident memory T cells ($CD4^+$ Trm)	$CD4^+CD69^+CD103^{+/-}$

Other approaches to augmenting HCT grafts for antileukemic activity include isolation of T cells targeting HA-1 and other LAAs using Streptamer technology to infuse very small numbers of unmanipulated antigen-specific T cells (44, 45), or infusion of minor H antigen–specific T cell lines or clones (NCT03091933) (46, 47) (Figure 3B). Previously, Warren and colleagues infused T cell clones specific for minor H antigens into recipients of HLA-matched sibling donor HCT who developed post-HCT relapse (46) and observed complete remissions (CRs) in 5 of 7 patients. However, the CRs were transient, probably due to limited persistence of minor H antigen–specific T cell clones that were cultured in vitro for many weeks. Greater success is expected for strategies that target well-characterized hematopoietically restricted minor H antigens and use contemporary methods to rapidly produce genetically modified T cells targeting the antigen of interest (41). While current clinical trials targeting minor H antigens are evaluating minor H antigen–specific T cells as treatment for post-HCT leukemia recurrence to establish the T cell product safety profile, a longer-term goal is to deliver hematopoietically restricted minor H antigen–specific T cells with or soon after the HCT graft (Figure 3A) to augment GVL and prevent relapse.

An alternative approach to amplify the number of minor H antigen–specific T cells delivered to the patient would be to vaccinate the HCT donor against minor H antigens to generate a Tm response, and then infuse Tm, including minor H antigen–specific Tm, at the time of or after HCT (Figure 3C). Using a murine model, the Shlomchik group demonstrated that vaccination of donors with recipient minor H antigens and subsequent infusion of donor Tm transferred leukemia- and pathogen-specific immunity to murine bone marrow transplantation (BMT) recipients (48). The transferred Tm expanded markedly after BMT and augmented GVL. The effect required expression of the antigen on the leukemic cells but induced little GVHD, even when antigen was ubiquitously expressed in the recipient. However, GVL was diminished when the targeted minor H antigen was ubiquitous. Ultimately, donor vaccination and transfer of donor Tm, including hematopoietically restricted minor H antigen–specific Tm, may be a relatively simple and inexpensive alternative to adding back genetically modified or selected and cultured minor H antigen–specific T cells. One caveat is that donors must be vaccinated months before the intended infusion to allow the generation of Tcm. Clinical trials of vaccination of HCT recipients against minor H antigens have been completed without excess toxicity, providing reassurance of probable safety in HCT donors, although the observed efficacy of vaccinating HCT recipients was limited (49, 50). Small studies have shown that vaccinating HCT donors against neoantigens and other tumor antigens before HCT for patients with multiple myeloma is safe and potentially efficacious, setting a precedent for this type of strategy (51).

One challenge common to strategies targeting minor H antigens to augment GVL is the need to identify adequate numbers of hematopoietically restricted minor H antigens presented by diverse HLA types. Over 100 HLA class I– or II–restricted human minor H antigens have been identified and at least partially characterized. Of these, less than 10 appear to be highly hematopoietically restricted. However, many more suitable minor H antigens are likely yet to be discovered, given the large number of total nonsynonymous SNPs with a variant allele frequency between 0.1 and 0.9 across the human genome, a significant minority of which are encoded by genes with predominantly hematopoietically restricted expression. For example, Lansford et al. performed an in silico analysis to predict minor H antigens in a cohort of 101 HLA-matched HCT recipient donor pairs and identified 102 peptides with desirable properties for public, leukemia-associated minor H antigens, specifically (a) predicted high binding affinity to a common HLA molecule; (b) RNA expression in

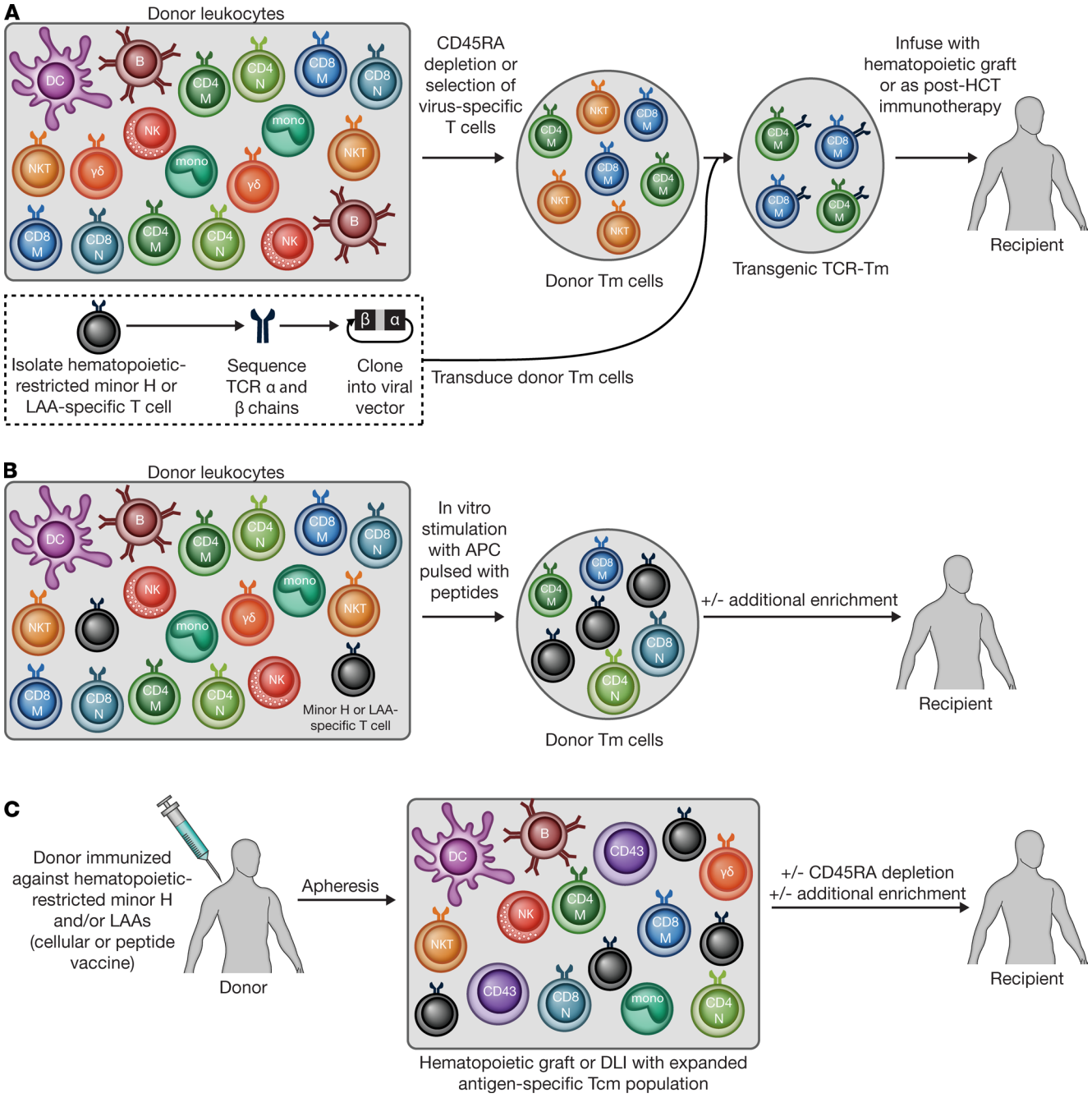

Figure 3. Strategies to augment donor LAA– and/or minor H antigen–specific T cell immunity. (**A**) Generation of transgenic antigen-specific T cells. Donor leukocytes are collected, enriched for Tm by CD45RA depletion or selection of virus-specific cells, transduced to express a transgenic TCR specific for a defined minor H antigen or LAA, purified, expanded, and infused into the recipient at the time of HCT or subsequently. The box depicts a schematic of transgenic TCR development (left to right): a T cell clone with a well-characterized high-affinity hematopoietically restricted minor H antigen– or LAA-specific TCR is identified and the α and β chains of the TCR are sequenced, and then cloned into a viral vector for transfer. (**B**) Primary in vitro stimulation of antigen-specific T cells. Donor leukocytes are stimulated with antigen-presenting cells (APCs) pulsed with peptides for one or multiple LAAs and/or minor H antigens to produce a T cell product with an expanded population of LAA- and/or minor H antigen–specific effector T cells for infusion into the recipient with or without additional enrichment. (**C**) In vivo expansion of antigen-specific T cells using vaccination. Donors are vaccinated against one or multiple LAAs and/or minor H antigens several months before HCT, using peptide- or cell-based vaccines, to allow formation of Tcm responses against the antigens. Antigen-specific Tcm are then transferred either with the PBSC graft at the time of HCT, or as DLI after HCT, with or without additional manipulation (e.g., further enrichment of Tcm or depletion of CD45RA$^+$ cells) to reduce the risk of GVHD. Illustrated by Rachel Davidowitz.

AML, but not in GVHD target organs; and (c) optimal allele frequencies to allow minor mismatches to be common (52). A proportion of these candidates would be expected to be naturally processed and presented on HLA molecules in leukemic cells and to elicit a T cell response. Of note, the Falkenburg group demonstrated that, in addition to tissue specificity, the magnitude and diversity of the immune response influence the balance between GVHD and GVL (53). Specifically, the investigators characterized alloreactive $CD8^+$ T cell responses in recipients of pan-TCD HLA-matched HCT who achieved CR and/or full donor chimerism after donor lymphocyte infusion (DLI). The frequency and diversity of minor H antigen–specific $CD8^+$ T cells were lower in patients with selective GVL compared with those with GVHD. Moreover, although activated and alloreactive T cells in patients with selective GVL were more likely to recognize minor H antigens that were presented only on hematopoietic tissue and not on a representative nonhematopoietic tissue, there were exceptions. These results imply that, from a safety perspective, minor H antigens targeted with T cells to augment GVL may not need to be absolutely hematopoietically restricted, particularly if T cell infusion occurs after the proinflammatory period immediately after HCT.

Class II–restricted, fairly ubiquitously expressed minor H antigens may be worth investigating for their potential to elicit selective GVL, given that HLA class II molecules are generally expressed at relatively low levels on nonhematopoietic cells under noninflammatory conditions; a recent publication suggests this approach has merit (54). Specifically, the authors observed that $CD4^+$ DLI from HLA-identical sibling donors could induce conversion from mixed to full donor chimerism in 4 HCT recipients, with GVL reactivity but without GVHD, by targeting HLA class II–restricted minor H antigens, some of which were associated with genes expressed in nonhematopoietic cells. However, T cells specific for ubiquitously expressed minor H antigens may be more prone to activation-induced cell death and/or T cell exhaustion after adoptive transfer compared with those specific for hematopoietically restricted minor H antigens and were less effective at mediating GVL in murine studies (48, 55). Moreover, HLA class II gene expression is often downregulated on leukemic cells after HCT, which could limit the utility of targeting HLA class II–restricted minor H antigens to augment GVL (56, 57).

Alloantigens and GVL

In HLA-mismatched HCT, alloreactive donor T cells directly or indirectly recognize discrepant recipient HLA molecules or complexes of peptide and discrepant recipient HLA, thereby mediating both GVHD and GVL. Alloreactive T cells contribute substantially to GVL in HLA-mismatched HCT, as evidenced by the dominant immune escape mechanism of uniparental disomy that contributes to relapse in mismatched HCT (58). HLA class II–restricted alloantigens have been examined for their potential to elicit selective GVL, because these molecules are expressed at relatively low levels in nonhematopoietic tissue under noninflammatory conditions (59–62). Although HLA class II–mismatched HCT is associated with GVHD and there are certainly HLA-DR–, HLA-DQ–, and HLA-DP–specific T cells that recognize both hematopoietic and nonhematopoietic tissues, the HLA-DP–restricted T cell repertoire also contains $CD4^+$ T cells with a restricted tissue recognition pattern, including HLA-DP–restricted T cells that respond to primary AML but not to other cell types (59). Thus, some HLA-DP–specific T cells may contribute to GVL without GVHD. As for class II–restricted minor H antigens, a possible barrier to effectively targeting mismatched DP molecules is downregulation of class II gene expression after HCT, which occurs in approximately 50% of post-HCT relapses (56, 57). Although downregulated HLA class II expression on leukemic cells can be reversed by IFN-γ treatment, IFN-γ also increases HLA on normal nonhematopoietic tissue. Thus, the therapeutic window for augmenting GVL by targeting a select set of class II alloantigens is narrow.

Nonpolymorphic antigens and neoantigens

Donor-derived αβ T cells have the potential to respond to other classes of LAAs, including self-antigens overexpressed on hematopoietic cells, cancer-testis antigens (CTAs), and neoantigens. Although there are documented examples of donor-derived T cell responses to certain overexpressed antigens and CTA after HCT, little is known about the prevalence and repertoire of donor-derived T cell responses to these classes of LAAs or the contribution of neoantigen-specific T cells in HCT.

Overexpressed antigens derive from wild-type proteins with relatively abundant expression in malignant cells. Wilm's tumor 1 (WT1) is a well-studied example that is highly expressed in acute leukemias (63) but limited in normal $CD34^+$ HSCs and other tissues. Donor-derived WT1-specific T cells have been observed in vivo after HCT and DLI (64–69). Adoptive transfer of donor-derived in vitro–stimulated (70)

or TCR-transduced (71) $CD8^+$ T cells specific for an HLA-A*02:01–restricted WT1 epitope reduced post-HCT AML relapse in phase I studies. PR1 is an immunogenic HLA-A*02:01–restricted epitope produced from elastase and proteinase-3, which are abundantly expressed in chronic myelogenous leukemia (CML) (72). Circulating donor-derived PR1-specific $CD8^+$ T cells were detected in CML patients achieving CR after HCT (73) and in an AML patient after HCT and DLI (69). PR1-specific T cells engineered with a synthetic receptor that binds the peptide-HLA complex are in development (74). Immune responses against other overexpressed antigens CML28 (75), CML66/NUDCD1 (76–78), NuSAP1 (79), survivin (69), and others (80) arising after HCT and DLI suggest additional potential therapeutic targets.

CTAs are normally expressed only in germline tissues but are aberrantly expressed by malignant cells. Donor-derived responses to an HLA-A*02:01–restricted epitope of the CTA preferentially expressed antigen of melanoma (PRAME) have been identified after HCT (81) and DLI (69, 82). The use of in vitro–stimulated HCT donor T cells against PRAME and other LAAs (WT1, NY-ESO-1, and survivin) for prevention or treatment of relapse after HCT is under investigation (NCT02494167, NCT02203903, and NCT02475707).

Compared with solid tumors, leukemias have relatively few protein-coding mutations and gene fusions (83), corresponding with few potential neoantigens. However, neoantigens could still play a critical role in effective GVL responses, as donor T cell responses directed against an epitope derived from a single driver mutation or fusion essential for the malignant phenotype could eradicate the founding clone (84). $CD8^+$ T cell responses to neoantigens derived from patient-specific mutations were detected after HCT in 2 patients who achieved durable remissions from chronic lymphocytic leukemia in one study (85). In another case report, T cells binding nucleophosmin (NPM1) mutation–associated peptide/HLA tetramers were detected in a patient after DLI and HSC boosts for molecular recurrence of $NPM1^+$ AML (86). These cases suggest that further investigation into post-HCT neoantigen responses is warranted. Recently, normal donor T cells specific for a neoantigen derived from mutations in *NPM1* were discovered (87), and the corresponding TCRs are being employed to develop TCR T cell immunotherapy, which could be delivered before or after HCT to prevent relapse (Figure 3A). Phosphoantigens (pAgs), peptides derived from HLA-binding cancer phosphoproteins, are a type of neoantigen with potential broad applicability in hematologic malignancies, as some are expressed in acute leukemias but not normal tissues (88). Cobbold and colleagues demonstrated responses to pAgs in normal donors and HCT recipients but generally not in patients with AML prior to HCT, suggesting that pAg-specific T cell responses may contribute to GVL, with potential for augmentation.

Using donor-derived T cells may facilitate targeting neoantigens in acute leukemias, especially AML, where recipient T cells may be tolerized or suppressed (87). Infusion of donor-derived T cells targeting neoantigens to HCT is starting to be explored clinically. For example, Comoli and colleagues transferred in vitro–stimulated ^{p190}BCR-ABL1–specific donor $CD8^+$ and $CD4^+$ T cells to 2 patients with Ph^+-ALL and molecular MRD or morphological relapse following HCT, administration of DLI, and tyrosine kinase inhibitors; ^{p190}BCR-ABL1–specific T cell transfer showed possible antileukemic activity (89). As next-generation sequencing technology decreases in cost and whole-exome sequencing or targeted mutation screening becomes a standard of care for acute leukemia, more personalized or semipersonalized T cell immunotherapies targeting HCT recipient neoantigens with donor T cells are likely to be evaluated in clinical trials.

αβ T cells modified to express CARs

Immunotherapy employing T cells genetically modified to express a CD19-targeting CAR has demonstrated efficacy in ALL and non-Hodgkin lymphoma (90–92). CAR T cells targeting other cell surface molecules show promise for treating hematological malignancies in preclinical studies and early clinical trials (92). In the context of HCT, donor-originated T cells can be obtained by apheresis from HCT recipients or directly from the HCT donor, and then modified with CAR, with or without additional strategies to mitigate GVHD risk (93–100). Although CAR T cells are currently being investigated to treat disease relapse, they could also be infused prophylactically at the time of HCT to augment an antileukemic effect. Some groups are piloting this approach in the context of pan-TCD HCT. Incorporating CAR-modified T cells or transgenic TCRs in HCT grafts could deliver a potent antileukemic effect and be particularly advantageous for patients undergoing HCT with refractory or relapsed disease. However, avoiding toxicity while preserving the advantages of the polyclonal, multispecific T cell response inherent to HCT will be a challenge. Specifically, CAR T cells delivered with T cell–replete HCT could promote GVHD. For example, inflammatory cytokines such as IL-6 are elevated in patients with CAR T cell–associated cytokine-release syndrome (101,

102), and IL-6 contributes to the acute GVHD pathogenesis (102–104). If single-specificity CAR T cells are delivered in the context of pan-TCD HCT, opportunistic infections and leukemia escape by downregulation of the target molecule would be predicted. Ultimately, development of a multispecific hematopoietic cell graft incorporating CAR and/or TCR T cells will require sophisticated graft engineering.

Failure of GVL delivered by αβ T cells

HCT recipients may sustain remissions that last years after HCT but then relapse, consistent with the existence but ultimate failure of GVL activity. A growing body of literature reports several mechanisms of GVL failure, including adaptations of residual leukemic cells to evade T cell control and T cell changes consistent with increasing functional impairment over time in some HCT recipients (105–107). Understanding the mechanisms of GVL failure should enable the development of strategies to promote durable GVL.

Loss or repression of HLA genes. As αβ T cells recognize peptide antigens presented in association with HLA molecules, genomic HLA loss or downregulation of HLA expression after HCT are potential mechanisms of leukemia escape from donor T cell control. Genomic loss of mismatched HLA can occur through copy-neutral loss of heterozygosity, also known as uniparental disomy, in the context of HLA-mismatched HCT, such that mismatched HLA molecules are lost without decreasing the overall levels of HLA class I expression. Acquired uniparental disomy occurs in leukemic cells in approximately 30% of patients who relapse after haploidentical HCT (58). Genomic HLA loss has also been reported after HLA-matched HCT in some studies (108–110). The potential for genomic HLA loss after HLA-mismatched HCT should be considered in designing strategies to augment GVL. For example, employing T cells that target an epitope presented on an HLA allele that is shared between a donor and recipient is likely to be more advantageous than targeting an epitope on the nonshared HLA that has a high probability of being deleted and replaced.

Downregulation of HLA class II expression on leukemic cells is another mechanism of escape after HLA-matched and mismatched HCT (56). Using RNA-sequencing (RNA-seq), Christopher and colleagues observed that most HCT recipients who relapsed demonstrated a 3- to 12-fold reduction in HLA class II gene expression. At the protein level, class II downregulation was apparent by flow cytometry or immunohistochemistry in 17 of 34 HCT recipients. IFN-γ exposure could reverse class II downregulation, suggesting epigenetic regulation of class II molecules as an escape mechanism. Interestingly, there was no significant downregulation of HLA class I expression. Similarly, Toffalori and colleagues found that transcripts for HLA class II and the class II regulator CIITA were downregulated in AML blasts at post-HCT relapse and confirmed loss of HLA-DR and HLA-DP surface expression in 7 of 33 relapses, without HLA class I downregulation (57). Collectively, these studies imply that $CD4^+$ T cells specific for class II–restricted minor H antigens and possibly other LAAs play a major role in GVL, as the downregulation of class II molecules represents an adaptive response to immune pressure. We can also infer that employing T cells targeting class I–restricted LAAs to augment GVL may avoid this mechanism of escape. Additionally, IFN-γ is well known to upregulate HLA expression on cells, and two groups (56, 57) confirmed that IFN-γ exposure could restore class II expression on post-HCT leukemic cells in vitro, suggesting systemic IFN-γ administration may help maintain GVL. However, adjunctive IFN-γ therapy would carry a significant risk of GVHD induction, especially early after HCT (102, 111). Moreover, because IFN-γ also upregulates the expression of the inhibitory ligand PD-L1 on leukemic cells, it could also actually facilitate escape from GVL (111).

Upregulation of T cell inhibitory molecules. Alteration of cell surface molecules relevant to GVL on leukemic or T cells in patients experiencing post-HCT relapse has been described. Toffalori and colleagues examined transcriptional changes in AML blasts following HCT using genome-wide microarrays and observed downregulation of multiple activating ligand and adhesion molecules, such as LFA-1 (57). Upregulation of most inhibitory molecules in the AML blasts was not detected by microarrays, although a slightly higher proportion of cells expressed PD-L1. Interestingly, PD-L1 tended to be upregulated on AML blasts without downregulation of HLA class II molecules. Toffalori et al. also noted that PD-1 expression on T cells was increased in patients with post-HCT relapse compared with post-HCT patients in remission. All post-HCT groups had higher PD-1 expression on T cells than healthy volunteers. Similarly, Noviello and colleagues reported that, in patients who developed post-HCT AML relapse, more marrow-infiltrating Tcm and Tscm cells expressed PD-1 and other inhibitory receptors compared with those from patients in remission (112).

Checkpoint inhibitor (CPI) administration to block T cell inhibitory receptors or their ligands has been evaluated extensively for patients with solid tumors and to a lesser extent for hematological malignancies in the non-HCT setting. Relatively little clinical data exists about CPI in the context of HCT (113–118),

due to concerns about the risk of inducing severe GVHD. In one prospective study of the CTLA4 inhibitor ipilimumab in patients with relapsed hematological malignancies after HCT, 32% of patients achieved CR (23%) or partial response (9%), immune-related adverse events occurred in 21%, and GVHD, precluding further administration of ipilimumab, occurred in 14% (115). The median time from HCT to initial ipilimumab treatment was 675 days (range 198 to 1830) (115), so the risk of GVHD and immune-related adverse events may be higher in patients treated with CPI earlier after HCT. Additional clinical trials evaluating CPI in patients with leukemia, including those 6–12 weeks or more after HCT are ongoing (NCT02890329, NCT03600155). Combination strategies of CPI and hypomethylating agents are being evaluated in the clinic (119) and could represent a useful combination after HCT, as CPI may counter the upregulation of inhibitory molecules (e.g., PD-1, PL-L1) induced by hypomethylating agents (120), and hypomethylating agents may mitigate CPI-associated GVHD risk (121, 122).

γδ T cells

γδ TCR–expressing T cells represent 1%–5% of all postthymic $CD3^+$ T cells and participate in both innate and adaptive immunity (123). γδ T cells are similar to cytotoxic $CD8^+$ T cells and NK cells, producing large amounts of IFN-γ and TNF-α, and can exert cytotoxic effects on microbial pathogens and malignant cells. Indeed, in single-cell RNA-seq (scRNA-seq) studies, γδ T cells cluster between $CD8^+$ T cells and NK cells, reflecting a similar transcriptional profile (124). γδ T cells express CD16 and can induce antibody-dependent cellular cytotoxicity (ADCC). In addition, they can function as antigen-presenting cells (APCs), induce proliferation and IFN-γ production in αβ T cells, stimulate NK cell cytotoxicity, and induce MHC class I expression on tumor cells (125). In human tumors, a greater abundance of γδ T cells correlates with a favorable prognosis, and numerous studies demonstrate antileukemic activity by γδ T cells (126).

γδ T cells express somatically rearranged TCRs and can also be activated via NK cell receptors (NKRs), such as DNAM1 and NKG2D (127, 128). Although understanding of how γδ T cells recognize ligands is incomplete, γδ TCRs are known to bind soluble or membrane proteins and can bind CD1d on APCs presenting glycolipid and microbial lipids (128). Although some γδ T cells appear to recognize MHC peptide complexes (129, 130), their recognition of antigen is generally MHC independent such that HLA deletion or downregulation should not inhibit γδ T cell–mediated GVL. γδ T cells are divided into subsets (Vδ1, Vδ2, Vδ3, and Vδ5) based on the δ chain, (131), and further classified on the basis of γ chain expression. scRNA-seq shows that the two major γδ T cells subsets, Vδ1 and Vδ2, form close but distinct clusters, reflecting expression of shared and distinct genes, and are closely related to NK and $CD8^+$ T cell subsets, respectively (124).

Vγ9δ2 T cells comprise the major γδ T cell subset in the peripheral blood (132) and are activated by nonpeptidic pyrophosphate pAgs, such as isopentenyl pyrophosphate (IPP), which is produced in the mevalonate pathway (133). In neoplastic cells, mevalonate dysregulation leads to overproduction and Vγ9δ2 T cell activation (127). The mevalonate pathway can be manipulated pharmacologically to activate Vγ9δ2 T cells using bisphosphonates such as zoledronic acid (ZOL) (127). Recognition of pAg by γδ T cells depends on butyrophilin (BTN) family member A1 (BTN3A1, also known as CD277) and RhoB (134–136). Vγ9 receptor chains have restricted CDR3 sequences and many public clonotypes exist; Vδ2 is more diverse but requires specific hydrophobic amino acids at position 97 for pAg recognition (128).

Vδ1 T cells are the predominant γδ T cell type in epithelial tissues, where they recognize antigens from virus-infected and cancer cells (137). Like Vγ9δ2, Vδ1 T cells can be activated by their TCR or by NKR. Vδ1 T cells do not recognize pAgs and their TCR ligands remain largely unknown, although Vδ1 T cells can be activated through their TCR by members of the CD1 family (138). Interestingly, Vδ1 T cells that recognize the HLA-A*02:01–restricted MART1 antigen via their TCR have recently been described (129). Vδ1 T cells can also recognize tumor antigens via natural cytotoxicity receptors NKG2D (139), NKp30, and NKp44 (140). CMV-stimulated Vδ1 T cells also have antitumor reactivity (141, 142).

γδ T cells in HCT

After HCT, γδ T cells reconstitute within 30 to 60 days with a repertoire that remains quantitatively similar to normal adult γδ T cell repertoires but differs from the recipient's pre-HCT repertoire and from the donor repertoire, indicating de novo generation in the recipient's thymus (143). The γδ T cell repertoire remains stable after initial reconstitution, except in cases of CMV reactivation, during which some γδ T cell clones, mostly $Vδ2^-Vγ9^-$ (Vδ1, Vδ3), undergo proliferation (143). In one study of 153 partially TCD

HCT recipients with acute leukemia, an increased number of γδ T cells at day 60 or greater associated with improved 5-year DFS and OS, although relapse and GVHD did not differ significantly (144). In another report of 102 HCT recipients with leukemia, increased γδ T cell numbers associated with fewer infections and improved long-term event-free survival (145). γδ T cells may facilitate GVL but are unlikely to cause GVHD in humans, as the γδ T cell repertoire is not known to include alloantigen-specific T cells. Moreover, γδ T cells are generally not MHC restricted, do not proliferate in mixed lymphocyte cultures, and do not cause GVHD in murine HCT models, although they may participate in established GVHD (146–148).

γδ T cells in αβ-TCD HCT

One strategy to remove alloreactive T cells from the donor stem cell graft is αβ-TCD (Figure 2C), often coupled with depletion of $CD19^+$ B cells, to avoid EBV posttransplant lymphoproliferative disorder. This approach retains γδ T cells and NK cells, which both have the potential to protect against opportunistic pathogens and deliver an antileukemic effect (149–155). The outcomes of patients who received haploidentical αβ-TCD grafts have been encouraging, with 3-year DFS, relapse, severe acute (grade III–IV), and extensive chronic GVHD rates of 62%, 29%, 0%, and 1%, respectively, in a retrospective analysis of 98 pediatric patients with leukemia (155). Immune reconstitution studies in patients receiving αβ-TCD grafts found that γδ T cells represent the predominant T cell subset in the first 3 months after HCT and have demonstrable antileukemic activity in vitro (153). Vδ2 is the predominant subtype, and naive Vδ2 T cells increase between day 20 and 90, reflecting generation from HSCs. Vδ1 cells expanded in vivo in response to CMV reactivation, and antileukemic activity was greater in Vδ1 T cells from patients with CMV reactivation (153). The same group studied administration of ZOL every 28 days to αβ-TCD graft recipients with leukemia. ZOL treatment associated with increased cytotoxicity of both Vδ1 and Vδ2 cells against primary leukemia blasts, and patients given more than 3 ZOL infusions had improved OS compared with those given 1–2 infusions, although without a reported effect on relapse (156).

Augmenting grafts for γδ T cells

As γδ T cells can be expanded in vivo and ex vivo from HSC donors and recipients, strategies to deliver more γδ T cells have been published (157–162) and are being evaluated in ongoing clinical trials (NCT04008381, NCT03533816, NCT03790072, and NCT03885076). γδ T cells can be transduced with CAR or αβ TCRs of known specificity to deliver enhanced antileukemic activity and avoid potential GVHD associated with allogeneic αβ TCR T cells (163, 164). Alternatively, pAg-reactive Vγ9δ2 TCR can be transduced into αβ TCR T cells to transfer antitumor reactivity. Engineered γδ TCR–expressing αβ T cells are designed to overcome the challenges of working with polyclonal Vγ9δ2 T cells that have variably avid TCRs and express multiple innate receptors, including inhibitory receptors (165–167). The transduced γδ TCR is reported to compete successfully with the endogenous αβ TCR for CD3, thus suppressing αβ TCR expression and mitigating the risk of alloreactivity without the need for αβ chain knockout. Novel bispecific nanobody–based constructs targeting both Vγ9Vδ2 T cells and tumor antigens are also being explored in preclinical studies and can induce potent Vγ9Vδ2 T cell activation (168).

Some potential concerns about using γδ T cells to augment GVL should be considered. γδ T cells have functional plasticity, including the ability to change their cytokine profile to secrete IL-17, which can directly promote tumor growth (127) and contribute to GVHD pathogenesis (125). γδ T cells have also been reported to transdifferentiate extrathymically into αβ T cells, and it is unclear whether transdifferentiated cells are free of GVHD-causing potential (169). Last, the small minority of γδ T cells that do recognize HLA-restricted peptide antigens could contribute to GVHD if they recognize alloantigens.

Conclusions and future directions

Since GVL was initially described in the 1970s, T cells have been identified as a driving force of this phenomenon. Much remains to be learned about T cell subsets and target antigens that underlie effective GVL responses, as well as the mechanisms by which leukemia can escape. The key question is, what is the most potent way to augment and sustain GVL without inducing detrimental GVHD? New tools to identify T cell antigens (170, 171) from TCR sequences in an unbiased manner could reveal new GVL targets and facilitate assessment of an antigen's potential to induce toxicity. The results of clinical trials of graft engineering, vaccination, and adoptively transferred antigen-specific T cells will inform the therapeutic potential of specific antigens and classes of antigens. Advances in graft and T cell engineering will facilitate clinical transla-

tion of donor grafts and/or cell products that have undergone depletion or enrichment of cell populations, expansion of antigen-specific T cells, or engineering to induce expression of one or more specific receptors. New technology capable of advanced graft engineering including efficient enrichment of grafts with lymphocytes with antileukemic activity is under development (172). A clear definition of the mechanisms of immune escape and relapse after HCT will facilitate the successful implementation of therapeutic strategies to augment GVL, including the rational use of CPI. Ultimately, the ability to provide complete protection from post-HCT relapse and other causes of HCT failure will require development of a cohesive strategy that combines graft engineering to carefully craft the content of the donor product(s) administered during HCT and administration of other agents that can sustain the efficacy of beneficial T cells.

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Address correspondence to: Marie Bleakley, 1100 Fairview Avenue N Seattle, Washington 98109-98120, USA. Phone: 206.667.6572; Email: mbleakle@fredhutch.org.

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