Published in final edited form as:

Immunol Rev. 2014 January ; 257(1): . doi:10.1111/imr.12142.

Genetically engineered donor T cells to optimize graft-versus-tumor effects across MHC barriers

Arnab Ghosh^{1,2}, Amanda M. Holland³, and Marcel R.M. van den Brink¹

¹Department of Medicine and Immunology, Memorial Sloan-Kettering Cancer Center, New York, NY

²Department of Internal Medicine, Icahn School of Medicine at Mount Sinai, New York, NY

³MRC Centre for Immune Regulation, University of Birmingham, Birmingham, UK

Summary

Hematopoietic stem cell transplantation has been used for more than 50 years to combat hematologic malignancies. In addition to being the first stem cell therapy, transplantation has provided evidence for the potent anti-tumor effects of T cells. Facilitating T-cell-based immunity against malignancies requires a careful balancing act between generating a robust response and avoiding off-target killing of healthy tissues, which is difficult to accomplish using bulk donor T cells. To address these issues, several approaches have been developed, drawing on basic T-cell biology, to potentiate graft-versus-tumor activity while avoiding graft-versus-host disease. Current strategies for anti-tumor cell therapies include (i) selecting optimal T cells for transfer, (ii) engineering T cells to possess enhanced effector functions, and (iii) generating T-cell precursors that complete development after adoptive transfer. In this review, we assess the current state of the art in T-lineage cell therapy to treat malignancies in the context of allogeneic hematopoietic stem cell transplantation.

Keywords

precursor T cells; TRAIL; PLZF; GVHD; GVT; allo-HSCT

Introduction

The immunosurveillance hypothesis, proposed independently by Lewis Thomas and Macfarlane Burnet, posits that tumors arise at more frequent intervals than detected due to recognition and destruction of the nascent malignancies by host immune cells (1). Over the decades, evidence has accumulated to link immune incompetence with increased incidence of cancers. Increased incidences of anal and cervical carcinomas (2, 3), lymphomas (4), and Kaposi's sarcoma (5) in patients with human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) have been well-established. It is now known that some tumors express mutated self-proteins known as tumor-associated antigens (TAAs), capable of eliciting a tumor-specific immune response (6). Zinkernagel *et al.* (7) suggested that failure of the immune system to recognize the TAA leads to the evolution of manifest cancerous disease, a concept known as the immune-unresponsiveness hypothesis. Though Burnet and Zinkernagel were honored with Nobel prizes for their findings, scientists

Correspondence to: Marcel R.M. van den Brink, Department of Immunology and Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY 10065, Tel.: +1 646 8882304, Fax: +1 646 422 0452, vandenbm@mskcc.org.

The authors have no conflicts of interest to declare.

continue to pursue a fuller understanding of the interplay between the development of cancer and the immune system in order to develop effective anti-tumor immunotherapies (8). These studies have led to the development of the cancer immunoediting hypothesis, which unifies the tumor protective immunosurveillance and tumor promoting immune unresponsiveness aspects of immunity (9). A corollary of this hypothesis that the immune system shapes up tumor immunogenicity is that in order to be effective, cancer immunotherapies must involve large numbers of high quality effectors that can eliminate tumors and tumor-induced immune suppressions.

T cells, now known to be key effectors of anti-tumor effects, are attractive candidates for cancer immunotherapy (10). While results with adoptive therapy with unmodified autologous T cells have been encouraging in some solid tumors (11), its utility in the treatment of hematologic malignancies such as leukemia has been limited. Wider application of adoptive transfer of unmodified autologous T cells has been restricted by the difficulty in isolating TAA-specific T cells and the scarcity of functional T cells following conventional cancer therapies, though these constraints were partially overcome when hematologic cell replacement was propounded. Transplantation of hematopoietic cells from allogeneic donors supplemented with mature donor T cells can cure minimal residual disease and prevent leukemia relapse (12). In a path-breaking trial by Hans Kolb and colleagues (13), adoptively transferred peripheral blood mononuclear cells (PBMCs) derived from the peripheral blood of donors were used to treat three patients that suffered from CML relapse after bone marrow transplantation. All three patients achieved hematologic and cytogenetic remission persisting up to 7 months, thus documenting the first trial of allogeneic T-cell-based immunotherapy. Of note, two of the three patients treated with allogeneic PBMCs developed graft-versus-host disease (GVHD).

Allogeneic T-cell-based therapies of malignant conditions

Allogeneic hematopoietic stem cell transplantation

Hematopoietic cell replacement therapy in the form of allogeneic hematopoietic stem cell transplantation (allo-HSCT) was developed from research on possible consequences of a nuclear attack on American marines during the Cold War (14). Over the years, it has evolved from a cell replacement strategy following cytoreduction to an active immunotherapy option in combination with donor T-cell transfers. The availability of T-cell-depletion devices and immunosuppressive drugs has reduced, to a small extent, the requirement of HLA-matched donors and opened up the options to use HLA-mismatched and haploidentical grafts. Regimen-related toxicity, the reactivity of the immunocompetent donor cells towards the host (graft-versus-host reactions), malignant relapse, and slow immune recovery are the major limitations of present day mismatched allo-HSCT (15).

For successful outcomes of allo-HSCT, two components hold the keys (16). First, preparative regimens based on irradiation or cytotoxic drugs lead to myeloablation, destruction of the immune system, and elimination of cancer cells. However, this 'myeloablative' therapy is unlikely to kill every tumor cell, which results in a persistent risk of leukemia relapse. Intensification of myeloablative therapies can sometimes decrease the rates of relapse at the cost of increased treatment-related morbidity and mortality.

The second aspect associated with allo-HSCT is the recognition of the host-derived malignant cells by the cells of the transplanted immune system. This component termed as graft-versus-tumor (GVT) reaction was initially described as a desirable side effect of graft-versus-host disease (GVHD), based on observations that syngeneic HSCT is associated with a higher risk of tumor relapse (17). In addition, GVHD is associated with a lower risk of relapse and complete remissions were observed in patients with post-HSCT leukemia

relapse who had a flare-up of GVHD (reviewed in 16). Separating GVHD from GVT remains the Holy Grail of allo-HSCT.

Donor lymphocyte infusion

Depletion of T cells in the graft leads to a significant decrease in the incidence of GVHD (18). However, this also results in the loss of potentially beneficial effects of donor T cells, namely engraftment of donor hematopoietic cells and the T-cell-dependent GVT and anti-microbial effects. These losses may be overcome, however, by a subsequent transfer of donor-derived T cells, known as donor lymphocyte infusion (DLI), which is primarily used for post-transplant relapse.

In light of the indirect evidence that donor lymphocytes provide potent GVT effects following allo-HSCT, direct application of donor lymphocytes was attempted (19). The first direct evidence was obtained when donor-derived PBMC infusions were used to treat post-HSCT relapse of chronic myeloid leukemia (13). Further support for the role of T cells as effectors of GVT came from the utilization of clonal donor T cells stimulated *ex vivo* with leukemia cells to treat CML relapse (20). These and other studies furthered DLI-based approaches to treat relapsed malignancies following allo-HSCT (21).

When MHC-mismatched allo-HSCT is used as a platform for DLI, the GVT effects of the DLI are critically dependent on the presence of host APC (22, 23). Using naive donor T cells, these studies demonstrated a crucial role of host APC in priming donor-derived T cells leading to allo-recognition of host MHC (23). These studies identified that the success of DLI therapy with allo-HSCT was dependent on the continued presence of host APC. A further consequence of these studies was the further demonstration that GVT activity was dependent on similar factors as GVHD, thereby emphasizing the intricate linkage of the beneficial and deleterious effects of T cells in HSCT.

Attempts have been directed towards modulating the *in vivo* environment to make DLI more conducive to GVT effects while hampering the development of GVHD. One strategy was to control the inflammatory environment and the soluble factors, which lead to the development of GVHD. DLI given late after HSCT were shown to elicit GVT effects with a lower risk of GVHD (24). In addition, homing to non-lymphoid organs is a prerequisite for eliciting GVHD and trapping of T cells in lymphoid tissues can reduce the incidence and severity of GVHD (25). The above observations have now been explained by inflammatory checkpoints, absent after delayed DLI, which allow the migration of activated T cells to the GVHD non-lymphoid target organs (26).

Selecting the optimal T cell for GVT

While infusion of whole T-cell subsets of donor origin as in a donor lymphocyte infusion is expedient, matters of safety and increased effectiveness demand exploring the use of purified or potentiated subsets of T cells that can mount a strong GVT effect while suppressing or at least without causing GVHD. About 1–10% of mature T cells can recognize and react with foreign MHC (27). Until recently, it was not clear if the mechanism of alloreactivity was specific to a few antigens or explained by degeneracy. Some evidence suggest that alloreactive T cells interact with non-self-MHC in a peptide-specific manner. However, the interactions seem to be polyspecific, resulting in a degree of T-cell promiscuity (28, 29). The GVT effects of allogeneic T cells are at least in part dependent on specific recognition of tumor antigens. Following bone marrow transplantation in metastatic colon cancer, the development of a tumor-specific CD8⁺ T-cell population has been reported during the development of GVHD (30). The CD8⁺ T-cell population reactive to Carcino-embryonic antigen (a colorectal carcinoma-associated neoantigen) was then isolated and

found to have potent anti-tumor effects *in vivo*. Murine studies have also demonstrated that GVT effects can be elicited by CD8⁺ T cells, independent of the recognition of alloantigens on the tumor targets (31).

T cells are often directed towards specific target antigens uniquely or preferentially expressed on tumor cells. Two general categories of target antigen for T-cell therapy are under study (32): polymorphic minor histocompatibility antigens (mHAg)s and antigens associated with a malignant phenotype. Polymorphic mHAg)s show a limited tissue distribution and are recognized by alloreactive T cells. The mHAg)s HA-1 and HA-2, expressed exclusively on hematopoietic cells, can act as lymphohematopoietic targets of allogeneic CD8⁺ T cells, sparing the non-hematopoietic GVHD target organs (33, 34). HA-1-reactive T cells, cloned from patients treated with post-transplant DLI, have been shown to inhibit leukemia growth *in vitro* (35) and in murine models (36). Beads coated with HA-1/HA-2 have been used as artificial antigen-presenting constructs to enrich antigen-specific CD8⁺ T-cell clones (37). However, polymorphic mHAg)s like HA-1 and HA-2 have limited and differential expression, restricting the applicability of mHAg-directed T-cell therapy to a few regions and selected donor-recipient matches (38).

An alternate strategy is to develop clones against antigens associated with malignancy. In an allogeneic context, this approach has been proven effective in treating post-transplant viral infections. Monoculture-derived allogeneic CD8⁺ T cells directed against viral epitopes of EBV have been used as treatment or prophylaxis following HSCT (39). In the context of tumors, MHC-restricted allogeneic T cells can be raised against peptide epitopes that are preferentially expressed on tumors. In a murine study, cloned CD8⁺ T cells were cultured against mdm2, a protein expressed on tumor cell lines in an MHC-restricted manner. Adoptive transfer of these clones mediated specific reactivity against the mdm2-expressing tumors in mice but not host cells (40). From the point of practicality however, selection of such clones from a typically large T-cell repertoire for every donor-host combination is an onerous task. In experimental models, priming donor-type T cells *ex vivo* with recipient-derived DC loaded with leukemia lysate to generate a cell product with strong GVL properties circumvents the need of host APC for successful GVL effects (41). Adequate loading of leukemia-lysate and priming with APC of recipient origin was crucial to elicit substantial GVL effects. However, the *ex vivo* expanded product had considerable GVHD potential and induced lethal reactions when adoptively transferred early post-HSCT while the inflammatory reactants were abundant. Genetic engineering of T cells with T-cell receptors (TCRs) of known tumor reactivity has been used to enhance GVT (42). However, exogenously introduced TCR single chains can pair with endogenous TCR causing lethal GVHD (43).

In addition to GVHD potential, another important limitation of the above approaches is the requirement of an *a priori* knowledge of tumor-associated antigens or mHAg)s. This is elusive in most malignant conditions. The cognate tumor antigens are often poorly antigenic and elicit weak T-cell responses. Finally, the requirement of intense cell processing and clonal expansion makes these strategies costly and time consuming (44).

Genetic enhancement of T-cell cytolytic pathways

T cells have the ability to kill other cells via cascades of killing molecules that are activated through optimum stimulation of the T-cell receptor. Cytolytic molecules in both CD8⁺ and CD4⁺ T cells have a role in GVT and GVHD. For the purpose of genetic engineering, therefore, adoptive transfer of unfractionated T cell populations may be both more practical than isolating subsets and functionally superior (45).

Cytotoxic factors cause target cell death via multiple mechanisms, including direct destruction of cellular architecture or receptor-mediated activation of cell death pathways, which activate different caspases leading to unraveling of the target cells (46). Regardless of the specific cytolytic pathway that has been activated, T-cell-mediated toxicity is a focused and efficient method of eliminating target cells that leaves the causative T cell left unscathed to mediate further cytolysis. Although cytotoxic killing is unidirectional, responder T cells are susceptible to killing by similarly armed cytotoxic T cells through fratricide. Fratricide represents a unique self-regulation among cytotoxic T cells that maintains a tight check on the numbers of rapidly expanding lethal cells. Lack of fratricidal regulation leads to autoimmune disorders and unrestricted proliferation (47).

Perforin-granzyme

Perforin and granzymes are families of soluble cytolytic molecules that are delivered into the target cells and synergistically cause cell death. Perforin, stored in the lysosomal granules of killer cells, is biochemically and evolutionarily-related to complement (48). On release, perforin assembles at the membrane of the targets and perforates the cell membrane (49). Granzymes also reside in lysosomal granules of effector cells, which are distinct from those that contain perforins. They are a diverse group of serine proteases containing His-Asp-Ser catalytic triads. At least 11 granzymes have been described, of which humans have five (A, B, H, K, M) and mice have 10 (AG, K, M, N) (reviewed in 50). Following appropriate TCR stimulation, perforin and granzymes are released close to the synapse, where the cytolytic molecules work in synergy via an unknown mechanism to cause disassembly of the target cells. In one suggested mechanism, perforin forms pores through transmembrane attachments in the cytoplasm of the target cells. Granzyme is transferred into the cytoplasm of the target cells, leading to apoptosis. An alternative mechanism is that perforin disrupts the membrane of target cells, causing internalization of perforin and granzyme granules. Perforin subsequently acts on the granules releasing granzymes inside the target cells and causing apoptosis.

Among the different granzymes, granzyme B is by far the best studied. Granzyme B mediates cytolysis through specific cleavage of aspartic acid residues that are unique among eukaryotic serine proteases. In mice, induction of granzyme B cleaves caspases, leading to cell death. In humans, granzyme B additionally disrupts mitochondrial stability through Bid, which contributes to cell death. Granzyme B-mediated cytolysis participates in the rejection of allogeneic tumors and tissues. Mouse models deficient in granzyme B and its downstream granzymes have impaired clearance of allogeneic tumors, and the perforin-granzyme pathway contributes to graft-versus-tumor effects (51–53). However, there is also some evidence of fratricidal effects of granzyme B on other CD8⁺ T cells, which can impair GVT (54).

The role of perforin-granzyme pathways in the development of GVHD is nuanced. Early studies in mouse models of allo-HSCT showed that CD8⁺ T cells deficient in granzyme B have impaired GVHD, and the perforin-granzyme B pathway plays an important role in development of MHC class I-mediated GVHD (55, 56). This pathway has a relatively minor role in MHC class II-mediated GVHD (57) and minor histo-incompatibility-driven GVHD (58). Furthermore, perforin-deficient T cells did not elicit significant organ-specific GVHD, further supporting a role for perforin in driving GVHD (52, 58). In addition to its possible contributory role in the development of GVHD, a major hindrance to the use of T cells overexpressing perforin and granzymes is the lack of specificity. Almost any cell could potentially be a target of the perforin-granzyme pathway, rendering T cells over expressing perforin or granzyme highly unpredictable in their effects.

Ligands of death receptor family

Death receptors are members of the tumor necrosis factor receptor (TNFR) superfamily that contain a death domain (DD) in their intra-cytoplasmic portions. At least eight members of the death receptor family have been characterized: TNFR1 (also known as DR1, CD120a, p55, and p60), CD95 (also known as DR2, APO-1 and Fas), DR3 (also known as APO-3, LARD, TRAMP and WSL1), TNF-related apoptosis-inducing ligand receptor 1 (TRAILR1) (also known as DR4 and APO-2), TRAILR2 (also known as DR5, KILLER, and TRICK2), DR6, ectodysplasin A receptor (EDAR), and nerve growth factor receptor (NGFR)(59). Ligands of death receptors are a diverse set of molecules, many of which are expressed by lymphocytes including activated CD4⁺ and CD8⁺ T cells. Stimulation of DRs by their ligands does not automatically cause cell death, which is tightly regulated by cellular anti-apoptotic factors. Indeed, cancer cells often use these anti-apoptotic factors to escape apoptotic cell death mediated by the immune system. However, DRs are expressed specifically and tightly regulated in health and disease.

Based on their signal transduction, DRs can be grouped under two groups. Group one receptors, CD95 and TRAILR1/2, recruit death-inducing signaling complexes (DISC) on ligation that activate caspase-8, leading to apoptosis(60). Group two receptors utilize DISC-independent mechanisms. Ligation of TNFR1 induces of NFκB and the MAPK pathway activation, which ordinarily leads to proliferation and cellular survival. In the absence of optimum NFκB activation; however, TNFR1 signaling leads to unraveling of the cell, both through apoptosis and necrosis. Activated T cells use Fas-ligand and TRAIL to mediate cytotoxicity and play crucial roles in GVHD and GVT.

TRAIL

TRAIL (also known as Apo2 ligand) is expressed on activated CD4⁺ and CD8⁺ T cells. Ligation of TRAIL to its death receptors —DR4 (in humans) and DR5 (in humans and mice) — induces receptor trimerization, leading to the formation of a DISC. DISC results in recruitment of the adapter molecule Fas-associated death domain (FADD), which in turn recruits and activates caspase-8 to execute apoptosis (61). DR4 and DR5 are expressed on various TRAIL-sensitive malignancies, including head and neck tumors and certain hematologic disorders (62). In many other tumors, the expression of TRAIL receptors can be induced to enhance their susceptibility to TRAIL-mediated apoptosis. Proteasome inhibitors like Bortezomib have been shown to up regulate DR5 in murine lymphoma models by inhibiting the anti-apoptotic protein c-FLIP in target cells and enhancing TRAIL-mediated clearance (63, 64). In the context of allo-HSCT, TRAIL-deficient T cells show a significantly lower GVT activity than TRAIL-expressing T cells (65). By contrast, TRAIL played little or no role in the GVHD activity of donor T cells, and the TRAIL pathway was not required for GVHD target organ pathology. The ability of TRAIL to induce cell death in transformed cells and tumors in a manner that does not require the definition of individual tumor-specific antigens and its inconsequentiality in the development of GVHD make it an attractive candidate for allo-HSCT-based cancer immunotherapy across MHC barriers (61, 62).

TRAIL can be over-expressed in soluble or membrane-bound forms for use as an immunotherapeutic agent. Soluble forms are however, subjected to a rapid clearance, which can limit their clinical efficiency (66). Experiments on the sensitivity of murine TRAIL-susceptible tumor cell lines including lymphoma cell lines like and fibroblast sarcomas were performed to compare soluble TRAIL to membrane-bound TRAIL (2PK-3 cells transduced with TRAIL). 2PK-3-TRAIL cells could induce considerably higher levels of apoptosis than even the highest dose of soluble TRAIL (Marcel van den Brink, unpublished data). These findings are supported by studies on human cells where strong anti-tumor effects were

obtained by the use of membrane-bound TRAIL (67). The efficiency of soluble TRAIL forms is further restricted due to a widespread distribution of decoy receptors and sequestration of tumors (68).

In our recent study, we investigated the use of genetically enhanced TRAIL-overexpressing T cells of donor origin for cellular immunotherapy in mouse models of allo-HSCT (69). Adoptive transfer of donor TRAIL-overexpressing T cells mediated superior tumor-free survival in tumor-inoculated allo-HSCT recipients compared to recipients of control-transduced T cells, thus demonstrating an enhanced GVT effect. In addition to the strong GVT, mice treated with TRAIL over-expressing T cells showed lower GVHD-related morbidity and mortality. We investigated the mechanisms leading to lowered GVHD by studying host APC and alloreactive T cells where TRAIL over-expressing T cells may be acting. DR5 receptors were prevalent in host antigen presenting cells and activated donor T cells. We then used animals deficient in DR5 as hosts and donors of T cells and studied the development of GVHD. The incidence of GVHD was not significantly different between hosts with and without DR5 after adoptive transfer of TRAIL-overexpressing T cells. However, DR5-deficient donor T cells were not able to suppress GVHD. Our study showed that TRAIL-overexpressing T cells could suppress GVHD by fratricide of alloreactive T cells. Collectively, our data demonstrated that donor T cells genetically engineered to overexpress TRAIL could enhance GVT and suppress GVHD in recipients of allo-HSCT, and represent a powerful cell therapy strategy to treat TRAIL-susceptible tumors across MHC barriers.

Manipulating T-cell reactions to allo-reactivity

Th1/Th2/Th17 balance and GVHD

Activated CD4⁺ helper T cells mediate many of their functions through cascades of a series of cytokines, by which they are broadly classified into different subsets. Historically, CD4 responses were classified as Th1 and Th2, though this paradigm has been expanded in recent years with the emergence of several novel subsets including regulatory T cells, Th17, and T-follicular helper cells (70). The fates of the Th subsets are orchestrated by transcription factors that control their gene expression programs and act as master regulators of CD4⁺ T-cell functions. The Th1 subset is defined by T-bet, which is upregulated by IFN- γ and directly activates the Th1 program (71). The Th2 program is defined by the transcription factor GATA-3 and activated by IL-4 (72). The Th17 subset is defined by the transcription factor ROR- γ t. In addition to activating Th1, T-bet also represses GATA-3 and ROR- γ t transcription programs (73). The Th transcription factor and their cytokines weave a complex interplay of activating and regulatory networks, which makes it difficult to study the effect of Th subsets in isolation.

The role of Th subsets in the development of GVHD and mediation of GVT effects is nuanced. Following alloreactive stimulation, the Th1 program is initiated. CD4⁺ T cells produce IL-2, which drives proliferation and IFN- γ production by CD8⁺ T cells, providing positive feedback to the Th1 program. Activation of the Th1 program also causes repression of the GATA-3-driven Th2 program. Th1 can thus drive of acute GVHD reactions. However, in experimental models, deficiency of IFN- γ could trigger lethal GVHD (74, 75). Additionally, depletion of Th2-inducing IL-4 prevented GVHD, indicating that Th2 subsets also participate the development of GVHD (76). These paradoxes were further complicated with the question of the role of Th17 in GVHD pathology. It has been shown that Th17 cells can mediate acute GVHD pathology (70). However, its role in the development of GVHD is not clear. Allo-HSCT recipients of IL-17-deficient CD4⁺ T cells had moderately lower GVHD mortality and pathological scores, suggesting a minor role of donor IL-17 in the development of GVHD (77).

T-cell manipulation with forced PLZF expression

Although Th subsets have a remarkable role in the development of GVHD, the translation of this knowledge is complicated. For one, knocking down either one of these subsets could potentially enhance GVHD. Blocking individual cytokines resulted in reciprocal differentiation of Th1, Th2, and Th17 subsets that potentiate alloreactivity (78). We therefore explored the possibility of introducing genes that can cause concurrent production of Th1 and Th2 polarity associated cytokines.

PLZF is a member of the BTB-ZF (Broad complex, Tram track, Bric-à-brac-zinc finger) family of transcription factors, and controls development and cellular function across multiple lineages in mice and humans (79). It is expressed in invariant natural killer T cells (NKT) cells and natural killer (NK) cells but not conventional T cells. In NKT cells, PLZF controls key steps in development, activation and effector functions (80, 81). In NK cells, it regulates a subset of interferon stimulated genes and contributes to antiviral immunity (82). Ectopic expression of PLZF in conventional T cells can confer innate-like properties such as cytokine production on primary activation and an activated effector/memory phenotype in naive T cells (83, 84). Additionally, following stimulation of the T-cell receptor, PLZF expressing conventional T cells concurrently produce Th2 polarizing cytokines like IL-6 and proinflammatory IFN γ that are typically antagonistic.

To explore the potential of PLZF expressing T cells as immunotherapeutic agent in the context of allo-HSCT, we administered allo-HSCT recipient mice with PLZF transgenic (PLZF-TG) conventional T cells in murine GVHD models and found that PLZF-TG T cells did not elicit significant GVHD lethality or morbidity (85). Such an attenuated GVHD was associated with a hyperactivated state of alloreactive T cells that were highly susceptible to cell death. Additionally forced PLZF expression was associated with a skewing of the T cells towards Foxp3⁺ Treg subset following allo-activation. While the majority of alloactivated CD4⁺ T cells were T-bet expressing Th1 polarized, expression of GATA-3 signifying Th2 polarization and increased IL4 expression was also observed. Our study showed that PLZF-mediated attenuation of GVHD was multifactorial and Th1/Th2 repolarization, skewing towards Tregs and enhanced susceptibility to Fas-mediated fratricide regulated the alloreactive expansion of T cells and prevented GVHD. Despite decreased GVHD, PLZF-TG T cells had preserved GVT activity against lymphoma cell lines in murine GVT models attributable to enhanced IFN γ expression and Fas-mediated cytotoxicity. PLZF could thus represent a candidate gene for engineering donor T cells enhancing safety from GVHD, while retaining potent GVT efficacy.

Adoptive therapy with precursors of T cells

Adoptive therapy with mature conventional T cells may not be suitable or feasible for all patients, despite recent advances in the technology. Several barriers remain to the application of mature T-cell transfer: (i) the need for MHC matching to facilitate TCR recognition of peptide: MHC and, in allogeneic settings, to reduce the risk of graft-versus-host disease, (ii) difficulty in obtaining suitable numbers of cells for adoptive therapy, (iii) poor *in vivo* persistence, and (iv) inability to fully regenerate multiple T-cell lineages in immunodeficient recipients. CAR-based engineered T-cell therapies and the use of autologous T cells may reduce concerns about MHC-matching. Despite early evidence regarding the safety of CAR-transduced T cells with intact endogenous TCR (86), this is an ongoing field of study, and CARs are only viable in cancers with *bona fide* tumor antigens that can be targeted without adversely affecting healthy tissues (87). The use of autologous T cells eliminates the ability to stockpile cells for adoptive therapy, thereby increasing the time and cost involved in treating individual patients. The scarcity of functional tumor-specific T cells in patients, due to central and peripheral tolerance mechanisms, can make

isolation of suitable numbers from the peripheral blood an onerous undertaking, while expansion protocols can change the activation status of cells, potentially diminishing their tumor-killing capacity (86). Even as current T-cell therapies are improving the quality of and extending the lives of patients, complementary and alternative strategies must be developed to overcome the remaining barriers and reach underserved patient populations.

Recent advances in the understanding of T-cell development have fed forward into novel preclinical approaches for enhancing T-cell function using progenitor cells. In the years since the central role of Notch signaling in T-cell development has been appreciated (88, 89), several Notch-based approaches for generating T-lineage cells have been developed (90–92). The OP9-DL1 coculture method, and its successors (93), allow for the recapitulation of most steps of T-cell development *in vitro*. The lack of MHC class II expression in the cultures largely precludes development of CD4⁺ T cells, but the system efficiently drives the generation of thymocyte-like T-cell progenitors and mature CD8⁺ T cells from both murine and human hematopoietic precursors. These Notch-based systems have been exploited to generate cells for adoptive transfer.

Recent studies have demonstrated the feasibility of generating mature CD8⁺ T cells from murine hematopoietic precursors (94). T cells generated *in vitro* display similar cell surface and transcriptional profiles to thymic-derived CD8⁺ T cells, with the notable exception of increased expression of the exhaustion marker PD-1. The OP9-DL1 system produced a pool of self-tolerant CD8⁺ T cells capable of proliferative responses against third-party antigens; CD8⁺ T cells generated on OP9-DL1 underwent homeostatic proliferation after transfer into syngeneic immunodeficient mice. The *in vitro*-generated T-cell pool displayed a broad TCR repertoire. Activation by dendritic cells presenting tumor- or viral-specific antigens resulted in expansion CD8⁺ T cells that bound MHC class I:antigen tetramer. Together, these findings demonstrate the potential to generate a functional mature CD8⁺ T-cell pool *in vitro*, with the potential to expand and isolate antigen-specific cells for adoptive transfer.

These findings are supported by the ability to generate *de novo* mature conventional CD8⁺ T cells from human umbilical cord blood (UCB) hematopoietic stem cells (HSC). TCR^{hi}CD27^{hi}CD1a⁻ T cells generated on OP9-DL1 were phenotypically similar to *ex vivo* UCB CD8⁺ T cells. Upon TCR ligation, *in vitro*-generated CD8⁺ T cells proliferated, upregulated activation markers, and produced IFN- γ and granzyme B (95).

These studies provide an intriguing new direction for mature T-cell therapies, which may allow for *in vitro* generation and expansion of large numbers of CD8⁺ T cells with tumor-antigen specificity. While *in vitro*-generated mature T cells are suitable only for autologous or syngeneic transfer, with modifications to the stromal cells driving differentiation, T cells restricted to various MHC types could be produced and banked for enhanced accessibility and cost-effectiveness. However, several impediments remain to the broad usage of *in vitro*-generated mature CD8⁺ T cells. The long-term survival of the adoptively transferred cells and the *in vivo* functional capacity against established tumors remain to be investigated. The expression of PD-1, a marker of exhausted T cells with limited functional capacity, may lead to poor *in vivo* responses, regardless of T cell persistence. As with many mature T cell therapies, adoptive transfer of *in vitro*-generated CD8⁺ T cells would restore only a fraction of the T lineage, neglecting to repopulate the CD4⁺, innate, and regulatory T-cell pools. While CD8⁺ T cells have potent anti-tumor effects, the helper role of CD4⁺ T cells, as well as their newly appreciated cytotoxic anti-tumor capacity (96) makes them critical components of a fully functional T-cell response. Finally, although *in vitro*-generated CD8⁺ T cells are similar to *ex vivo* UCB T cells, a thorough analysis of the self-reactivity remains to be undertaken given their atypical central tolerance education.

The OP9-DL1 coculture system enables an alternative approach to mature T-cell transfer: the generation and infusion of T-cell-committed progenitor cells. One of the limitations of the Notch-based stromal cell coculture system, the difficulty in generating a full array of late stage thymocyte-like cells, can be utilized as an advantage. With high doses of IL-7 and frequent passaging, OP9-DL1 coculture with HSCs can yield large numbers of early stage CD4⁻CD8⁻ [double negative (DN)] T-cell precursors (preT) (97). PreT do not express TCRs due to their early development phenotype, largely DN2 (CD44⁺CD25⁺) and DN3 (CD44⁻CD25⁺). Upon adoptive transfer into lethally irradiated allogeneic bone marrow transplant recipients, preT engraft in the thymus, where they undergo the remaining stages of T-cell development and give rise to mature peripheral T cells (97). The absence of TCR expression at the transfer enables selection and education in the host thymus, resulting in productive host MHC recognition without a risk for GVHD in allogeneic recipients. Of particular interest, preT may be of third-party origin from both the donor marrow graft and the recipient, making them candidates for a true 'off-the-shelf' therapy, independent of MHC-matching concerns (98).

Adoptive transfer of *in vitro*-generated precursors results in the development of preT-derived conventional TCRαβ⁺ CD4⁺ and CD8⁺ T cells, intestinal intraepithelial lymphocytes, and TCRγδ⁺ T cells *in vivo* (97). Conventional preT-derived T cells in euthymic BMT recipients primarily have a naïve phenotype with a broad TCR repertoire. They proliferate and produce IFN-γ and TNF-α in response to polyclonal TCR stimulation *ex vivo*. PreT recipients also have superior T cell-mediated immunity *in vivo* including enhanced memory responses against *Listeria monocytogenes* (97). Importantly, preT transferred across MHC barriers confer anti-tumor immunity in recipients in the absence of GVHD. The malignancy killing capacity of preT can be dramatically improved using CARs to target tumor antigens (98). These findings, while promising, did not address the critical clinical issue of progenitor transfer and development in a recipient with limited thymic function.

Upon adoptive transfer into athymic BMT recipients, preT generate TCRαβ⁺ and TCRγδ⁺ T cells (99). Athymic preT recipients have significantly increased T-cell counts after BMT. As in euthymic recipients, preT-derived CD8⁺ T cells display a diverse Vβ repertoire, while CD4⁺ T cells feature an oligoclonal repertoire. T cells from preT recipients respond appropriately to *ex vivo* TCR stimulation, as measured by proliferation and cytokine secretion. Finally, genetically engineered preT mediate significantly improved anti-viral immunity compared to BM only recipients. While significant questions remain regarding the efficacy of anti-tumor responses in athymic recipients and the basis for preT education and selection, these data suggest that preT may represent a powerful weapon in the anti-tumor arsenal.

Conclusions

As our understanding of T-cell biology grows, the armories of T-cell weapons primed to wage battle against hematologic malignancies swell. Strategies to select and administer optimal T cell or precursor subsets, with or without genetic engineering, further the decades long goal of separating graft-versus-host and graft-versus-tumor activities. Although significant challenges to T-cell-based anti-tumor therapies remain, the expanding array of cell therapies continues to increase the odds that a given clinical situation can be successfully resolved with an appropriate adoptive T-cell intervention.

Acknowledgments

This research was supported by National Institutes of Health award numbers R01-HL069929 (MvdB), R01-CA107096 (MvdB), R01-AI080455 (MvdB), P01-CA33049 (MvdB), and R01-HL095075 (MvdB). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Support was also received from the Ryan Gibson Foundation, The Lymphoma Foundation and Alex's Lemonade Stand. AG is a fellow of the Lymphoma Research Foundation.

References

1. Burnet FM. The concept of immunological surveillance. *Prog Exp Tumor Res.* 1970; 13:1–27. [PubMed: 4921480]
2. Schafer A, Friedmann W, Mielke M, Schwartlander B, Koch MA. The increased frequency of cervical dysplasianeoplasia in women infected with the human immunodeficiency virus is related to the degree of immunosuppression. *American journal of obstetrics and gynecology.* 1991; 164:593–599. [PubMed: 1992708]
3. Caussy D, et al. Interaction of human immunodeficiency and papilloma viruses: association with anal epithelial abnormality in homosexual men. *International journal of cancer Journal international du cancer.* 1990; 46:214–219. [PubMed: 2166710]
4. Cesarman E. Pathology of lymphoma in HIV. *Current opinion in oncology.* 2013; 25:487–494. [PubMed: 23942293]
5. Chang Y, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science.* 1994; 266:1865–1869. [PubMed: 7997879]
6. Zitvogel L, Tesniere A, Kroemer G. Cancer despite immunosurveillance: immunoselection and immunosubversion. *Nat Rev Immunol.* 2006; 6:715–727. [PubMed: 16977338]
7. Pardoll D. Does the immune system see tumors as foreign or self? *Annu Rev Immunol.* 2003; 21:807–839. [PubMed: 12615893]
8. Willimsky G, Blankenstein T. Sporadic immunogenic tumours avoid destruction by inducing T-cell tolerance. *Nature.* 2005; 437:141–146. [PubMed: 16136144]
9. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science.* 2011; 331:1565–1570. [PubMed: 21436444]
10. Rosenberg SA, Spiess P, Lafreniere R. A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. *Science.* 1986; 233:1318–1321. [PubMed: 3489291]
11. Rosenberg SA, Dudley ME. Cancer regression in patients with metastatic melanoma after the transfer of autologous antitumor lymphocytes. *PNAS.* 2004; 101:14639–14645. [PubMed: 15381769]
12. Kolb HJ, Schmid C, Barrett AJ, Schendel DJ. Graft-versus-leukemia reactions in allogeneic chimeras. *Blood.* 2004; 103:767–776. [PubMed: 12958064]
13. Kolb HJ, et al. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood.* 1990; 76:2462–2465. [PubMed: 2265242]
14. Welniak LA, Blazar BR, Murphy WJ. Immunobiology of Allogeneic Hematopoietic Stem Cell Transplantation. *Annu Rev Immunol.* 2007; 25:139–170. [PubMed: 17129175]
15. Gooley TA, et al. Reduced mortality after allogeneic hematopoietic-cell transplantation. *The New England journal of medicine.* 2010; 363:2091–2101. [PubMed: 21105791]
16. Porter DL, Antin JH. The graft-versus-leukemia effects of allogeneic cell therapy. *Annual Review of Medicine.* 1999; 50:369–386.
17. Odom LF, et al. Remission of relapsed leukaemia during a graft-versus-host reaction. A "graft-versus-leukaemia reaction" in man? *Lancet.* 1978; 2:537–540.
18. Sykes M, Chester CH, Sundt TM, Romick ML, Hoyles KA, Sachs DH. Effects of T cell depletion in radiation bone marrow chimeras. III. Characterization of allogeneic bone marrow cell populations that increase allogeneic chimerism independently of graft-vs-host disease in mixed marrow recipients. *J Immunol.* 1989; 143:3503–3511. [PubMed: 2584703]
19. Slavin S, Morecki S, Weiss L, Or R. Donor Lymphocyte Infusion: The Use of Alloreactive and Tumor-Reactive Lymphocytes for Immunotherapy of Malignant and Nonmalignant Diseases in

- Conjunction with Allogeneic Stem Cell Transplantation. *Journal of Hematotherapy & Stem Cell Research*. 2002; 11:265–276. [PubMed: 11983098]
20. Falkenburg JH, et al. Complete remission of accelerated phase chronic myeloid leukemia by treatment with leukemia-reactive cytotoxic T lymphocytes. *Blood*. 1999; 94:1201–1208. [PubMed: 10438707]
 21. Loren AW, Porter DL. Donor leukocyte infusions for the treatment of relapsed acute leukemia after allogeneic stem cell transplantation. *Bone marrow transplantation*. 2008; 41:483–493. [PubMed: 18026156]
 22. Reddy P, Maeda Y, Liu C, Krijanovski OI, Korngold R, Ferrara JL. A crucial role for antigen-presenting cells and alloantigen expression in graft-versus-leukemia responses. *Nat Med*. 2005; 11:1244–1249. [PubMed: 16227991]
 23. Mapara MY, Kim YM, Wang SP, Bronson R, Sachs DH, Sykes M. Donor lymphocyte infusions mediate superior graft-versus-leukemia effects in mixed compared to fully allogeneic chimeras: a critical role for host antigen-presenting cells. *Blood*. 2002; 100:1903–1909. [PubMed: 12176915]
 24. Johnson B, Truitt R. Delayed infusion of immunocompetent donor cells after bone marrow transplantation breaks graft-host tolerance allows for persistent antileukemic reactivity without severe graft-versus-host disease. *Blood*. 1995; 85:3302–3312. [PubMed: 7756664]
 25. Kim YM, Sachs T, Asavaroengchai W, Bronson R, Sykes M. Graft-versus-host disease can be separated from graft-versus-lymphoma effects by control of lymphocyte trafficking with FTY720. *J Clin Invest*. 2003; 111:659–669. [PubMed: 12618520]
 26. Chakraverty R, et al. An inflammatory checkpoint regulates recruitment of graft-versus- host reactive T cells to peripheral tissues. *J Exp Med*. 2006; 203:2021–2031. [PubMed: 16880259]
 27. Rudolph MG, Stanfield RL, Wilson IA. How TCRs bind MHCs, peptides, and coreceptors. *Annu Rev Immunol*. 2006; 24:419–466. [PubMed: 16551255]
 28. Felix NJ, et al. Alloreactive T cells respond specifically to multiple distinct peptide-MHC complexes. 2007; 8:388–397.
 29. Oosten LEM, Blokland E, Kester MGD, Falkenburg JHF, van Halteren AGS, Goulmy E. Promiscuity of the AlloHLA-A2 Restricted T Cell Repertoire Hampers the Generation of Minor Histocompatibility Antigen-specific Cytotoxic T Cells across HLA Barriers. *Biology of Blood and Marrow Transplantation*. 2007; 13:151–163. [PubMed: 17241921]
 30. Carnevale-Schianca F, et al. Allogeneic nonmyeloablative hematopoietic cell transplantation in metastatic colon cancer: tumor-specific T cells directed to a tumor-associated antigen are generated in vivo during GVHD. *Blood*. 2006; 107:3795–3803. [PubMed: 16403911]
 31. Stelljes M, et al. Graft-versus-host disease after allogeneic hematopoietic stem cell transplantation induces a CD8+ T cell-mediated graft-versus-tumor effect that is independent of the recognition of alloantigenic tumor targets. *Blood*. 2004; 104:1210–1216. [PubMed: 15090450]
 32. Appelbaum FR. Haematopoietic cell transplantation as immunotherapy. *Nature*. 2001; 411:385–389. [PubMed: 11357147]
 33. Mutis T, Verdijk R, Schrama E, Esendam B, Brand A, Goulmy E. Feasibility of Immunotherapy of Relapsed Leukemia With Ex Vivo-Generated Cytotoxic T Lymphocytes Specific for Hematopoietic System-Restricted Minor Histocompatibility Antigens. *Blood*. 1999; 93:2336–2341. [PubMed: 10090944]
 34. de Witte MA, Toebes M, Song JY, Wolkers MC, Schumacher TN. Effective graft depletion of MiHAg T-cell specificities and consequences for graft-versus-host disease. *Blood*. 2007; 109:3830–3838. [PubMed: 17202318]
 35. Kuhns MS, Davis MM, Garcia KC. Deconstructing the Form and Function of the TCR/CD3 Complex. *Immunity*. 2006; 24:133–139. [PubMed: 16473826]
 36. Hambach L, et al. Human cytotoxic T lymphocytes specific for a single minor histocompatibility antigen HA-1 are effective against human lymphoblastic leukaemia in NOD/scid mice. *Leukemia*. 2006; 20:371–374. [PubMed: 16357839]
 37. Oosten LE, et al. Artificial antigen-presenting constructs efficiently stimulate minor histocompatibility antigen-specific cytotoxic T lymphocytes. *Blood*. 2004; 104:224–226. [PubMed: 15031203]

38. Di Terlizzi S, et al. Therapeutic and diagnostic applications of minor histocompatibility antigen HA-1 and HA-2 disparities in allogeneic hematopoietic stem cell transplantation: a survey of different populations. *Biol Blood Marrow Transplant*. 2006; 12:95–101. [PubMed: 16399573]
39. Leen AM, et al. Monoculture-derived T lymphocytes specific for multiple viruses expand and produce clinically relevant effects in immunocompromised individuals. *Nat Med*. 2006; 12:1160–1166. [PubMed: 16998485]
40. Gao L, Yang TH, Tourdot S, Sadovnikova E, Hasserjian R, Stauss HJ. Allo-major histocompatibility complex-restricted cytotoxic T lymphocytes engraft in bone marrow transplant recipients without causing graft-versus-host disease. *Blood*. 1999; 94:2999–3006. [PubMed: 10556182]
41. Ghosh A, et al. Donor T cells primed on leukemia lysate-pulsed recipient APCs mediate strong graft-versus-leukemia effects across MHC barriers in full chimeras. *Blood*. 2009; 113:4440–4448. [PubMed: 19182207]
42. de Witte MA, et al. Targeting self-antigens through allogeneic TCR gene transfer. *Blood*. 2006; 108:870–877. [PubMed: 16861342]
43. Bendle GM, et al. Lethal graft-versus-host disease in mouse models of T cell receptor gene therapy. *Nature medicine*. 2010; 16:565–570. 561p following 570.
44. Porter D, Levine J. Graft-versus-host disease and graft-versus-leukemia after donor leukocyte infusion. *Semin Hematol*. 2006; 43:53–61. [PubMed: 16412789]
45. Jiang Z, Podack E, Levy RB. Major histocompatibility complex-mismatched allogeneic bone marrow transplantation using perforin and/or Fas ligand double-defective CD4(+) donor T cells: involvement of cytotoxic function by donor lymphocytes prior to graft-versus-host disease pathogenesis. *Blood*. 2001; 98:390–397. [PubMed: 11435308]
46. Chavez-Galan L, Arenas-Del Angel MC, Zenteno E, Chavez R, Lascurain R. Cell death mechanisms induced by cytotoxic lymphocytes. *Cellular & molecular immunology*. 2009; 6:15–25. [PubMed: 19254476]
47. Mateo V, et al. Perforin-dependent apoptosis functionally compensates Fas deficiency in activation-induced cell death of human T lymphocytes. *Blood*. 2007; 110:4285–4292. [PubMed: 17724145]
48. Law RH, et al. The structural basis for membrane binding and pore formation by lymphocyte perforin. *Nature*. 2010; 468:447–451. [PubMed: 21037563]
49. Lopez JA, Brennan AJ, Whisstock JC, Voskoboinik I, Trapani JA. Protecting a serial killer: pathways for perforin trafficking and self-defence ensure sequential target cell death. *Trends in immunology*. 2012; 33:406–412. [PubMed: 22608996]
50. Ewen CL, Kane KP, Bleackley RC. A quarter century of granzymes. *Cell death and differentiation*. 2012; 19:28–35. [PubMed: 22052191]
51. Hartmann N, et al. Recombinant CD95-Fc (APG101) prevents graft-versus-host disease in mice without disabling antitumor cytotoxicity and T-cell functions. *Blood*. 2013; 121:556–565. [PubMed: 23203823]
52. Schmaltz C, et al. Differential use of Fas ligand and perforin cytotoxic pathways by donor T cells in graft-versus-host disease and graft-versus-leukemia effect. *Blood*. 2001; 97:2886–2895. [PubMed: 11313285]
53. Hsieh MH, Korngold R. Differential use of FasL- and perforin-mediated cytolytic mechanisms by T-cell subsets involved in graft-versus-myeloid leukemia responses. *Blood*. 2000; 96:1047–1055. [PubMed: 10910921]
54. Bian G, et al. Granzyme B-mediated damage of CD8+ T cells impairs graft-versus-tumor effect. *Journal of immunology*. 2013; 190:1341–1350.
55. Graubert TA, Russell JH, Ley TJ. The role of granzyme B in murine models of acute graft-versus-host disease and graft rejection. *Blood*. 1996; 87:1232–1237. [PubMed: 8608209]
56. Blazar BR, Taylor PA, Vallera DA. CD4+ and CD8+ T cells each can utilize a perforin-dependent pathway to mediate lethal graft-versus-host disease in major histocompatibility complex-disparate recipients. *Transplantation*. 1997; 64:571–576. [PubMed: 9293867]
57. Graubert TA, DiPersio JF, Russell JH, Ley TJ. Perforin/granzyme-dependent and independent mechanisms are both important for the development of graft-versus-host disease after murine bone

- marrow transplantation. *The Journal of clinical investigation*. 1997; 100:904–911. [PubMed: 9259590]
58. Baker MB, Altman NH, Podack ER, Levy RB. The role of cell-mediated cytotoxicity in acute GVHD after MHC-matched allogeneic bone marrow transplantation in mice. *The Journal of experimental medicine*. 1996; 183:2645–2656. [PubMed: 8676085]
 59. Lavrik I, Golks A, Krammer PH. Death receptor signaling. *Journal of cell science*. 2005; 118:265–267. [PubMed: 15654015]
 60. Schleich K, Krammer PH, Lavrik IN. The chains of death: a new view on caspase-8 activation at the DISC. *Cell cycle*. 2013; 12:193–194. [PubMed: 23287476]
 61. Takeda K, Stagg J, Yagita H, Okumura K, Smyth MJ. Targeting death-inducing receptors in cancer therapy. *Oncogene*. 2007; 26:3745–3757. [PubMed: 17530027]
 62. Smyth MJ, Takeda K, Hayakawa Y, Peschon JJ, van den Brink MR, Yagita H. Nature's TRAIL--on a path to cancer immunotherapy. *Immunity*. 2003; 18:1–6. [PubMed: 12530970]
 63. Hallett WH, et al. Sensitization of tumor cells to NK cell-mediated killing by proteasome inhibition. *Journal of immunology*. 2008; 180:163–170.
 64. Sayers TJ, et al. The proteasome inhibitor PS-341 sensitizes neoplastic cells to TRAIL-mediated apoptosis by reducing levels of c-FLIP. *Blood*. 2003; 102:303–310. [PubMed: 12637321]
 65. Schmaltz C, et al. T cells require TRAIL for optimal graft-versus-tumor activity. *Nat Med*. 2002; 8:1433–1437. [PubMed: 12426560]
 66. Kelley SK, et al. Preclinical studies to predict the disposition of Apo2L/tumor necrosis factor-related apoptosis-inducing ligand in humans: characterization of in vivo efficacy, pharmacokinetics, and safety. *J Pharmacol Exp Ther*. 2001; 299:31–38. [PubMed: 11561060]
 67. Carlo-Stella C, et al. Antitumor activity of human CD34+ cells expressing membrane-bound tumor necrosis factor-related apoptosis-inducing ligand. *Hum Gene Ther*. 2006; 17:1225–1240. [PubMed: 17107337]
 68. Walczak H, et al. Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. *Nat Med*. 1999; 5:157–163. [PubMed: 9930862]
 69. Ghosh A, et al. Adoptively transferred TRAIL+ T cells suppress GVHD and augment antitumor activity. *The Journal of clinical investigation*. 2013; 123:2654–2662. [PubMed: 23676461]
 70. Serody JS, Hill GR. The IL-17 differentiation pathway and its role in transplant outcome. *Biology of blood and marrow transplantation: journal of the American Society for Blood and Marrow Transplantation*. 2012; 18:S56–61. [PubMed: 22226114]
 71. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell*. 2000; 100:655–669. [PubMed: 10761931]
 72. Das J, Chen CH, Yang L, Cohn L, Ray P, Ray A. A critical role for NF-kappa B in GATA3 expression and Th2 differentiation in allergic airway inflammation. *Nature immunology*. 2001; 2:45–50. [PubMed: 11135577]
 73. Ivanov, et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell*. 2006; 126:1121–1133. [PubMed: 16990136]
 74. Murphy WJ, et al. Differential effects of the absence of interferon-gamma and IL-4 in acute graft-versus-host disease after allogeneic bone marrow transplantation in mice. *The Journal of clinical investigation*. 1998; 102:1742–1748. [PubMed: 9802888]
 75. Yang YG, Dey BR, Sergio JJ, Pearson DA, Sykes M. Donor-derived interferon gamma is required for inhibition of acute graft-versus-host disease by interleukin 12. *The Journal of clinical investigation*. 1998; 102:2126–2135. [PubMed: 9854048]
 76. Ushiyama C, Hirano T, Miyajima H, Okumura K, Ovary Z, Hashimoto H. Anti-IL-4 antibody prevents graft-versus-host disease in mice after bone marrow transplantation. The IgE allotype is an important marker of graft-versus-host disease. *Journal of immunology*. 1995; 154:2687–2696.
 77. Kappel LW, et al. IL-17 contributes to CD4-mediated graft-versus-host disease. *Blood*. 2009; 113:945–952. [PubMed: 18931341]
 78. Yi T, et al. Reciprocal differentiation and tissue-specific pathogenesis of Th1, Th2, and Th17 cells in graft-versus-host disease. *Blood*. 2009; 114:3101–3112. [PubMed: 19602708]

79. Kelly KF, Daniel JM. POZ for effect--POZ-ZF transcription factors in cancer and development. *Trends Cell Biol.* 2006; 16:578–587. [PubMed: 16996269]
80. Kovalovsky D, et al. The BTB-zinc finger transcriptional regulator PLZF controls the development of invariant natural killer T cell effector functions. *Nat Immunol.* 2008; 9:1055–1064. [PubMed: 18660811]
81. Savage AK, et al. The transcription factor PLZF directs the effector program of the NKT cell lineage. *Immunity.* 2008; 29:391–403. [PubMed: 18703361]
82. Xu D, et al. Promyelocytic leukemia zinc finger protein regulates interferon-mediated innate immunity. *Immunity.* 2009; 30:802–816. [PubMed: 19523849]
83. Raberger J, et al. The transcriptional regulator PLZF induces the development of CD44 high memory phenotype T cells. *Proceedings of the National Academy of Sciences of the United States of America.* 2008; 105:17919–17924. [PubMed: 19004789]
84. Kovalovsky D, Alonzo ES, Uche OU, Eidson M, Nichols KE, Sant'Angelo DB. PLZF induces the spontaneous acquisition of memory/effector functions in T cells independently of NKT cell-related signals. *J Immunol.* 2010; 184:6746–6755. [PubMed: 20495068]
85. Ghosh A, et al. PLZF Confers Effector Functions to Donor T Cells That Preserve Graft-versus-Tumor Effects while Attenuating GVHD. *Cancer research.* 2013; 73:4687–4696. [PubMed: 23733752]
86. Vacchelli E, et al. Trial Watch: Adoptive cell transfer for anticancer immunotherapy. *Oncoimmunology.* 2:e24238. [PubMed: 23762803]
87. Stauss HJ, Morris EC. Immunotherapy with gene-modified T cells: limiting side effects provides new challenges. *Gene Ther.*
88. Radtke F, et al. Deficient T cell fate specification in mice with an induced inactivation of Notch1. *Immunity.* 1999; 10:547–558. [PubMed: 10367900]
89. Wilson A, MacDonald HR, Radtke F. Notch 1-deficient common lymphoid precursors adopt a B cell fate in the thymus. *J Exp Med.* 2001; 194:1003–1012. [PubMed: 11581321]
90. Schmitt TM, Zuniga-Pflucker JC. Induction of T cell development from hematopoietic progenitor cells by delta-like-1 in vitro. *Immunity.* 2002; 17:749–756. [PubMed: 12479821]
91. Schmitt TM, de Pooter RF, Gronski MA, Cho SK, Ohashi PS, Zuniga-Pflucker JC. Induction of T cell development and establishment of T cell competence from embryonic stem cells differentiated in vitro. *Nat Immunol.* 2004; 5:410–417. [PubMed: 15034575]
92. Ohishi K, Varnum-Finney B, Bernstein ID. Delta-1 enhances marrow and thymus repopulating ability of human CD34(+)CD38(-) cord blood cells. *J Clin Invest.* 2002; 110:1165–1174. [PubMed: 12393852]
93. Mohtashami M, Shah DK, Nakase H, Kianizad K, Petrie HT, Zuniga-Pflucker JC. Direct comparison of Dll1- and Dll4-mediated Notch activation levels shows differential lymphomyeloid lineage commitment outcomes. *J Immunol.* 185:867–876. [PubMed: 20548034]
94. Dervovic DD, Ciofani M, Kianizad K, Zuniga-Pflucker JC. Comparative and functional evaluation of in vitro generated to ex vivo CD8 T cells. *J Immunol.* 189:3411–3420. [PubMed: 22925927]
95. Awong G, Herer E, La Motte-Mohs RN, Zuniga-Pflucker JC. Human CD8 T cells generated in vitro from hematopoietic stem cells are functionally mature. *BMC Immunol.* 12:22. [PubMed: 21429219]
96. Quezada SA, et al. Tumor-reactive CD4(+) T cells develop cytotoxic activity and eradicate large established melanoma after transfer into lymphopenic hosts. *J Exp Med.* 207:637–650. [PubMed: 20156971]
97. Zakrzewski JL, et al. Adoptive transfer of T-cell precursors enhances T-cell reconstitution after allogeneic hematopoietic stem cell transplantation. *Nat Med.* 2006; 12:1039–1047. [PubMed: 16936725]
98. Zakrzewski JL, et al. Tumor immunotherapy across MHC barriers using allogeneic T-cell precursors. *Nat Biotechnol.* 2008; 26:453–461. [PubMed: 18376399]
99. Holland AM, et al. Extrathymic development of murine T cells after bone marrow transplantation. *J Clin Invest.* 122:4716–4726. [PubMed: 23160195]