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Graft engineering comes of age in allogeneic hematopoietic cell transplant

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Abstract

Graft-versus-host disease (GVHD) has been a barrier to safely conducting allogeneic hematopoietic cell transplant (HCT). Immunosuppressive therapies (IST) mitigate the risk of GVHD, but these pharmacologic agents can cause significant toxicities and breakthrough severe GVHD may still occur. Graft engineering has emerged as an alternate GVHD prevention strategy, reducing the need for ISTs and potentially decreasing toxicity. Early approaches to graft engineering uniformly focused on broad T cell depletion. Since then, refined understanding of transplant immunology has resulted in more rational graft designs. Meanwhile, advances in cell selection, sorting, depletion, and expansion have made precision engineered grafts a reality. In this review, we summarize established graft engineering strategies for the prevention of GVHD and highlight promising approaches under active investigation. Modern graft engineering has immense potential, offering an exciting new path to eradicating GVHD, reducing non-relapse mortality, and potentially transforming the field of allogeneic HCT.

Introduction

The curative potential of allogeneic hematopoietic cell transplant (HCT) is well recognized. However, the therapeutic benefits of HCT are limited by potential complications including post-transplant relapse, graft-versus-host disease (GVHD), infection, and graft rejection. Progress has been challenging as strategies to improve one outcome may unavoidably worsen another. The clearest example is GVHD prevention, which traditionally requires immunosuppressive therapies (IST) that inherently lead to a higher risk of disease relapse and infection by diminishing graft-versus-tumor (GVT) and impairing post-HCT immune reconstitution.(1) The holy grail of HCT involves decoupling GVHD from GVT while preserving immune reconstitution.

Historically, allogeneic HCT has required pharmacologic IST to prevent GVHD. The traditional calcineurin inhibitor (CNI)-based regimens have remained in use for decades. At many centers, CNI-based GVHD prophylactic regimens have been supplanted by post-transplant cyclophosphamide (PTCy), which has demonstrated superior GVHD prevention and GVHD-free, relapse free survival in prospective, phase III trials.(2, 3) Meanwhile, CNI-based regimens continue to evolve through incorporation of effective agents such as anti-thymocyte globulin (ATG), abatacept, or ruxolitinib.(4-6) Despite significant improvements in GVHD incidence, these pharmacologic agents result in reduced post-HCT T cell diversity and are marred by notable toxicities such as infections, renal injury, cardiac toxicity, hemorrhagic cystitis, and potentially secondary malignancies.(7)

Alternatively, graft engineering, in which the allogeneic donor cells are modified ex vivo, can mimic the benefits of pharmacologic IST while theoretically limiting associated toxicities. A more detailed understanding of transplant immunology has allowed for manipulation of the immune system beyond pharmacologic immune suppression, instead targeting clinically detrimental immune cell populations while sparing or enhancing beneficial populations. Advances in selective cell depletion, sorting, and expansion have led to precision engineered grafts that are turning the preclinical promise of graft engineering into a clinical reality.

Principles of Graft Engineering

Hematopoietic cell populations can be identified by characteristic surface antigen expression. Graft engineering leverages techniques that target these antigens to sort cells and create a desired graft composition (Figure 1). Early strategies were developed in the 1980s with a focus on *ex vivo* T cell depletion (TCD) using soybean agglutination and sheep red blood cell resetting.(8, 9) This practice evolved with the advent of targeted monoclonal antibodies that function through complement-mediated killing.(10, 11) While the remnant surviving cell population remains intact for clinical use, a drawback of this approach is that the depleted target cells are no longer viable, eliminating the potential for controlled partial depletion or adding depleted cells back to the graft.

Immunomagnetic selection marked a significant breakthrough in graft engineering. This approach uses monoclonal antibody-coated magnetic microbeads to bind specific cell populations, which are then isolated by passage through a magnetic column.(12, 13)

This physical separation preserves the viability of bound and unbound cells, allowing for both positive and negative selection. Immunomagnetic selection also enables selective targeting of T cell subsets rather than broad TCD. By separating grafts into viable component parts, immunomagnetic selection opened the possibility of precision engineered grafts with prespecified doses of isolated cell populations.(14)

Because immunomagnetic sorting generally targets one antigen at a time, effective isolation of multiple graft components requires sequential selection. Fluorescence-activated cell sorting (FACS) has helped overcome this limitation by using multiple parameters to identify and separate phenotypically distinct cell populations in parallel, thus enabling rapid and precise generation of defined cell products.(15-17)

Donor Graft Cell Populations

The fundamental concept of graft engineering relies on understanding the function of individual immune cell populations to rationally design a cellular “recipe” to optimize transplant outcomes. The major components of the allogeneic HCT graft include hematopoietic stem cells (HSCs), T cells, B cells, and natural killer (NK) cells, each comprising functionally distinct subpopulations as described below (Figure 2).

Hematopoietic Stem Cells

Hematopoietic stem and progenitor cells (HSPCs) are bone marrow progenitor cells essential for engraftment and subsequent hematopoiesis.(18) HSPCs express CD34, which can be used as a marker for cell selection.(19, 20) The donor CD34+ cell dose is routinely used to assess graft adequacy with $2x10^6$ CD34+ cells/kg generally

considered to be the minimum acceptable dose, although higher doses have been associated with improved engraftment and survival.(21-23)

T Cells

T cells are necessary to facilitate engraftment and mediate alloreactivity against host tissues and malignant cells, contributing to both GVT and GVHD.(24, 25) Donor T cells, identified by CD3 expression, comprise diverse populations with distinct roles after allogeneic HCT. Beyond the major $CD4^+$ helper and $CD8^+$ cytotoxic T-cell populations, differences in T cell receptor (TCR, αβ versus γδ), differentiation state (naïve versus memory), and regulatory function (Tregs) influence their contributions to immune reconstitution, GVT, and GVHD as follows:

- αβ T cells comprise the majority of mature donor T cells in the donor graft and recognize HLA presented antigens through the αβ TCR.(26, 27) Their HLA-dependent alloreactivity contributes to both GVT and GVHD, while their distinct TCR provides a target for selective depletion from the graft.(28)
- γδ T cells are part of the innate immune system. They lack CD4 and CD8 co-receptors, are not alloreactive, and do not cause GVHD.(29) Instead, they provide antitumor immunity through recognition of stress induced ligands such as major histocompatibility complex class I related chain A and B (MICA/B), NKGD2 ligands, and toll-like receptor activation. Their limited alloreactivity allows them to provide anti-tumor immunity without substantially contributing to GVHD, making them a useful cell population in selectively engineered grafts.(29)

- Naïve T cells migrate from the bone marrow to the thymus and mature, but have not yet encountered cognate antigens.(30) This naïve state is characterized by CD45RA expression.(31) At this phase of development, naïve T cells express broad TCR diversity and are easily activated by a wide array of antigen stimuli, which contributes heavily to GVHD.(32) Thus, selective depletion of naïve T cells has been an important graft engineering strategy.(33)
- Memory T cells (Tmems) arise when naitve T cell interact with their cognate antigen and develop into Tmems. This progression leads to loss of CD45RA expression, upregulation of CD45RO, and loss of TCR diversity. As such, Tmems react briskly when exposed to the cognate antigen. Compared with naïve T cells, Tmems have lower alloreactive potential while retaining infectious and tumor-specific immunity.(34, 35) Tmems comprise central memory (Tcm), effector memory (Tem), and terminally differentiated effector memory (TEMRA) populations with distinct proliferative and effector functions.(36)
- Regulatory T cells suppress immune activation and, in allogeneic HCT, limit alloreactivity and GVHD.(37-39) Their regulatory function is controlled by the transcription factor FOXP3, and Tregs can be identified by a surface expression CD4+CD25+CD127lo phenotype.(40, 41) Preservation or enrichment of Tregs while maintaining conventional T cell immunity has become as an important strategy in graft engineering, as discussed below.

B Cells

Allogeneic grafts contain naïve and memory B cells that contribute to the gradual recovery of humoral immunity after allogeneic HCT, including antimicrobial and vaccine

responses.(42-44) However, donor-derived B cells can contribute to chronic GVHD and antibody-mediated complications such as autoimmune hemolytic anemia.(45-47) Uniform expression of CD19 provides a targetable surface marker for selective B cell manipulation during graft engineering.(48)

Natural Killer Cells

NK cells are innate lymphocytes that reconstitute rapidly after allogeneic HCT and provide both antitumor and antiviral immunity. (49, 50) NK cells are activated through a balance of inhibitory and activating ligands receptors including killer cell immunoglobulin like receptor ligands (KIR) and NKG2D, which trigger cytotoxicity through the perforin/granzyme release and cytokine production.(51, 52) Because NK cell responses are largely independent of antigen-specific HLA recognition, NK cells can theoretically provide GVT activity with limited potential to cause GVHD.(53, 54)

Clinical Graft Engineering

T Cell Depletion

Early clinical graft engineering strategies focused on broad TCD, using negative selection to remove $CD3^+$ T cells while preserving other graft components (Figure 3). Initial clinical trials in HLA matched donors demonstrated low rates of GVHD (~10-20%), but this benefit was offset by high rates of graft failure, disease relapse, and infection.(55-58) A phase III randomized trial was conducted that compared TCD grafts to calcineurin-inhibitor (CNI) based prophylaxis in patients receiving myeloablative

conditioning (MAC) HLA matched HCT. Although TCD resulted in lower rates of grade III-IV acute (aGVHD, 18% versus 37%, p<0.01), a higher incidence of relapse and infection were noted, leading to no improvement in survival outcomes.(59)

The low incidence of GVHD observed with TCD enabled the extension of this concept to haploidentical HCT. Aversa et al. were able to show excellent engraftment without acute or chronic GVHD in 43 recipients of TCD haploidentical grafts, although infection contributed to an exceedingly high non-relapse mortality (NRM) of 40%.(60) These early studies established the potential of graft engineering, but also highlighted the need to retain beneficial donor immunity in the engineered graft.

CD34+ Selection

Like TCD grafts, CD34+ selection achieves substantial T cell depletion, but through positive selection of CD34+ HSPCs.(61) Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 0303 demonstrated that CD34+ selected grafts were associated with lower chronic GVHD (19% versus 50%) and higher 2-year GVHD-free survival (41% versus 19%) compared with T-cell replete grafts (Table 1).(61, 62) These findings led to BMT CTN 1301, a phase 3 randomized trial comparing CD34+ selected peripheral blood stem cell (PBSC) grafts without IST to T-cell replete bone marrow grafts with either tacrolimus/methotrexate or PTCy in patients receiving MAC HLA matched donor HCT.(63) While PBSC grafts generally confer a higher risk of chronic GVHD, CD34+ selected PBSC transplant resulted in less severe chronic GVHD than marrow grafts with tacrolimus/methotrexate. However, infection-related NRM was significantly higher, resulting in inferior OS (HR, 1.74; *P*=0.02).

Collectively, TCD and CD34+ selection showed that broad removal of donor T cells can effectively prevent GVHD. However, as donor T cells are critical in eliminating residual recipient T cells that can cause graft rejection, these TCD approaches can increase the risk of graft failure especially when combined with reduced intensity conditioning (RIC) regimens or HLA mismatched donors. Further, TCD leads to slower immune reconstitution, higher infection risk, and possibly higher NRM. These limitations provided the rationale for more selective graft engineering strategies designed to preserve GVT and infection control while preventing GVHD.

Selective Depletion Strategies

Selective depletion of specific immune cell populations represented the next advance in graft engineering. Approaches ranging from targeted depletion of individual cell subsets to increasingly sophisticated graft-manipulation platforms have enabled greater control over graft composition and function.

CD8 Depletion

As the cytotoxic effectors of the T cell compartment, selective $CD8^+$ T-cell depletion has been explored as a rational strategy to reduce GVHD while preserving $CD4^+$ T cells for immune reconstitution and GVT. In a prospective randomized trial of 38 patients receiving CD8+ depleted bone marrow versus standard bone marrow grafts, CD8+ depletion reduced rates of grade II-IV aGVHD to 20% compared to 80% ($p < 0.004$), but was associated with two cases of graft failure and no improvement in DFS or OS. (64) A subsequent study of partial CD8+ depletion in 41 patients demonstrated strikingly high

rates of both acute and chronic GVHD. (65) Thus, CD8+ depletion did not consistently achieve the desired effect across studies.

αβ depletion

αβ T-cell depletion selectively removes alloreactive αβ T cells while preserving γδ T cells and is often combined with $CD19^{+}$ depletion as retained B cells may contribute to tissue injury and post-transplant lymphoproliferative disorders with this strategy.(66, 67) Beyond solely eliminating αβ T cells, αβ depletion appears to result in more rapid post-transplant immune reconstitution of both γδ T cells and NK cells, thus mitigating infection risk compared to broad T cell depletion.(68, 69)

The clinical development of αβ depletion has been particularly successful in pediatric haploidentical HCT. Early studies demonstrated low rates of GVHD with and without pharmacologic IST, although graft rejection remained a concern.(28, 70) Building on these successes, a prospective trial in Italy treated 213 children with acute leukemias with MAC followed by an αβ/CD19+ B cell depleted haploidentical graft and no IST.(71) The incidence of grade II-IV acute and chronic GVHD were low at 15% and 8% respectively. Long-term NRM and relapse were 5.2% and 23%, respectively, yielding 5-year DFS of 75%. The authors concluded these results in haploidentical HCT were comparable to historical outcomes with HLA matched donors.

Experience with αβ depletion in adults has been relatively limited, with studies demonstrating feasibility and low rates of GVHD, but concerns regarding NRM, relapse, and infections.(69, 72) Thus, αβ T cell depletion remains an established pediatric graft engineering strategy that has improved the outcomes of haploidentical HCT in children.

Naïve T Cell Depletion

Naïve T cell depletion selectively removes CD45RA+ naïve T cells implicated in GVHD while preserving Tmems important for GVT and infection control. Because $CD34^+$ progenitor cells may also express CD45RA, this strategy involves a two-step process where the CD34+ cells are first positively selected and then the remaining CD34- fraction undergoes naïve T cell depletion using anti-CD45RA monoclonal antibody and immunomagnetic selection.

In the largest experience to date, Bleakley, et al. reported combined results from three phase II trials including 138 acute leukemia patients who received naïve T cell depleted HCT from HLA matched donors.(73) All patients received the same engineered graft with $\geq 5 \times 10^6$ CD34+ cells/kg, 10^7 CD3+ T cells/kg, and $\leq 7.5 \times 10^4$ naive T cells/kg. Grafts were confirmed to contain CD3+ CD45RA- CD45RO+ Tmems. Two trials included MAC and one was mixed MAC/RIC, and all trials used tacrolimus (two with methotrexate). Grade III–IV acute GVHD and moderate-severe chronic GVHD (cGVHD) occurred in only 4% and 1%, respectively. Three-year NRM was only 8%, while the incidence of relapse was 27%, and RFS was 69%. Engraftment and infectious outcomes were favorable. Limited studies have also evaluated naïve T cell depletion for haploidentical HCT and demonstrated low rates of aGVHD and infections with rapid post-transplant Tmem recovery.(74)

Thus, naïve T cell depletion shows promise as a strategy to reduce GVHD while preserving GVT and infection control. The favorable early outcomes have prompted a

randomized phase III clinical trial comparing naïve T cell depletion to PTCy (NCT03779854) that is currently underway.

Precision Immune Composition

The next evolution in graft engineering moves beyond selective depletion to creating precise immune composition. By independently isolating and recombining specific cell populations at defined doses, the allogeneic donor graft can be purposefully designed rather than simply depleted of unwanted cells. With modern advances, multiple cell populations can be selected, isolated, or depleted to create a customized graft.

Regulatory T Cell Grafts

The role of Tregs in suppressing autoimmune disease fostered the hypothesis that enhancing donor Tregs may reduce GVHD. Because Tregs are rare in circulation, clinical application requires strategies to enrich Tregs either through *ex vivo* expansion or cell sorting to control their ratio to conventional T cells (Tcon).

Expanded Treg Grafts

Early clinical studies explored expanded Tregs in umbilical cord blood transplant (UCBT). Investigators at the University of Minnesota devised a method to isolate Tregs from cryopreserved UCB grafts via anti-CD25 immunomagnetic positive selection followed by expansion through co-culturing with anti-CD3/anti-CD28 monoclonal antibody coated beads and interleukin-2 (IL-2). In a phase I trial, 23 patients were treated with UCBT supplemented by Tregs (0.1-30 x 10^5 Tregs/kg) expanded from a separate UCB graft.(75) Clinical outcomes were similar to historical cohorts with lower rates of grade II-IV aGVHD observed in patients receiving Treg expansion (43% versus

61%, p=0.05). A subsequent study in HLA matched sibling donors used a slightly modified technique but also demonstrated that expanded Tregs ($4x10^5$ Tregs/kg) were feasible and resulted in low rates of acute and chronic GVHD.(76)

Treg Selected Grafts

At the same time, Treg selection using CD25+ immunomagnetic bead selection was being tested in haploidentical HCT in Italy. In an early study, Pierini et al. reported outcomes among 50 patients treated with CD25+ selected Tregs ($2x10^6$/kg) infused on day -4 followed by Tcons on day -1 and CD34+ stem cells on day 0 with no pharmacologic IST.(77) While cGVHD was infrequent, 15 patients developed aGVHD and NRM was high at 21% including three due to GVHD.

Recognizing that CD25+ bead selection may result in dose inconsistencies, the group from Stanford University developed a high purity method of selecting CD4+CD25+CD127low Tregs using immunomagnetic selection and high-speed flow cytometric sorting.(78, 79) They further identified that the ratio of these Tregs to conventional T cells (Tcons) contributed to potential GVHD prevention benefits.(79) The technique was evaluated in a phase I dose-finding trial that confirmed a preferred dosing regimen of $>2x10^6$ CD34+ cells/kg and $2\text{-}3x10^6$ Tregs/kg on day 0 followed by $3x10^6$ Tcons/kg on day +2 with single agent tacrolimus.(80)

Meyer, et al. reported results of a single center phase II trial of this Treg graft with tacrolimus in 44 patients with hematologic malignancies receiving MAC and predominantly HLA matched donors.(81) The study met its pre-specified endpoint, with a 1-year GVHD-free relapse free survival (GRFS) of 64% prompting pharmaceutical industry interest in this approach.

Orca Bio Grafts

The favorable outcomes with the Stanford Treg engineered graft provided the foundation for the development of Orca-T (Orca Bio, Sacramento, CA). Orca-T similarly separates the donor graft into three defined components (HSPCs, high-purity Tregs, and Tcons), delivered in the same sequence and in a similar ratio as the Stanford Treg graft. Mobilized donor cells are shipped to Orca Bio for centralized manufacturing and then distributed to the treating center for infusion.

On June 30, 2026, Orca-T obtained US FDA approval for adults with hematologic malignancies as an engineered cellular therapy from HLA matched donors to be administered after MAC. This approval came on the heels of the positive Precision-T trial, a multicenter randomized phase 3 trial comparing Orca-T to unmanipulated PBSC grafts with tacrolimus/methotrexate.(82) The primary endpoint of 1-year cGVHD-free survival (cGFS) was significantly higher in the Orca-T arm (78% versus 38%, $p<0.01$), attributed to lower moderate-to-severe cGVHD (12.6% versus 44%, $p<0.01$) and exceptionally low NRM (3.4% versus 13.2%, $p=0.03$). While OS did not significantly differ between the arms (94% versus 83%), the trend favored Orca-T. Importantly, the incidence of infections was not higher among patients treated with Orca-T, signifying an important step forward in engineered grafts. Preliminary correlative analyses from Precision-T demonstrated distinct T-cell reconstitution with Orca-T compared with tacrolimus/methotrexate, characterized by greater relative Treg representation and early expansion of effector memory T cells that persisted beyond 6 months.(83) Notably, to complete transit and manufacturing within the 72-hour vein-to-vein time required, the trial was limited to centers in the United States and donor collections in North America.

For commercial use, Orca-T availability will therefore be limited to regions with suitable Orca manufacturing sites and infrastructure.

Ongoing trials are evaluating new indications for Orca-T including Serene-T, which is testing Orca-T with various RIC regimens (NCT07216443). Orca Bio has also developed a second-generation product, Orca-Q, that similarly includes Treg enrichment, but also incorporates proprietary Tcon modifications. Orca-Q is currently in trials using HLA matched, mismatched, and haploidentical donors (NCT03802695).

Emerging Platforms and Future Directions

Graft engineering has historically focused on preventing GVHD. The next frontier is engineering donor immunity not only to prevent GVHD and maintain GVT, but to enhance GVT to reduce post-HCT relapse.

Natural Killer Augmentation

NK cells contribute to GVT and infection control but are both quantitatively reduced and functionally impaired in the early post-transplant period.(49, 84-87) Graft engineering methods therefore aim to isolate and expand donor NK cells, often for post-transplant infusion.(88) In a phase 1/2 trial of haploidentical HCT with PTCy, infusion of donor NK cells that were expanded using membrane-bound IL-21 resulted in robust NK-cell recovery and a reduction in relapse from 38% to 4% (P=0.014).(88) An alternative approach used IL-12, IL-15, and IL-18 to induce a memory like phenotype with enhanced function and persistence. In 15 patients with active AML, infusion of these cells on day +7 post-haploidentical HCT resulted in an 87% CR rate and detectable

cells beyond 2 months, although most patients ultimately relapsed.(89) Together, these studies demonstrate the feasibility of engineering NK cells to augment post-transplant GVT.

γδ Augmentation

αβ T cell depletion preserves γδ T cells, which reconstitute rapidly and may contribute to GVT; however, their low number limits positive selection for clinical use. It has been observed that *in vivo* administration of zoledronic acid can promote γδ T cell recovery after αβ T cell depleted haploidentical HCT, while *ex vivo* culture with zoledronic acid, IL-2, and artificial antigen-presenting cells achieved >600-fold expansion.(90) A phase I trial is evaluating haploidentical HCT with PTCy followed by expanded, donor-derived γδ T cells (NCT05015426).(91) Preliminary results suggest no significant toxicity, with 10/12 (83%) patients remaining in minimal residual disease (MRD) negative remission at one year.

Targeted Cellular Therapies

Chimeric antigen receptor (CAR) T cells have transformed the treatment of relapsed/refractory lymphoid malignancies, and incorporation of donor-derived CAR T cells into the allogeneic graft represents a natural extension of graft engineering. A phase 1 trial in adults with high-risk B-cell acute lymphoblastic leukemia incorporated donor-derived CD19/CD22 CAR T cells into the Orca-T graft, demonstrating feasibility and low rates of immune effector cell toxicity and GVHD.(92) At >1.5 years of follow-up, EFS and OS were both 100%, with donor CAR T cells demonstrating robust expansion

and prolonged persistence relative to the same CAR platform in the autologous setting.(93)

Unlike CAR T cells which target surface antigens, engineered T cell receptors (TCR) can target intracellular peptides presented by specific HLA molecules. This HLA restriction presents an opportunity to selectively target recipient hematopoietic cells while sparing an HLA mismatched graft that lacks the targeted HLA allele.(94) An ongoing multicenter trial is evaluating this concept in haploidentical and mismatched unrelated donor (MMUD) HCT using engineered TCRs that target the minor histocompatibility antigens HA-1 and HA-2 when presented on HLA A 02:01 recipients (NCT07702578).

Current Challenges

The incredible progress in graft engineering is met with a number of ongoing challenges. While precision engineered grafts results in low rates of GVHD, infection, and NRM, the emergence of PTCy as an economical, uncomplicated, and effective GVHD prevention strategy highlights the need for direct, prospective comparisons. Confirmatory studies of engineered graft techniques using HLA mismatched and haploidentical donors are warranted to improve the access and reach of these therapies. Rigorous cost-effectiveness analyses will be necessary to understand and support the higher up-front cost of graft engineering, and to extend these strategies beyond the United States market. Finally, comparative correlative analyses will help to elucidate biological mechanisms associated with clinical outcomes and are critical to discovering the next generation of graft engineering to promote GVT. Addressing these

challenges are critical in order to continue to accelerate the field and for mainstream adoption of this sophisticated technology.

Conclusions

The promise of graft engineering is increasingly being realized with precision platforms that efficiently and effectively reduce GVHD while preserving GVT and donor immunity. The field has evolved immensely from early pan-T cell depletion towards rationally designed grafts with defined immune composition and function. The next frontier will extend beyond GVHD prevention to focus on GVT, potentially through integration of engineered grafts with adoptive cellular therapies. Expanding these approaches across donor types and into lower-risk malignancies and nonmalignant disorders is also of great interest. Prospective comparisons to contemporary pharmacologic regimens, such as PTCy, are lacking; such trials are necessary to confirm clinical benefit and justify the added costs associated with graft engineering. Ultimately, precision graft engineering offers the possibility of redesigning allogeneic HCT to enhance its therapeutic potential while minimizing its defining complication: GVHD.

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Tables

Study	Phase	Population	GVHD Prophylaxis Strategy	N	Donor Type	Grade III-IV aGVHD	Chronic GVHD	NRM	Relapse	DFS	OS
Devine et al. Blood 2011 (BMT CTN 0303)	II	Adults, AML	CD34-selected graft	44	MSD/MUD	5%	19%	14%	24%	53% (3-y DFS)	56% (3-y)
Luznik et al. JCO 2022 (BMT CTN 1301)	III	Adults, AML/MDS/ALL	CD34-selected graft	114	MSD/MUD	3%	9%	22%	21%	57% (2-y)	60% (2-y)
			PTCy	114	MSD/MUD	10%	27%	16%	14%	70%	76%
			CNI/Methotrexate	118	MSD/MUD	4%	34%	8%	26%	67%	76%
Merli et al. Blood 2024	I/II	Pediatric, Acute Leukemia	TCRαβ/CD19-depletion	213	Haploidentical	NR	8%	5%	23%	72% (10-y)	75% (10-y)
de Witte et al. Blood Adv. 2021	II	Adults, Heme malignancies	TCRαβ/CD19-depletion	35	Haploidentical	17%	23%	32%	29%	40% (2-y)	54% (2-y)
Bleakley et al. JCO 2022	II	Adults, AML/MDS/ALL	Naïve T-cell depletion (CD45RA depletion)	138	MSD/MUD	4%	7%	8%	23%	69% (3-y)	77% (3-y)
Meyer et al. Blood 2026 (PRECISION-T)	III	Adults, AML/MDS/ALL	Orca-T	93	MSD/MUD	6%	13%	3%	21%	75% (1-y)	94% (1-y)
			Tac/MTX	94	MSD/MUD	17%	44%	13%	13%	74% (1-y)	83% (1-y)

Table 1: Summary of results of landmark graft engineering clinical trials.

Abbreviations: acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphoblastic leukemia (ALL), matched sibling donor (MSD), matched unrelated donor (MUD), tacrolimus (Tac), methotrexate (MTX)

Figure Legends

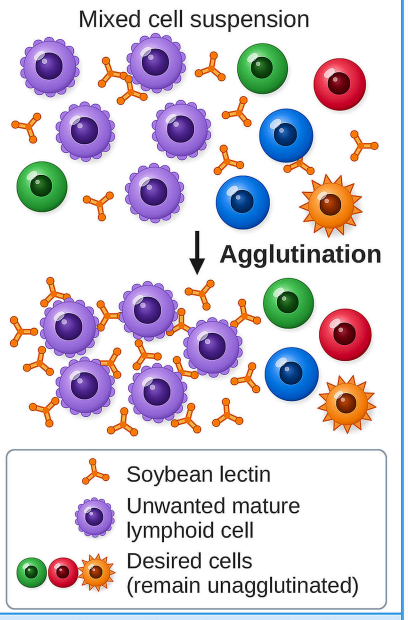
Figure 1: Summary of technical approaches to cell selection

Figure 2: Allogeneic hematopoietic cell graft composition and function of different cell types

Figure 3: Overview of engineered graft strategies used in the clinical setting

Major cell separation approaches in graft engineering

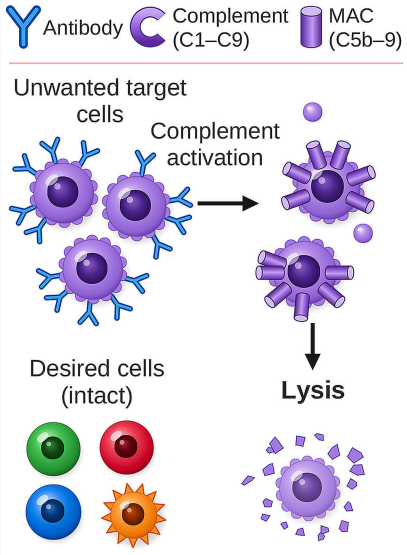
1 Soybean agglutination



Lectin-mediated agglutination enables physical separation of selected cells.

Historical bulk separation method

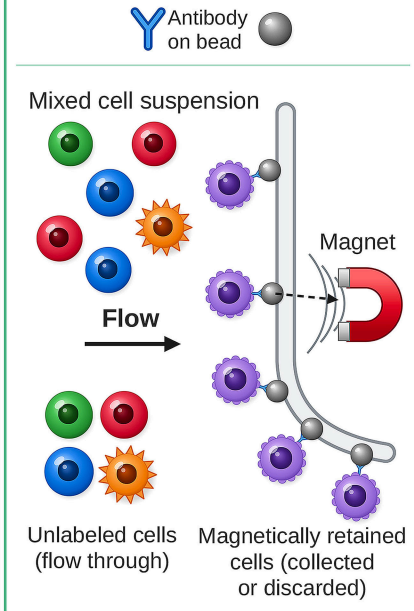
2 Antibody depletion / complement killing



Antibody-coated unwanted cells are eliminated by complement-mediated lysis.

Selective depletion

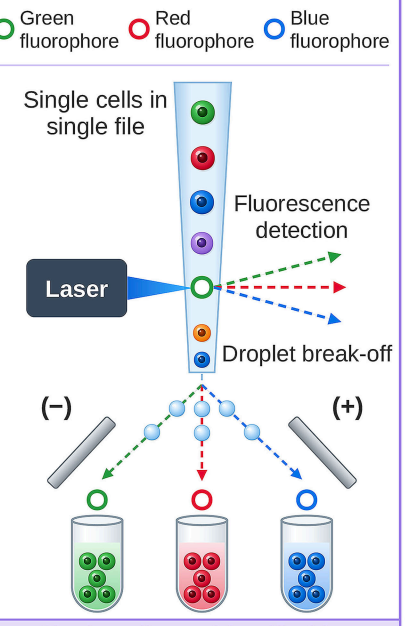
3 Immunomagnetic selection



Magnet bead-labeled cells are retained or removed using a magnetic field.

Clinical-scale GMP platform

4 FACS sorting



Single cells are identified by fluorescence and sorted into collection streams.

High-dimensional multiparameter purification

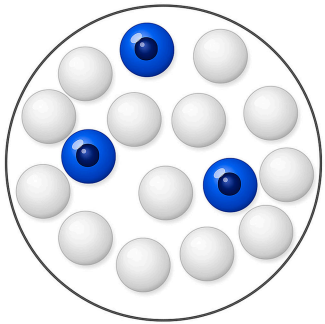
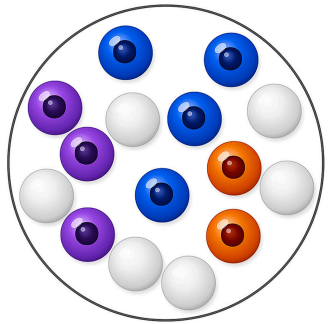
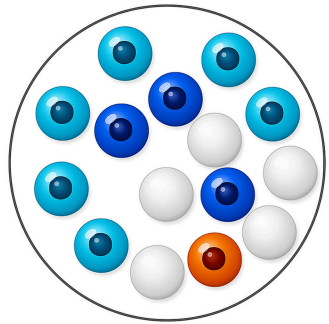
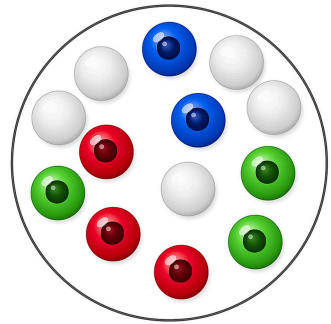
	1 Soybean agglutination	2 Antibody depletion / complement killing	3 Immunomagnetic selection	4 FACS sorting
Specificity	Low specificity	Moderate specificity	High specificity	Very high specificity
Scalability	Moderate scalability	Moderate scalability	High scalability	Lower scalability
Multiparameter capability	Low	Low	Low-to-moderate	Very high
Historical / modern use	Historical	Historical to limited modern use	Modern standard	Emerging clinical use

Cell type	Engraftment	Graft-versus-tumor (GVT)	Immune reconstitution (anti-infective immunity)	Graft-versus-host disease (GVHD)
CD34⁺ stem cells	↑↑↑	—	—	—
Conventional αβ T cells (CD4/CD8)	↑	↑↑↑	↑↑↑	↑↑↑
Regulatory T cells (Tregs)	↑	↑	↑	↓↓↓
γδ T cells	—	↑↑	↑↑	↓
Natural killer cells (NK)	—	↑↑	↑↑	↓
Naïve T cells (CD45RA⁺)	—	↑↑	↑	↑↑↑
Memory T cells (CD45RO⁺)	—	↑↑	↑↑↑	↑
B cells	—	↑	↑↑	↑↑

↑↑↑ = strong increase
↑↑ = moderate increase
↑ = slight increase / limited role

— = minimal / none

↓ = slight decrease
↓↓ = moderate decrease
↓↓↓ = strong decrease

	CD34⁺ SELECTION (Broad depletion)	αβ T-CELL DEPLETION (Selective depletion)	NAÏVE T-CELL DEPLETION (CD45RA⁺ depletion)	PRECISION IMMUNE ENGINEERING (Orca-T)
	 = CD34⁺ stem cell	 = CD34⁺ stem cell = γδ T cell = NK cell	 = CD34⁺ stem cell = Memory T cell = NK cell	 = CD34⁺ stem cell = Treg = Conventional T cell (Tcon)
Manipulation	Broad immune cell depletion	Remove αβ T cells	Remove CD45RA⁺ naïve T cells	Add defined cell populations
Primary cells in graft	CD34⁺ stem cells	CD34⁺ + γδ T cells + NK cells ± others	CD34⁺ + memory T cells ± other subsets	CD34⁺ + Tregs + conventional T cells
Key advantage	Lowest GVHD	Fast immune recovery with low GVHD	Preserves antiviral and immune memory	Balanced GVHD prevention and immunity
Major limitation	Delayed immune reconstitution	Complex manufacturing; residual alloreactivity	Limited clinical data; possible GVHD	Manufacturing complexity and cost
Clinical experience	High (decades)	High	Emerging	Emerging