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The composition and function of peripheral blood stem cell grafts and the impact on clinical outcome

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Cover illustration: Peripheral blood stem cell graft, cells derived from the graft – Tengyu Wang 2026

The composition and function of peripheral blood stem cell grafts and the impact on clinical outcome

Thesis for Doctoral Degree (Ph.D.)

By

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Life is like a bag of stem cells,

you never know what you're gonna get.

Populärvetenskaplig sammanfattning

En stamcellstransplantation innebär att man tar en frisk donators benmärgsstamceller och överför dem till en sjuk patient. På detta sätt kan man bota sjukdomar genom att ersätta sjuka celler med friska donatorsceller. Hur går detta till i praktiken och vilka utmaningar finns det för att genomföra en lyckad transplantation? I den här sammanfattningen kommer ni att få veta mer om hur en stamcellsdonation går till och vilka faktorer som kan påverka utfallet av en transplantation. Vi kommer sedan att fokusera på några viktiga aspekter som rör vad som händer med stamcellsprodukten när den befinner sig mellan donatorn och patienten.

Det hela börjar med att donatorn och patienten måste ”matcha” genetiskt. Vid en sådan matchning bekräftas det att donatorn är tillräckligt frisk för att kunna donera stamceller. Idag samlas de flesta stamceller direkt via blodet, men det går även att samla via benmärgsdragning. I denna avhandling fokuserar vi enbart på stamceller som samlas direkt från blodet.

För att starta en stamcellssamling måste donatorn först ta en medicin för att få ut stamceller från benmärgen ut till blodet. Sedan låter man donatorns blod passera en så kallad aferesmaskin som avskiljer olika delar av blodet. När vi samlar stamceller från blodet kommer det ofta med en hel del vita blodkroppar. I studie I har vi jämfört andelen av specifika vita blodkroppar i en stamcellspåse när vi samlar celler med två olika aferesmaskiner. Där har man sett att det finns en betydligt högre andel vita blodkroppar i stamcellsprodukter från en äldre maskin. Tittar man vidare på vad som händer efter transplantationen har det visat sig att produkter från dessa aferessamlingar med höga andel vita blodkroppar oftare attackerade patienten efter transplantationen. Detta gav oss inblick i hur olika inställningar i själva samlingsprocessen kan påverka utfallet hos patienterna.

Det är sällan bra att ha för mycket eller för lite av det goda. Samma sak gäller för stamceller och andra vita blodkroppar i en stamcellspåse. I studie II har vi gått igenom historiska data om olika celltyper i stamcellspåsen och jämfört hur ofta patienter får olika problem efter stamcellstransplantation. Det visade sig att för mycket av vissa vita blodkroppar var kopplat till vissa komplikationer. Lite

oväntat var också att högre doser stamceller också var förknippat med sämre utfall jämfört med de som fick en ”lagom” dos.

Man kan ofta behöva transportera stamceller mellan sjukhus, eftersom donatorn och patienten inte alltid befinner sig på samma plats. Ibland kan donatorn också befinna sig på en annan kontinent, vilket innebär betydande resetider. Man har tidigare sett att stamceller och vita blodkroppar dör när de förvaras under lång tid utanför kroppen. Vi har i studie III mätt hur många celler i stamcellsprodukten som har dött eller håller på att dö efter transporten. Där har man sett att längre restider innebär fler döda celler och att en högre andel döda celler i stamcellsprodukten var förknippat med sämre ettårsöverlevnad för patienter.

I den sista studien, studie IV, ville vi inte bara undersöka antalet celler som en markör för hur bra stamcellsprodukten var utan också om cellerna i produkten kunde göra sitt jobb. En funktionell studie handlar om att aktivera celler och se hur väl de kan växa i en odling. Resultatet visade att patienter som fick stamcellsprodukter med bättre tillväxt hade färre fall av en viss virusinfektion. Ett annat fynd var att ett visst svar efter stimulering kunde vara förknippat med en högre risk för att cancersjukdomen skulle återkomma.

Sammanfattningsvis har studie I-IV visat att stamcellssamlingen, transporten, cellsammansättningen och funktionen av stamcellsprodukten spelar en roll i utfallet av en stamcellstransplantation. Vi behöver förbättra vår kunskap om de faktorer som kan påverka stamcellsprodukten. Framöver kommer det också att komma fler cell- och genterapier där aferesmaskiner, logistik och cellsammansättning kan komma att spela en avgörande roll för framgången för dessa behandlingar.

Abstract

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) involves the collection of a stem cell graft from a healthy donor and its infusion into a patient. The graft contains functional immune cells that may confer beneficial effects, including relapse control through the graft-versus-leukemia (GvL) effect, or adverse effects, such as graft-versus-host disease (GvHD). In clinical practice, peripheral blood stem cell (PBSC) grafts are commonly characterized by their $CD34^+$ cell dose, even though stem cells typically constitute less than 2% of the total graft cell content. Consequently, information on graft composition is often limited, and there is no clear consensus on how such data should guide clinical practice. The studies presented here investigate how graft composition, viability, and function influence clinical outcomes following allo-HSCT.

In Study I, we evaluated the effects of changing apheresis devices on graft composition and clinical outcomes in 71 HLA-matched sibling donors at Karolinska. The introduction of the Optia device (MNC program) produced a significantly lower lymphocyte yield than the older Cobe Spectra device, following the addition of an elutriation step. Recipients of Optia-collected grafts had lower rates of acute GvHD (aGvHD) grades II–IV and lower transplant-related mortality. Unexpectedly, chronic GvHD (cGvHD) was more frequent after Optia grafts, while relapse and overall survival did not differ. These findings indicate that a small but significant change in apheresis settings can alter graft characteristics.

Graft composition was further investigated in Study II. The study included 299 adults who underwent transplantation with PBSC grafts from sibling donors (n = 97) or unrelated donors (n = 202). Graft lymphocyte subsets were quantified by flow cytometry and assessed in relation to clinical outcomes. In unrelated donor grafts, a higher $CD8^+$ T cell dose was associated with a lower incidence of relapse, with the lowest relapse incidence observed in the highest quartile. In contrast, a higher $CD34^+$ dose was associated with increased transplant-related mortality (TRM) and a higher incidence of relapse. Among recipients of sibling donor grafts, a higher $CD19^+$ dose was associated with severe aGvHD and TRM, while a higher CD4/CD8 ratio was also associated with aGvHD. Although these data are significant, several other studies report contradictory findings. The relationship between graft composition and clinical outcomes is complex, and many confounding factors are difficult to control for.

Transporting the graft is critical, as viability declines with longer ex vivo duration. In Study III, we assessed graft viability using Annexin V, an apoptosis marker, in combination with 7-AAD. Grafts transported for two days had lower viability than those transported for one day (median Annexin V/7-AAD viability, 83.2% vs. 86.4%). Recipients of grafts with viability below the median (85.1%) had lower 1-year survival (63.7% vs. 88.9%). Low graft viability remained an independent predictor of TRM in the multivariable analysis. Overall, Annexin V and 7-AAD measurements of graft viability were associated with a significant effect on 1-year survival.

The third important aspect of the graft is its function. In Study IV, we investigated whether graft responses to pokeweed mitogen (PWM), staphylococcal enterotoxin A (SEA), or specific stimuli such as CMV, EBV, adenovirus, candida, and zoledronate ($\gamma\delta$ T cell response) were associated with infectious complications, relapse, and GvHD. In unrelated-donor grafts, low proliferative responses to PWM and SEA were associated with increased CMV reactivation. Interestingly, the graft response to CMV-specific stimuli was not associated with CMV reactivation. After PWM stimulation, high PD-1 expression on activated $CD8^+$ T cell blasts was associated with relapse and inferior overall survival. This finding may have a mechanistic explanation, as PD-1 is often expressed on activated T cells and is part of an inhibitory signaling pathway that is highly expressed on exhausted cells. To better understand this finding, further experimental studies are needed.

In conclusion, these studies highlight different aspects of the PBSC graft and the complex ways it is handled, which ultimately affect graft quality and clinical outcomes. As cell therapies become an increasingly common treatment option, a better understanding of the starting material may lead to small but meaningful clinical improvements.

List of scientific papers

I. **Wang T**, Remberger M, Axdorph Nygell U, Sundin M, Björklund A, Mattsson J, Uhlin M, Watz E. Change of apheresis device decreased the incidence of severe acute graft-versus-host disease among patients after allogeneic stem cell transplantation with sibling donors. Transfusion. 2018 Jun;58(6):1442-1451. doi: 10.1111/trf.14579. Epub 2018 Mar 13. PMID: 29536557.

II. Svenberg P, **Wang T**, Uhlin M, Watz E, Remberger M, Ringden O, Mattsson J, Uzunel M. The importance of graft cell composition in outcome after allogeneic stem cell transplantation in patients with malignant disease. Clin Transplant. 2019 Jun;33(6):e13537. doi: 10.1111/ctr.13537. Epub 2019 May 7. PMID: 30873642.

III. **Wang T**, Remberger M, Björklund A, Watz E. The impact of transportation time on apoptosis in allogeneic stem cell grafts and the clinical outcome in malignant patients with unrelated donors. Cytotherapy. 2022 May;24(5):508-515. doi: 10.1016/j.jcyt.2021.11.008. Epub 2022 Feb 21. PMID: 35210189.

IV. Söderström A*, **Wang T***, Törlén J, Lindahl H, Valentini D, Mielke S, Högelin KA, Robertson RA, Uhlin M. The lymphocyte function of allogeneic peripheral blood stem cell grafts is associated with relapse and cytomegalovirus reactivation. Transfusion. 2026 Aug 20. doi: 10.1111/trf.70374. PMID: 42619477.

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List of abbreviations

7-AAD	7-aminoactinomycin D
ACD	Acid citrate dextrose-A
AIM	Automatic Interface Monitoring (Optia system)
AML	Acute myeloid leukemia
APC	Antigen-presenting cell
ATG	Antithymocyte globulin
BAFF	B-cell activating factor
BM	Bone marrow
CAR	Chimeric antigen receptor
CAST	Cell therapy and Allogeneic Stem cell Transplantation
CCE	Counterflow centrifugation elutriation
CD	Cluster of differentiation
CE1/CE2	Collection efficiency 1/2
CFU	Colony-forming unit
cMNC	Optia continious mononuclear cell program
CMV	Cytomegalovirus
CsA	Cyclosporine A
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
CXCL12/SDF-1	C-X-C motif chemokine ligand 12 / stromal cell-derived factor 1
CXCR4	C-X-C chemokine receptor type 4
DLI	Donor lymphocyte infusion
EBV	Epstein-Barr virus
EVF	Erythrocyte volume fraction
FASCIA	Flow cytometric assay of specific cell-mediated immune response in activated whole blood
FasL	Fas ligand

G-CSF	Granulocyte colony-stimulating factor
GvHD	Graft-versus-host disease
GvL	Graft-versus-leukemia
HLA	Human leukocyte antigen
HSPC	Hematopoietic stem and progenitor cell
HSCT	Hematopoietic stem cell transplantation
IFN-γ	Interferon-gamma
IL	Interleukin
ISHAGE	International Society for Hematotherapy and Graft Engineering
LAG3	Lymphocyte-activation gene 3
LPS	Lipopolysaccharide
MAC	Myeloablative conditioning
MAGIC	Mount Sinai Acute GvHD International Consortium staging algorithm
MHC	Major histocompatibility complex
MRD	Measurable residual disease
MTX	Methotrexate
NGS	Next-generation sequencing
NK	Natural killer
NKG2D	Natural killer group 2 member D
NMDP	National Marrow Donor Program
OS	Overall survival
PBSC	Peripheral blood stem cell
PCR	Polymerase chain reaction
PD-1 / PD-L1	Programmed death-1 / programmed death ligand 1
PE	Phycoerythrin
PEG-G-CSF	Pegylated granulocyte colony-stimulating factor
PS	Phosphatidylserine

PTLD	Post-transplant lymphoproliferative disorder
PWM	Pokeweed mitogen
RIC	Reduced-intensity conditioning
SEA	Staphylococcal enterotoxin A
SSO	Sequence-specific oligonucleotide
SSP	Sequence-specific priming
TBI	Total body irradiation
TBV	Total blood volume
TCR	T cell receptor
TGF-β	Transforming growth factor beta
Th1/Th2/Th17	T helper 1/2/17 cells
TIM3	T cell immunoglobulin and mucin domain 3
TNF-α	Tumor necrosis factor alpha
TRM	Transplant-related mortality
UCB	Umbilical cord blood
URD	Unrelated donor
WBC	White blood cell
WMDA	World Marrow Donor Association

1 Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a well-established therapeutic modality for treating a variety of malignant and non-malignant hematological diseases and genetic disorders [1,2]. The advent and progressive refinement of multiple graft sources—including peripheral blood stem cells (PBSC), bone marrow (BM), and umbilical cord blood (UCB)—have significantly expanded the utility and success of allo-HSCT. Each graft source has a distinct cellular composition and functional profile, influencing engraftment kinetics, immune reconstitution, and post-transplant complications [3].

Among available graft types, PBSC grafts have become the preferred source in many clinical settings due to their ease of collection and rapid engraftment potential [4]. Mobilization of hematopoietic stem and progenitor cells (HSPCs), predominantly characterized by CD34 cell surface expression, into the peripheral circulation is primarily achieved via granulocyte colony-stimulating factor (G-CSF) administration, followed by collection through leukapheresis. While the procedural framework of PBSC mobilization and collection is well established, significant variability remains in graft composition, which comprises not only HSPCs but also mature immune cells and accessory populations. These cellular constituents collectively define graft function and ultimately impact clinical outcomes post-transplant.

Beyond graft composition, critical procedural factors such as graft transportation and storage conditions, and various graft manipulation strategies—including cell selection and depletion—may profoundly influence graft viability and functional integrity. The logistics of stem cell transport, inter-facility transfer timelines, and storage modalities present operational challenges that can affect cell survival, potency, and patient outcomes. Similarly, evolving graft-processing techniques aim to optimize graft quality, minimize the risk of graft-versus-host disease (GvHD), and enhance overall transplant success [5–7].

This thesis aims to provide a niched appraisal of the biology and clinical relevance of stem cell grafts with a particular focus on PBSCs. Emphasis is placed on the cellular composition and functional properties of grafts, the influence of apheresis methodologies, the impact of transportation and storage on graft quality, and the implications of functional graft assays. By addressing these facets, this work aims to elucidate the determinants of graft function that

inform transplantation outcomes and to develop strategies for improving graft handling and clinical management.

1.1 Allogeneic stem cell transplantation

1.1.1 Transplantation indications and patient selection

Hematological malignancies constitute the majority of indications for allo-HSCT, accounting for more than 60% of transplantations in Europe, with myeloid malignancies — led by acute myeloid leukemia (AML), which alone accounted for 42% of all transplantations in the 2024 EBMT survey — as the largest group. Non-malignant disorders, including severe aplastic anemia, inherited disorders of hematopoiesis, hemoglobinopathies, and immunodeficiencies, make up the remaining indications [8].

For an individual patient, selection is guided by two principal assessments. The first is disease-related risk: the probability of cure following transplantation varies according to diagnosis, disease stage, and cytogenetic or molecular risk profile. Formal instruments, including the Disease Risk Index and the EBMT risk score, stratify this risk. Patients undergoing transplantation for advanced or relapsed disease have substantially higher relapse risk than those transplanted in remission [9,10]. The second assessment concerns patient fitness. Transplant-related mortality (TRM) is predicted by comorbidity burden, and by age. Although age is no longer an absolute contraindication, transplantation rates among patients older than 70 continue to increase, and outcomes improve with reduced-intensity conditioning, it remains associated with higher non-relapse mortality [11,12].

Despite the expanding availability of novel therapies, including CAR T cell therapy and targeted agents, the demand for allo-HSCT continues to increase. Allo-HSCT activity increased steadily in recent survey years, reaching its highest annual volume in 2024 [8,13] (Fig. 1). This trend reflects an increase in demand and in patient eligibility. Targeted agents have improved remission rates and survival in AML; however, they rarely achieve durable disease control in patients with biologically high-risk disease. Consequently, patients with adverse cytogenetic features, persistent measurable residual disease (MRD), or relapse after these therapies remain standard candidates for transplantation [14,15]. At the same time, advances in supportive care and reduced-intensity conditioning (RIC), along with broader donor availability, including haploidentical donors, have made transplantation feasible for those with comorbidities who were previously considered ineligible [13].

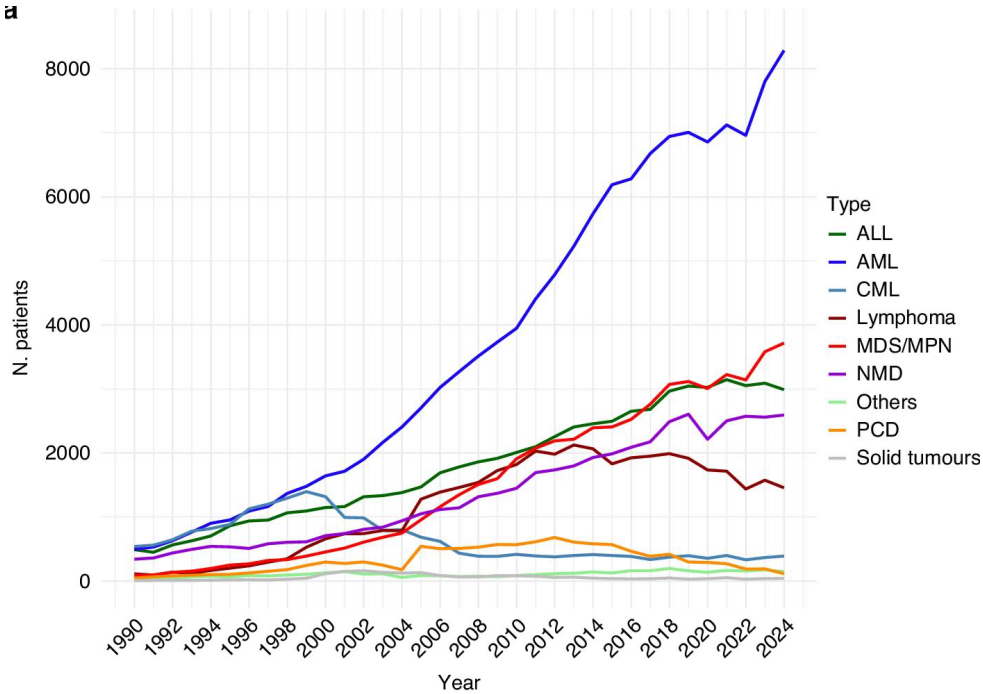


Figure 1. *Number of allo-HSCT in Europe. Type: ALL – Acute lymphoblastic leukemia , AML - Acute myeloid leukemia, CML - Chronic myeloid leukemia, MDS - Myelodysplastic syndrome, MPN - Myeloproliferative neoplasm, NPD - Non-malignant disease, PCD - Plasma cell disease. Source: Greco R. et al. The 2024 EBMT activity report: crossing one million HCTs and 20,000 CAR-T. A landmark in cellular therapy. Bone Marrow Transplant. 2026. CC-license.*

1.1.2 Donor selection

Allogeneic stem cell donation is a voluntary process in which donors provide stem cells to recipients with whom they may be related or unrelated. Donations are typically collected via PBSC collection or BM harvest. Unrelated donors (URD) are identified through national or international registries, which coordinate human leukocyte antigen (HLA) typing and administrative procedures related to donation.

More than 44 million donors are currently registered worldwide. The World Marrow Donor Association (WMDA) facilitates international exchanges between donor and transplant centers [16]. Despite the large number of registered donors, some patients still never find a suitable match [17]. A person's ethnicity affects their chances of finding a donor, as the largest registries are in North America (NMDP) and Europe (DKMS) [18]. By comparison, Sweden's registry,

Tobiasregistret, has more than 280,000 registered donors, representing about 3 percent of the Swedish population [19].

A key component of donor selection is the assessment of potential risks to both the donor and recipient. In PBSC donation, donor risks are primarily associated with G-CSF stimulation or the apheresis procedure. Approximately 90% of donors experience bone pain, which is typically mild to moderate and responds well to paracetamol. Other common adverse effects include headache, flu-like symptoms, fatigue, mild gastrointestinal complaints, and injection-site reactions. Most side effects resolve soon after G-CSF is discontinued [20]. Although studies have demonstrated that donation is generally safe, cases of severe adverse events and, rarely, life-threatening complications have been reported [21,22].

1.1.3 HLA matching and CMV matching

HLA matching has been central to donor selection since the first successful transplant, performed between HLA-identical siblings in 1968 [23]. URD can now be matched at ten alleles across HLA-A, -B, -C, -DRB1, and -DQB1 (a 10/10 match). Registry analyses have shown that mismatches at class I and class II loci increase the risks of GvHD and mortality [24–26]. HLA matching does not guarantee freedom from GvHD: minor histocompatibility antigens can sustain GvHD even in well matched donors/recipients [27]. With post-transplant cyclophosphamide or αβ/CD19 depleted grafts, haploidentical donors have also become acceptable options [28,29]. Despite advances in HLA typing and donor matching, HLA matching remains complex, and HLA mismatches may confound studies involving URDs. In the current era of haplo-transplantation, the role of HLA matching is also a topic for further discussion. Studies I-IV only include HLA-matched sibling donors or URDs while haplotransplantations were excluded.

The importance of CMV in allo-HSCT has previously been described in detail, matching donor and recipient CMV status is standard clinical practice [30]. CMV-positive donors in general have sustained CMV-specific T cell clones in circulation that contributes to controlling the virus. Matching CMV-positive recipients with CMV-positive donors gives the recipient viral control during the early phases after HSCT, while a mismatch, especially with CMV-negative donors to CMV-positive recipients, can increase the risk of CMV reactivation [31,32].

Complications associated with CMV reactivation and treatment is discussed in section 1.1.7

1.1.4 Age/sex/timing

Younger donor age has been associated with better survival with lower relapse risk [33,34]. Donor age is among the few donor characteristics clearly associated with survival after allo-HSCT. In large registry analyses of HLA-matched URD transplantation, each ten-year increment in donor age increased the mortality hazard, and younger age was the only donor factor that remained significant when other characteristics were tested [33,34]. The effect is not uniform across settings. In a study with mixed myeloablative and non-myeloablative conditioning transplantation using PBSC graft, donors aged sixty years or older did not adversely affect engraftment, GvHD, or TRM [35]. Donor age did not influence overall survival in Australian URD transplantations up to donor age 59 [36]. Donor age should therefore be weighed against donor availability, CMV serostatus, and logistical urgency rather than treated as an absolute criterion. Female donor to male recipient is also a known risk factor for GvHD, caused by immunization against minor antigens during pregnancy, for instance, the anti-H-Y antibody [37,38]. Therefore, male donors to male recipients are preferred if all other factors are equal.

1.1.5 Conditioning

Before allo-HSCT, conditioning is required to clear residual cancer, create space for engraftment, and reduce the risk of rejection of the transplanted HSCs. Conditioning regimens have evolved continuously over the past 60 years since the introduction of HSCT. A wide range of regimens is available to optimize therapeutic effects while minimizing toxicity to non-malignant cells. Myeloablative conditioning (MAC), a classical regimen developed during the early era of transplantation, aims to achieve complete marrow ablation. It may involve total body irradiation (TBI) combined with alkylating agents, primarily cyclophosphamide [39]. Later, reduced-intensity conditioning (RIC) was introduced in the 1990s, with reduced exposure to alkylators and TBI [40], enabling transplantation for older patients and those with comorbidities who were previously excluded from allo-HSCT due to the toxicity of myeloablative

regimens [41]. For the patient, conditioning is the principal treatment-related toxicity of transplantation. Myeloablative regimens cause severe cytopenias, prolonged neutropenia, and mucosal barrier injury, increasing infection risk during the pre-engraftment phase. TBI-based regimens can also lead to late endocrine and gonadal complications [42]. RIC reduces, but does not eliminate, the above-mentioned toxicities. Its reduced cytoreduction is offset by greater reliance on the GvL effect, potentially increasing relapse risk for some diagnosis [43].

1.1.6 Immune reconstitution

Allo-HSCT replaces the patient's immune system and entire hematopoiesis. The immune system requires time to be reconstituted post-HSCT. The origin of reconstituting immune cells is partly from the graft and partly from de novo production of immunological cells post-infusion [44–48]. The first wave is mediated by mature lymphocytes infused with the graft, which expand in the lymphopenic host through homeostatic peripheral expansion, providing early but incomplete defense against pathogens and GvL activity. The second wave, de novo lymphopoiesis from donor stem cells, depends on thymic function to generate a more diverse naïve repertoire. Full T cell regeneration takes one to two years, with B-cell recovery often slower still [46] (Fig. 2).

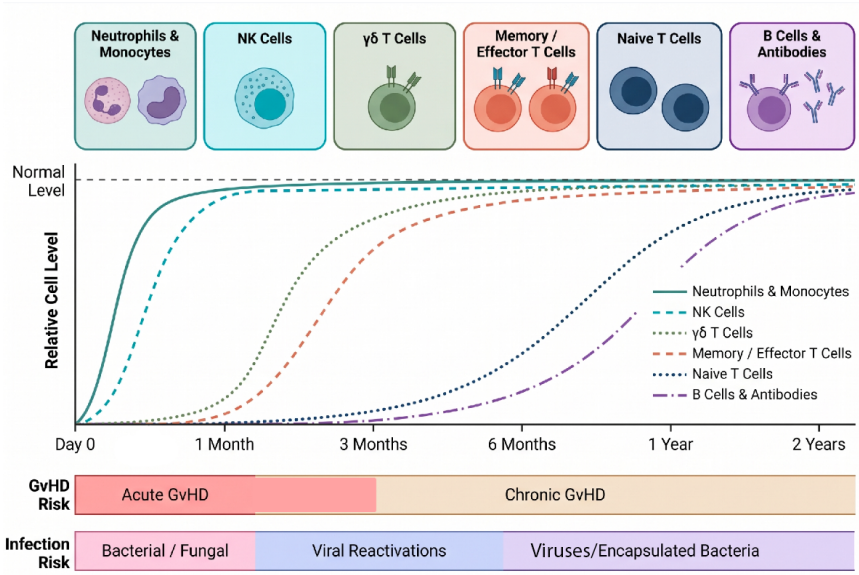


Figure 2. *Schematic overview of the timing of immune reconstitution after allo-HSCT. The risk of infection follows the reconstitution dynamics. Other factors, such as poor graft function, ATG use, and GvHD, can alter the reconstitution dynamics. Created with Biorender.com*

Reconstitution tempo differs sharply by lineage. Myeloid and NK cells recover within weeks, whereas T cells require months to years, particularly in older recipients with age-related thymic involution. γδ T cells reconstitute earliest among T cells and mediate anti-infective and anti-leukemic surveillance without provoking GvHD [49,50]. Memory αβ T cells reappear after that, largely from the graft-derived compartment, whereas naïve cells depend on thymopoiesis. It has earlier been shown that $CD4^+$ recovery above 50 cells/μL at day 100 is associated with faster viral clearance and superior survival [47]. B-cell recovery is the slowest, with hypogammaglobulinemia and impaired vaccine responses persisting one to two years, especially after GvHD [46].

Several other factors shape reconstitution such as; recipient age and thymic capacity, HLA disparity, conditioning intensity, CMV reactivation, and GvHD [51]. Pharmacological GvHD prevention is likewise a major determinant: anti thymocyte globulin (ATG) delays T cell recovery for up to six months and depletes the naïve compartment, and exposure-guided dosing improves immune recovery and survival [52,53].

Delayed T cell recovery increases the risk of opportunistic infections, most prominently CMV reactivation, and increased TRM, whereas early recovery of lymphocyte compartments predicts superior survival [54,55]. Antimicrobial prophylaxis shifts but does not abolish this risk, which is largely determined by the pace of reconstitution [55]. Most relevant to this thesis, the cells of the first wave are supplied by the graft: the number, viability, and function of the infused lymphocytes define the immunological starting point of the transplantation.

1.1.7 Complications

1.1.7.1 Infections

Infectious complications are a common cause of TRM and morbidity after allo-HSCT. Immune reconstitution after allo-HSCT is a dynamic process, and infection risk depends on which immunological compartment is defective or suppressed at the time [56]. During the pre-engraftment phase of severe neutropenia and mucosal barrier injury, bacterial bloodstream infections can be caused by Gram-negative organisms, often drug resistant [57]. From engraftment through the first months, the dominant deficit is cellular, such as delayed T cell reconstitution. This T cell deficiency can also be aggravated by GvHD and the associated immunosuppressive treatment. This deficiency permits invasive mold infections and reactivation of the herpesviruses, of which CMV is clinically the most important [58,59]. In the late phase, defective humoral immunity and cGvHD predispose to infections with encapsulated bacteria and to late viral disease [59,60].

CMV infection after allo-HSCT ranges from asymptomatic reactivation to severe CMV end-organ disease. CMV syndrome presents with fever, leukopenia, and thrombocytopenia. More serious end-organ disease includes pneumonitis and gastrointestinal involvement, with colitis, and, less frequently, hepatitis, encephalitis, or retinitis [59]. Beyond direct tissue injury, CMV reactivation can amplify the risk of graft failure, worsen GvHD through its immunomodulatory effects, and is independently associated with increased TRM [30,59]. Reactivation occurs in a large proportion of seropositive recipients, and the donor-recipient serostatus combination determines much of this risk [30,61]. Prevention changed decisively with the introduction of letermovir prophylaxis, which reduced the incidence of clinically significant CMV infection and closed the mortality gap between seropositive and seronegative recipients [62,63].

Prophylaxis reshapes immunity as well: CMV-specific T cell reconstitution is delayed under drug pressure, and infections cluster after prophylaxis cessation, driven by aGvHD and by grafts from CMV-seronegative donors, which lack the pre-formed virus-specific T cells that seropositive grafts carry [64,65]. Established treatment consists of ganciclovir or valganciclovir as first-line therapy, with foscarnet and cidofovir reserved for resistant disease. These agents have toxicities, nephrotoxicity and myelosuppression, and generate further complications. [59]. Studies I–III were conducted before the introduction of letermovir and maribavir; in the current treatment era, CMV reactivation rates have declined [66].

1.1.7.2 GvHD prophylaxis and ATG

GvHD is the principal immunological complication of allo-HSCT and a leading cause of TRM. It develops when immunocompetent donor lymphocytes, particularly T cells, that recognize recipient tissues as foreign and attack both healthy and malignant cells [67]. Despite HLA matching, GvHD affects a substantial proportion of recipients because alloreactivity is driven not only by mismatched HLA but also by minor histocompatibility antigens and inflammatory amplification [68]. In recent years, the role of the microbiota in GvHD has been widely discussed [69,70]. Although this topic warrants consideration, it falls outside the scope of these studies.

GvHD is divided into acute and chronic forms, nowadays more defined by clinical features rather than time since transplantation [71]. aGvHD affects the skin, gastrointestinal tract, and liver within the first months post HSCT. In contrast, cGvHD presents later as a multisystem, autoimmune-like disease with tissue fibrosis as its hallmark. Commonly used aGvHD gradings are the Glucksberg scale or MAGIC per-organ staging [72].

aGvHD is a common and potentially serious complication in allo-HSCT [73]. It typically presents within the first months after transplantation, classically before day 100, as a clinical syndrome of maculopapular rash, nausea, vomiting, diarrhea, and hyperbilirubinemia, reflecting inflammation of the skin, the gastrointestinal tract, and the liver [68]. Severity is graded from the degree of skin, gut, and liver involvement. The staging scale was formulated by Glucksberg in 1974 and still remains the basis for the grade I–IV classification used in most

studies [74]. Modern consensus criteria separate acute from chronic disease based on clinical features rather than on the 100-day limit alone [71].

cGvHD is the leading cause of late TRM after allo-HSCT. It typically emerges months after transplantation, and it behaves as a multisystem, autoimmune-like disorder in which inflammation progresses toward fibrosis [69]. The clinical spectrum includes sclerotic skin with joint contractures, sicca and mucosal disease, gastrointestinal involvement, and bronchiolitis obliterans with progressive breathlessness [75]. Severity is staged by the National Institutes of Health consensus criteria [71]. Treatment requires years of immunosuppression, with infection and metabolic complications as its cost; corticosteroids are first-line, and ruxolitinib as second line treatment option [76]. Extracorporal photopheresis is a safe treatment for refractory cGvHD based on ex vivo extraction of lymphocytes, followed by a selective induction of apoptosis in a subset of mononuclear cells before reinfusion of the modified cells to the patient. The basic idea is that apoptotic cells will be processed by DC and induce a more tolerogenic response in the recipient [77]. For many patients, cGvHD converts a curative transplantation into a years-long chronic illness, and reducing its incidence without sacrificing GvL activity remains an unmet clinical need [69].

Prophylaxis and treatment of GvHD rely on broad immunosuppression, and every strategy involves the same central trade-off between GvHD and GvL activity [78]. Standard prophylaxis combines a calcineurin inhibitor, ciclosporin or tacrolimus, with short-course methotrexate. This combination has improved GvHD disease-free, relapse-free survival in randomized comparisons [79].

ATG is widely used for in vivo T cell depletion, particularly in patients receiving grafts from URDs [80]. Polyclonal ATG antibodies bind multiple T cell surface antigens and deplete circulating T cells through complement-mediated lysis, antibody-dependent cell cytotoxicity, and Fas/FasL-triggered apoptosis. This targets naïve T cells most heavily while sparing some memory populations, which is why it acts as in vivo T cell depletion that prevents GvHD but delays immune reconstitution. Several randomized trials and meta-analyses have shown reduced incidence of acute and chronic GvHD with ATG, particularly in URD PBSC [80]. However, OS has not been uniformly improved and both graft failure and relapse remain concerns [78]. ATG is central to part of Study II – IV where all URD with malignant diseases receive ATG at Karolinska.

1.1.7.3 Relapse

Relapse is now the leading cause of treatment failure after allo-HSCT, a direct consequence of decades of success against TRM [81,82]. The GvL effect, mediated by donor T cells recognizing minor histocompatibility and leukemia-associated antigens is a strong immunological defense against relapse. Relapse can arise through antigen-presentation escape, clonal evolution, and progressive dysfunction of donor T cells in the post-transplant marrow [83,84]. Since the lymphocytes that mediate GvL early post HSCT are infused as part of the graft, the quality of that graft — its cellular composition, its viability after collection and transport, and the functional state of its lymphocytes — defines the immunological starting point of the transplant.

Because relapse begins below the threshold of clinical detection, surveillance depends on laboratory monitoring rather than symptoms. The disease-specific instrument is measurable residual disease (MRD) monitoring, which quantifies malignant cells through fusion transcripts, aberrant immunophenotypes, or mutation burden, and defines molecular relapse often weeks before overt disease [85]. Rising MRD feeds directly into clinical decisions: tapering immunosuppression and pre-emptive donor lymphocyte infusion are considered while disease burden remains subclinical. MRD markers, however, are not available for every patient, detection depends on the disease carrying a trackable lesion. Where no suitable MRD marker exists, lineage-specific chimerism can serve as the surrogate. Donor–recipient hematopoiesis is quantified by PCR-based markers on sorted cell subsets, and rising recipient fractions within the disease-relevant lineage signal imminent relapse in the same window that MRD would [86]. However, because recipient-derived cells are not necessarily malignant, chimerism should be interpreted alongside disease-specific MRD assessments whenever possible [86]. Both modalities are used to guide clinical interventions such as tapering immunosuppression or pre-emptive DLI ahead of overt hematological relapse.

DLI re-arms the GvL effect by infusing unmodified lymphocytes from the donor [87,88]. Because the T cells that mediate GvL also mediate GvHD, DLI is typically given as sequential escalating doses, each step increased only after assessing response and toxicity, so that the lowest dose clearing MRD is found before the dose causing GvHD [89,90].

1.2 Transplantation immunology

The following section outlines basic immunological functions that are important for understanding some of the challenges associated with allo-HSCT. It begins with the distinction between self and non-self and then examines specific components of the adaptive immune system relevant to these studies. Immune activation is tightly regulated to prevent unintended damage to healthy cells. This section also highlights that transplantation immunology encompasses far more than the topics addressed in this thesis. Other aspects of the immune system, such as innate immune activation, thymic function, and stem cell niches, are equally important but were omitted from this introduction due to space and time limitations. Nevertheless, the topics discussed below should provide an introduction to concepts relevant to Studies I-IV.

1.2.1 Antigen presentation and HLA

The discovery of HLA and understanding its role in antigen presentation, along with its polymorphic nature, has been a cornerstone of transplantation immunology [91]. Understanding antigen presentation and its restrictions helps explain which signals modify the immune system in the transplantation setting, in immune reconstitution, in infections and overall disease susceptibility [92].

The classical HLA genes are located on chromosome 6p21 within the major histocompatibility complex [93]. The polymorphism relevant to transplantation is concentrated to the exons encoding the peptide-binding groove: exons 2 and 3 of the class I genes (encoding the α1 and α2 domains) and exon 2 of the class II β-chain genes (encoding the β1 domain) — the antigen recognition domain. Conventional typing older techniques only targeted these exons. Now with the introduction of next-generation sequencing which resolves full gene sequences, it reduces ambiguities that arise when alleles are identical across the antigen recognition domain but differ elsewhere.

HLA class I molecules consist of a heavy membrane-spanning α-chain, β_2-microglobulin, and the bound peptide. Together, these components form a heterodimer or heterotrimer (with the peptide). Without the peptide in the groove, HLA class I is unstable on the cell surface [94]. Normally, an 8-10 amino acid peptide can bind to the class I groove, but there are variations in the structure based on the HLA type, resulting in variation in the binding preferences

of different amino acids [95]. Almost all nucleated cells in the body present HLA class I, which in turn presents all proteins produced in the cell.

HLA class II, also a heterodimer with an alpha and beta chain, is usually only present on professional antigen-presenting cells (APC). The target peptides presented by HLA class II are usually externally produced peptides that have been phagocytosed or endocytosed. Some of the most common APCs that will not be discussed further here are dendritic cells, macrophages, B cells, and thymic epithelial cells [96].

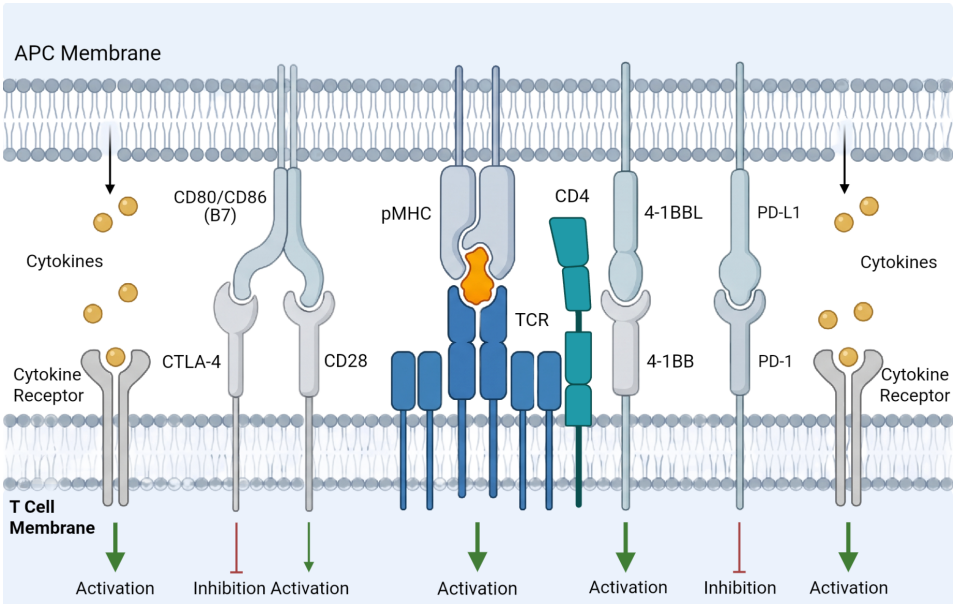


***Figure 3**. TCR activation with signal 1 via TCR, signal 2 costimulatory/inhibitory signal, signal 3 cytokine signaling. Created with Biorender.com.*

1.2.2 T cells

T cells are some of the most well studied cells in the transplantation setting [97]. This is due to the central role in the coordination of tolerance or severe immunological effector function, able to damage both host cells and pathogens.

T lymphocytes are conventionally divided by their antigen-recognition module and their differentiation status. The αβ T cell compartment separates into $CD8^+$ cytotoxic T cells, which recognize peptides presented on HLA class I and kill

infected or malignant target cells through perforin–granzyme-mediated lysis and Fas–FasL engagement, and $CD4^+$ helper T cells, which recognize peptides on HLA class II and coordinate the immune response through cytokine secretion and licensing of antigen-presenting cells. Naïve $CD4^+$ cells differentiate into functionally specialized lineages under the direction of polarizing cytokines and lineage-defining transcription factors — Th1 (IFN-γ, cellular immunity), Th2 (IL-4/IL-5/IL-13, humoral and anti-helminth immunity), Th17 (IL-17, mucosal and fungal immunity) and T follicular helper cells (B-cell support in germinal centres) [98].

Gamma delta (γδ) T cells constitute a small, atypical T cell lineage important for transplantation outcomes for several reasons. The γδ T cells respond rapidly, to stressed and transformed cells. They can mediate cytotoxicity through NKG2D and perforin/granzyme pathways and producing IFN-γ early after transplantation. Anti-leukemic activity against AML and other hematological malignancies has been demonstrated both preclinically and in clinical correlative studies [99]. Their reactivity is largely directed against infected and malignant cells rather than healthy alloantigens. γδ T cells have been associated with antiviral protection, particularly against CMV, with comparatively little evidence of a pathogenic role in GvHD [100]. Consistent with this favorable ratio of GvL to GvHD, γδ T cells are among the earliest lymphocyte populations to reconstitute after transplantation.

1.2.3 T cell activation, co-stimulation and co-inhibition

T cell activation requires three converging signals. Signal 1 is antigen-specific: the T cell receptor recognizes its peptide presented on HLA, with CD4 or CD8 stabilizing the interaction and transmitting early tyrosine-kinase signals through the CD3 complex. Signal 1 alone is insufficient and, without further instruction, induces anergy. Signal 2 is co-stimulation, delivered mainly by CD28 on the T cell engaging CD80/CD86 on an activated antigen-presenting cell, it licenses survival, interleukin-2 production and proliferation; inhibitory receptors such as CTLA-4 and PD-1 compete at this step to set the response ceiling. Signal 3 is the surrounding cytokine environment: IL-12, IL-6, IL-2 and other polarizing cytokines direct the activated cell toward effector lineages, Th1, Th17, cytotoxic or regulatory, thereby determining not only whether the T cell responds, but what kind of response it delivers.

After initial antigen encounter, T cells diversify into subsets with distinct migration and function. Upon activation, some become short-lived effector cells that mediate immediate defense; others persist as memory cells. Memory T cells are conventionally divided into central memory cells (T_{CM}, $CCR7^{+}CD45RA^{-}$), which home to lymph nodes and replenish the pool, and effector memory cells (T_{EM}, $CCR7^{-}$ $CD45RA^{-}$), which reside in tissues and exert rapid cytotoxic or cytokine responses [101]. With repeated stimulation, T_{EM} cells can further lose CD27 and CD28 and acquire a late, terminally differentiated phenotype (T_{EMRA}), a state reached fastest under the pressure of persistent viral infections [102]. T_{reg} cells also play a role in inducing tolerance and regulate the system [103]. This heterogeneity means that a total T cell count conceals functionally distinct populations, both in the graft and in the patient.

1.2.4 B cells

B cells derive from hematopoietic stem cells and mature through an ordered sequence of immunoglobulin-gene rearrangements in the bone marrow [104,105]. Rearrangement of the immunoglobulin heavy-chain locus at the pro-B-cell stage generates the pre-B-cell receptor, whose expression marks productive heavy-chain assembly and drives clonal expansion. Subsequent light-chain rearrangement yields the surface immunoglobulin B-cell receptor of the immature B cell. B cell precursors failing to express a functional receptor are eliminated, ensuring that only B cells carrying an intact antigen receptor proceed. Immature B cells then undergo central tolerance, receptor editing or deletion of autoreactive clones, before emigrating to the periphery as transitional B cells. There they complete maturation into follicular and marginal-zone subsets under the survival control of BAFF-R signaling.

Upon antigen encounter with T cell help, follicular B cells enter germinal centers, where their immunoglobulin genes undergo somatic hypermutation and class-switch recombination. Clonal selection on antigen affinity and T cell co-stimulation determines survival. The process yields two outputs: memory B cells, which mediate rapid anamnestic responses, and plasma cells. Antibody production is therefore not a single event but the endpoint of a developmental program spanning receptor assembly, tolerance, affinity maturation, and terminal differentiation.

Peripheral blood contains circulating naïve and memory B cells, together with transitional B cells and variable numbers of plasmablasts, but does not fully represent germinal-center, tissue-resident memory, or long-lived plasma-cell compartments. This is important for understanding how the B cell compartment of a PBSC graft influences early humoral reconstitution [106,107].

1.2.5 Exhaustion and anergy

The three-signal T cell activation framework requires a specific strength and duration to produce a positive signal. However, insufficient or chronic activation in a transplantation setting can lead to different outcomes. Two distinct states of functional failure dominate this discussion. Anergy is the acute, antigen-specific unresponsiveness that follows signal 1 without signal 2. Without co-stimulation, the T cell neither proliferates nor functions upon re-encounter with antigen [108]. Exhaustion is the progressive loss of effector function that develops under chronic antigen stimulation, characterized by sustained expression of inhibitory checkpoints PD-1, TIM-3, and LAG-3 [108,109]. The allogeneic recipient post transplantation provides ideal conditions for both. Persistent antigen is unavoidable, from residual leukemia, CMV reactivation and other inflammatory states [109]. Lymphopenia drives homeostatic proliferation of graft-derived memory cells toward exhaustion-like differentiation. Marrow memory T cells from relapsing patients carry an exhausted epigenetic signature [110]. Exhaustion is clinically consequential: exhausted cytotoxicity associates with relapse, and its reversal by early donor lymphocyte infusion predicts effective anti-leukemic responses [111,112]. Most relevant to this thesis, the exhaustion program is measurable in the graft before infusion. PD-1 expression on activated $CD8^+$ T cell blasts in **Study IV** identified recipients at relapse risk at the time of transplantation.

1.2.6 Apoptosis and necrosis – immunological effects

Cell death occurs through mechanistically distinct pathways with different immunological consequences. Apoptosis is caspase-dependent and preserves membrane integrity: phosphatidylserine (PS) is externalized as an eat-me signal. Apoptotic cells are cleared before their contents are released, and the engulfment process itself is actively anti-inflammatory, shifting antigen

presentation toward deletion and regulation rather than priming [113,114]. Phagocytosis of apoptotic cells drives the production of anti-inflammatory cytokines such as TGF-β and IL-10, promotes deletion of T cells recognizing antigens presented in this context, and supports the expansion of regulatory T cells. Apoptosis is therefore not merely a passive way for a cell to disappear, but an active tolerogenic program that the immune system reads as a signal to stay quiet [115].

Necrosis is the opposite situation: uncontrolled lysis and regulated lytic pathways release damage-associated molecular patterns (DAMPs) such as HMGB1, extracellular ATP, and mitochondrial DNA. These molecules engage pattern-recognition receptors on APCs and provide the inflammatory costimulation that matures dendritic cells and licenses them to prime rather than delete. In the allogeneic setting, where alloantigen is abundant from the moment of infusion, DAMP exposure supplies the second signal that converts harmless antigen presentation into priming, and drives the inflammatory first phase of aGvHD, for example [116,117].

There is no clear cut between apoptosis and necrosis. Apoptotic cells that escape timely clearance undergo secondary necrosis and convert from tolerogenic to pro-inflammatory stimuli, whereas cells approaching the apoptotic threshold may recover if the initiating stimulus is removed [118]. The immunological outcome of a dying cell is therefore decided dynamically, by the balance between the speed of clearance and the progression of membrane injury, rather than by the initiating pathway alone [115]. These considerations apply directly to graft handling. Transportation injury in **Study III** produced a predominantly early-apoptotic, PS signature rather than membrane loss. The immunological fate of apoptotic graft cells after infusion, silent clearance, recovery, or secondary necrosis with DAMP release, remains unclear.

1.3 Stem cell graft

1.3.1 Peripheral stem cell graft

PBSC grafts are heterogeneous, comprising a mixture of hematopoietic cells at various stages of maturation rather than an isolated population of stem cells [119,120]. $CD34^+$ hematopoietic stem and progenitor cells constitute only a small fraction of the collected cells, whereas the majority are mature myeloid and lymphoid cells [119]. Nevertheless, the $CD34^+$ cell dose remains the principal quantitative measure used to assess graft adequacy and guide collection, reflecting its established importance for hematopoietic engraftment [121,122]. Other graft components have received less systematic attention, despite substantial variation in their abundance. The relative proportions of granulocytes and lymphocytes vary between grafts and are influenced by mobilization and collection conditions [123]. PBSC graft composition should be considered beyond the $CD34^+$ compartment, particularly when investigating the potential contributions of other infused cell populations to clinical outcomes related to the immunological function of the graft.

Experience with graft manipulation highlights the importance of cellular composition in balancing the beneficial and adverse effects of allo-HSCT. Positive selection of $CD34^+$ cells markedly reduces the number of donor T cells and can lower the risk of GvHD. However, extensive T cell depletion may compromise immune reconstitution, and its effects on engraftment and disease control depend on the transplantation setting. More selective approaches, such as depletion of $CD45RA^+$ naïve T cells from DLI, aim to reduce alloreactivity while retaining memory T cells and their potential contribution to immune protection and GvL activity. Early clinical studies have demonstrated feasibility and a low incidence of DLI-associated GvHD, although preservation of the GvL effect requires further evaluation [124]. These observations illustrate that altering the cellular composition of an infused product can influence distinct clinical outcomes, rather than simply reducing its overall immunological activity.

1.3.2 Mobilization

In healthy donors, circulating $CD34^+$ hematopoietic stem and progenitor cells in the peripheral blood are scarce, typically numbering $1–3\times10^6$ cells/L or fewer.

Without mobilization, these low concentrations generally preclude collecting a clinically sufficient cell dose by apheresis [125]. Mobilization is a critical step to access PBSC, and the extent of mobilization also influences the ratio of peripheral blood cells to stem cells in the graft.

Granulocyte colony-stimulating factor (G-CSF) is an endogenous cytokine that regulates neutrophil production by promoting the proliferation, differentiation, and survival of granulocytic precursors. It also supports the survival and functional activity of mature neutrophils. G-CSF is produced by several cell types, including fibroblasts, endothelial cells, monocytes, and macrophages. Its production increases in response to microbial components, such as lipopolysaccharide (LPS), and inflammatory mediators, including interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α), thereby linking inflammatory signalling to increased granulopoiesis [126].

G-CSF mobilizes $CD34^+$ hematopoietic stem and progenitor cells primarily through indirect effects on the bone marrow microenvironment. These effects reduce stem cell retention and weaken adhesive interactions between the cells and the BM niche. Proliferation of myeloid cells, such as granulocytes, also releases proteases and other enzymes that affect the BM niche and the CXCR4-SDF-1 (CXCL12) interaction, resulting in the release of progenitors into peripheral circulation. Plerixafor, a more recent mobilizing agent, is a direct antagonist of CXCR4, blocking its interaction with SDF-1. For simplicity, all grafts were mobilized using only G-CSF. Plerixafor was recently approved for healthy donors and was not an option during Study I-IV [127].

G-CSF is typically administered subcutaneously at a total daily dose of 1 ME/kg for four days before PBSC collection. The daily dose may be divided into two administrations to enhance mobilization, although once-daily dosing is also used. Pegylated G-CSF (PEG-G-CSF) has a longer elimination half-life than non-pegylated G-CSF, allowing less frequent administration. However, a comparative study found no significant improvement in stem cell mobilization with the pegylated formulation [128].

1.3.3 Collection

The donor will arrive at the collection center after mobilization. The main requirements for a successful PBSC collection are as follows:

- Sufficient CD34⁺ cell count in peripheral blood, typically exceeding 10 million CD34⁺ cells per liter.
- Well-established venous accesses, through peripheral or central lines
- Hemodynamically stable donor without ongoing infections

Most donors will meet these criteria, and collection begins on day 1. A measurement of the CD34⁺ cell dose in the peripheral blood will be performed after starting the apheresis. Based on the CD34⁺ values in the peripheral blood, a prediction can be made regarding how many liters of blood need to be processed before reaching the target cell dose. Two methods for calculating collection efficiency can be used.

Collection efficiency 1 (CE1)

$$CE1 = \frac{Total\ CD34^{+} cells\ collected}{(Pre + Post)/2 \times TBV\ processed}$$

Collection efficiency 2 (CE2)

$$CE2 = \frac{Total\ CD34^{+} cells\ collected}{Pre\ \times\ TBV\ processed}$$

Pre – The number of CD34⁺ cells in the peripheral blood before apheresis.
Post – The number of CD34⁺ cells in the peripheral blood after apheresis.
TBV – Total blood volume

Most centers use CE2 because CD34⁺ cells are not measured after apheresis collection. The CE2 will omit dynamics in the CD34⁺ in peripheral blood during the day. Depending on the kinetics of the donor's G-CSF response, the CD34⁺ cell concentration in peripheral blood may change significantly during the day. CE2 will be artificially high when the CD34+ count is increasing during the day and artificially low when the donor's CD34 count is decreasing during the day. However, CE2 is still a viable prediction, especially for predicting when target cell doses are reached [129].

1.3.4 Transportation and storage

Storage of stem cell grafts can be categorized into two main types. The first is short-term storage used during transport between the collection and transplantation centers. The second is long-term cryopreservation of grafts using DMSO. Cryopreservation was more commonly employed during the COVID pandemic while there were uncertainties about donor availability [130].

The recommended temperature for transporting fresh PBSC grafts is 2–8 °C, with a validated optimal temperature of 4 °C [131]. Several other studies have separately validated the storage temperature of other stem cells with similar conclusions [132,133]. Current practice recommends transport in a cooled cryo-transportation box with thermal properties to maintain temperature for the duration of the transportation [134]. A temperature log accompanies the product at all times, and transportation conditions are evaluated upon arrival at the transplantation center. Deviations from the recommended temperature intervals are investigated and discussed with the transplantation unit. The option of not using the graft or changing the donor is rarely available, but viability and cell dose can provide information about possible engraftment time and risk for graft failure [135].

Cell concentration in the graft and transportation temperature are two key variables influencing cell viability [136]. These factors are also controllable at the donor center through validated transportation and dilution of the final graft with donor plasma.

1.3.5 Graft manipulation

Clinical studies evaluating various ex vivo graft manipulations provide the clearest evidence that graft composition can influence clinical outcomes. The earliest and most direct intervention was T cell depletion. In early randomized comparisons of monoclonal antibody-treated marrow, T cell depletion reduced GvHD but increased graft failure and relapse [137]. Another commonly used graft manipulation technique is the positive selection of $CD34^+$ cells in the graft, which removes most co-contaminating cells. $CD34^+$ selection could reduce T cells to fewer than 10^4/kg, enabling transplantation from fully haplotype-mismatched family donors. However, this practice was associated with the risks of delayed T cell reconstitution, opportunistic infection, and relapse [138]. The current

refinement of this approach is to deplete only the αβ T cell receptor population together with $CD19^+$ B cells, which preserves NK and γδ T cells. In pediatric haploidentical transplantation, this produces outcomes approaching those of HLA-matched donors [139]. This also demonstrates that GvHD protection can be separated from immune competence when the depletion is precise.

Another example is the depletion of naïve $CD45RA^+$ T cells from the graft was developed to mitigate the risk of GvHD while preserving antiviral and antileukemic activity. This approach targets the naïve T cell population that mediates alloreactivity against minor histocompatibility antigens, while sparing the memory compartment that carries antiviral and antileukemic activity. This produced lower cGvHD in phase II trials: a 3-year cumulative incidence of 7%, with no severe disease and no apparent excess of relapse [140]. These results show that different cell subsets carry different clinical liabilities, and removing one shifts the risk profile rather than simply diminishing immune function [141]. In this sense, selecting and depleting different subsets in the graft highlight the clinical meaning of graft composition and that much of allo-HSCT is a balancing act between sufficient and harmful immune responses.

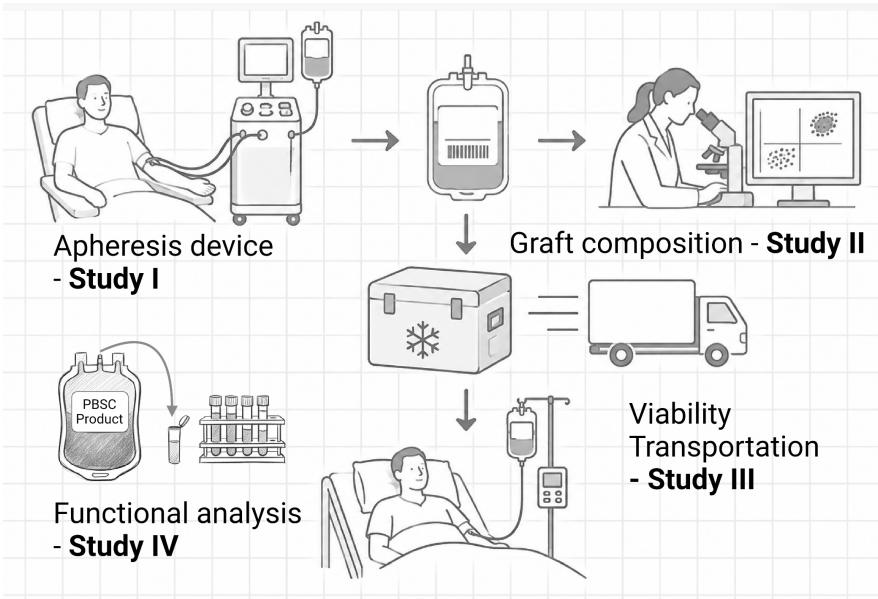


Figure 4. *Schematic overview of the studies in this thesis.*

2 Research aims

The overall aim of this thesis was to gain a deeper understanding of how the composition, handling, and function of PBSC grafts affect outcomes after allo-HSCT. Focus was placed on steps of the graft process that were less studied — apheresis collection and transportation — and on whether functional analyses of the graft could complement cell counting as quality measures.

- STUDY I: To investigate how different apheresis collection methods affect the cellular composition of PBSC grafts and whether the collection method influences clinical outcomes.

- STUDY II: To explore how lymphocyte subsets in the PBSC graft correlate with OS, TRM, GvHD, and relapse, including potentially adverse dose effects.

- STUDY III: To evaluate the impact of transportation time on apoptosis and viability in PBSC grafts, and whether graft viability affects clinical outcome.

- STUDY IV: To evaluate functional assays of PBSC graft lymphocytes using in vitro proliferative responses to mitogen and antigen stimulation. To find possible associations between the functional assay and the clinical outcome

Table 1. Descriptive overview of the study populations in Studies I–IV

Variable	Study I Apheresis device 2018	Study II Graft composition 2019	Study III Graft viability 2022	Study IV Graft function 2026
Number of patients	71	299	91	53
Study period	2011–2015	2006–2013	2014–2017	2020–2022
Donor type	Sibling 100%	Sibling 97 / URD 202	URD 100%	URD 100%
Diagnosis	70 Malignant, 1 non-malignant	Malignant only	Malignant only	51 Malignant, 2 non-malignant
Acute leukemia	32 (AML 23, ALL 9)	161 (Sib 50 [52%] / URD 111 [55%])	48 (AML 35, ALL 13)	17 (AML 14, ALL 3)
Chronic leukemia	6 (3 CML, 3 CLL)	30 (Sib 13 [13%] / URD 17 [8%])	4 (CML)	3 (CML 2, CMML 1)
Lymphoma	4	31 (Sib 10 [10%] / URD 21 [10%])	10 (lymphoma+CLL)	7 (lymphoma 4, myelosarcoma 2)
Plasma cell disease	9	14 (Sib 7 [7%] / URD 7 [3%])	9 (myeloma/Waldenström)	1 (MM)
Myelodysplastic/myeloproliferative	19	63 (Sib 17 [18%] / URD 46 [23%])	20 (MDS/MF)	24 (MDS 22, MF 2)
Non-malignant	1	0	0	2 (AA 1, SCID 1)
Recipient age, median	49 (19–71) Cobe / 51 (11–72) Optia	49 (15–71) Sib / 52 (4–71) URD	59 (14–75) low-viab / 54 (1–73) high-viab	58 (0–74)
Donor age, median	48 (21–70) / 48 (15–71)	48 (17–71) Sib / 29 (18–54) URD	28 (19–53) / 26 (19–54)	29 (19–54)
Sex (M/F)	19/12 Cobe, 22/18 Optia	53/44 Sib, 127/75 URD	24/22 / 30/15	34/64; donor 38/15
Conditioning	MAC 22 / RIC 49	Sib MAC 47/ RIC50; URD MAC 85/RIC 117	MAC 16 / RIC 76	MAC 9 / RIC 41 / other 3
ATG	1 (Cobe) / 0 (Optia)	0 Sib / 197 (98%) URD	44 / 44	—
GvHD prophylaxis	CsA/MTX 61; tacro+sirolimus 9; other 1	CsA/MTX 74+148; tacro+sirolimus 22+50; other 1+4	CsA+MTX 87; tacro+MTX 1; other 3	Cs/MTX 49; MMF/siro 1; MTX 1; siro 1
HLA match	All HLA-identical siblings	Sib HLA-identical; URD 6/8 n=4, 7/8 n=42, 8/8 n=156	6/6 matched 88/91; 3 DR1-MM	10/10 n = 48, 9/10 n = 5
CD34 dose $\times 10^6$/kg, median	8.6 (1.6–13.3) / 7.3 (2.4–10.0), p=0.05	8.1 (2.5–15.3) Sib / 7.8 (2.1–22.8) URD (p=0.54)	7.2 (3.6–10) / 7.5 (3.2–23)	7.2 (2.9–10.0)
T cells $\times 10^6$/kg	Figure 7	$CD3^+$ 386 / 321	—	$CD3^+$ 273.2 (132–653); $CD4^+$ 165 (55–371)
B cells / NK $\times 10^6$/kg	Figure 7	$CD19^+$ 83.8 / 72.2; NK 56.8 / 35.5	—	$CD19^+$ 70.2 (24–229); NK 32.3 (8–73)
TNC $\times 10^8$/kg, median	12.7 (2.4–36.4) Cobe / 13.3 (5.2–35.5) Optia	—	9.0 (4.6–19.6) / 10.0 (4.6–36.3)	—
Follow-up, median days	1461 (852–2039) Cobe / 670 (304–1339) Optia	—	313 (22–1322) / 478 (136–1336)	825 (72-1360)

3 Materials and methods

3.1 Patients, donors and clinical data

All patients and donors were managed at Karolinska University Hospital, where both the apheresis collection and the transplant procedure were performed. At the time of apheresis, standard flow cytometry panels quantified stem cell content in the donor's peripheral blood and, subsequently, in the graft component; these analyses were performed at the Stem Cell Laboratory. We retrieved clinical data from the quality registry administered by the transplantation department (CAST). The registry encompasses transplant-related variables, including diagnosis, conditioning regimen, engraftment, GvHD, relapse, and OS, as well as other clinically relevant parameters. See table 1.

3.2 Ethical implications

The Regional Ethics Review Board in Stockholm region approved all studies in this thesis in accordance with the Declaration of Helsinki. All studies were retrospective, and data were acquired within standard clinical practice. No additional sampling or invasive procedures were performed on the recipient or the donor. We maintained strict donor-recipient confidentiality during the studies, and we did not report individual donor-recipient pairs in the results. To balance ethical considerations, these studies aim to improve transplant outcomes, which would directly benefit the patient group, not the individual included in the study, by mitigating complications and reducing the need for DLI from future donors.

3.3 HLA typing

Currently at Karolinska, donors matched at 10/10 HLA loci (HLA-A, HLA-B, HLA-C, HLA-DR, and HLA-DQ) are considered fully matched. Previously low-resolution SSO typing was used for initial screening, followed by confirmatory SSP typing at four-digit resolution. NGS typing was introduced in the past years, replacing SSP for confirmatory typing. Given the time span between Studies I-IV, we used several HLA typing methods, and the definition of a fully matched donor shifted toward higher-resolution typing and better matching over the course of the

thesis. HLA matching was taken into consideration in multivariable analysis and mismatch was accounted for as a confounding factor.

3.4 Mobilization and apheresis collection

In Study I, all steps of the mobilization and apheresis process were documented in a registry within the apheresis unit. Donors received as close as possible 1 ME/kg/day of G-CSF, with dosage intervals limited by prefilled injectors containing 30 ME or 48 ME per injection. No donors received plerixafor in this study. For Studies II-IV, external collection centers handled the mobilization and donation process without our knowledge of any local methodologies for donor mobilization or apheresis device preferences.

Either two peripheral venous accesses or a single double-lumen femoral dialysis catheter could be used for apheresis, ensuring sufficient flow to establish a stable buffy-coat layer. We measured pre-collection $CD34^+$ values in peripheral blood in the morning before apheresis began, along with basic blood status parameters, to provide the apheresis operator with input parameters to adjust apheresis settings. Normal inlet flow was usually 50–70 mL/min, depending on venous access quality and donor body weight, ensuring the ACD infusion rate stayed below 1.2 mL/min/L TBV, below the level where citrate toxicity could affect the donor. On the Cobe Spectra (Terumo BCT), manual interface positioning was used to achieve a colorogram value of around 3–5% EVF, while on the Spectra Optia (Terumo BCT), a collection preference value was used to adjust the layer using the AIM system.

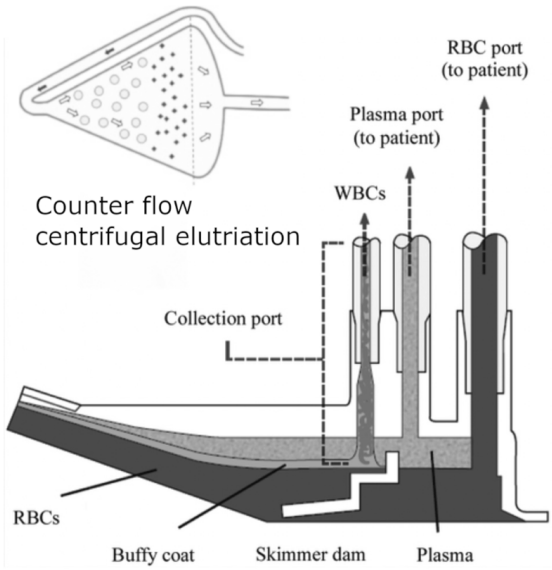


Figure 5. *Spectra Optia collection interface. The WBC port used for collection during the MNC program. The counter flow centrifugal elutriation is built in the tubing set and centrifugal forces are in the opposite direction as the flow.*

The counterflow centrifugation elutriation (CCE) in the MNC program/kit is designed to target cells based on cell size rather than relying solely on the density gradient at the Cobe interface (Fig. 5). The dual-step method, with density selection followed by size-based elutriation, was, in theory, intended to increase collection efficiency by using the highly packed buffy coat layer and then returning some of the packed platelets to the patient via elutriation. However, handling this two-step method and the time spent waiting while the elutriation chamber was flushed reduced collection speed. This is one of the main reasons why the graft composition differs between Cobe and the Optia MNC program. A newer Optia program, called cMNC, returned the apheresis process to something similar to Cobe, removing the elutriation step which was important for the MNC program.

An important note: throughout this thesis, Optia refers to the Optia MNC program. For more than 5 years, many centers have run Optia with the cMNC program, which functions differently from MNC. These results should be interpreted with the MNC program as the background, recognizing that the cMNC lacks the elutriation step, which is important for the functionalities and

graft composition acquired with MNC. Nevertheless, Study I should provide an understanding of how changes in apheresis programs can result in differences in the PBSC graft.

3.5 Transportation and storage

One of the most important aspects of Study III is transportation time and its effect on cell viability after different transport times. For all transports, a personal courier was assigned to the graft and handled the product between the sending and receiving sites. The transportation boxes used were validated for the task and designed to maintain a standard temperature of 4°C. A temperature log was mandatory for all transports and was checked upon arrival. If the graft arrived after late office hours, it was stored in a validated refrigerator in transfusion medicine overnight. Transplantation was then performed the following day. When requesting cells from an external collection center, we do not specify the transportation WBC limit. Other centers set the upper limit to between 200-300 x 10^9 leucocytes/L. At our site, PBSC requested with storage and transportation will be diluted with donor plasma to <300x10^9 leukocytes/L.

3.6 Flow cytometry – composition and viability

Flow cytometry is a core method used in these studies to describe the cellular composition of peripheral blood or graft [142]. The basics of the method involve labeling cells with fluorochromes that emit different wavelengths when excited by a specific laser. A sheath fluid creates a laminar flow, and cells pass a laser and detector in a single-cell stream, recording emissions from various cells. Size and granularity are recorded through forward and side scatter. In combination with detected extracellular or intracellular markers, cells can then be categorized through a series of gating strategies.

Graft cellular composition and viability were assessed by flow cytometry at the Stem Cell Laboratory, Karolinska University Hospital, Huddinge. Leukocyte counts in the graft samples were measured on an automated blood cell counter (Sysmex) to determine the absolute nucleated cell content before staining.

$CD34^+$ cells were enumerated by single-platform flow cytometry using the ISHAGE gating strategy (International Society for Hematotherapy and Graft

Engineering) for all the studies [143]. The absolute $CD34^+$ count was calculated relative to the bead count and reported as $CD34^+$ cells × 10^6/L in peripheral blood, and $CD34^+$ cells × 10^6/kg recipient body weight in the product.

Lymphocyte subsets were quantified in the graft using slightly different methods across studies due to time differences and an upgrade in flow cytometer from FC500 to DxFlex in the clinical routine. The primary lymphocyte panel was the T B NK cell panel with CD45 /CD56 /CD19/CD3/CD16. Regulatory T cells was defined as $CD4^+CD127^{low}$ $CD25^{high}$ (CD3, CD4, CD127, CD25) and measured in the grafts for Study II. Study IV used a nine-color panel (CD3, CD16, CD56, CD45, CD4, CD19, CD8, CD25, CD127) which simplified the staining and acquisition with better resolution between colors with more modern equipment with multiple lasers (acquired on a DxFlex, Beckman Coulter).

Viability was measured for Study III with two dyes marking different stages of cell death: early apoptosis is detected by Annexin V binding to PS translocated to the outer plasma membrane, while loss of membrane integrity is marked by 7-AAD intercalation in dying (necrotic or late-apoptotic) cells [144]. Graft viability was defined as the percentage of cells double-negative for Annexin V and 7-AAD, early apoptotic cells as Annexin V-positive/7-AAD-negative, and necrotic cells as all 7-AAD-positive cells (Fig. 6). The distinction between necrotic and early apoptotic cells is not clear-cut but reflects markers of accumulated cell damage during graft handling and transport.

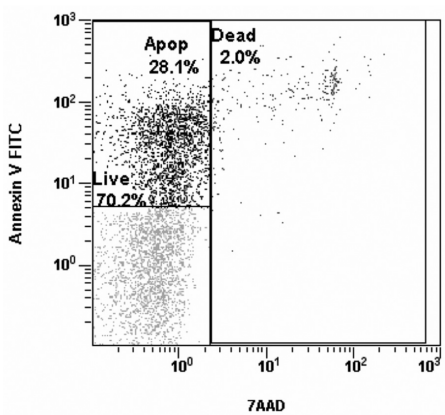


Figure 6*. Gating strategy for viability markers. Lower left gate: Live cells (Annexin V negative, 7-AAD negative). Upper left gate: Apoptotic cells (Annexin V positive, 7-AAD negative). Right gate: necrotic cells (Annexin V +/-, 7-AAD positive).*

In addition to phenotyping, graft lymphocyte function was assessed in vitro by blast formation upon mitogen and superantigen stimulation, expression of activation markers (PD-1 and HLA-DR), and cytokine production — the assay details are given under "Functional graft assay."

3.7 Functional graft assay

The functional assay involves stimulating the PBSC graft for 7 days with various mitogens/antigens, with blast count serving as a readout of proliferation. Supernatant from the stimulated cells was stored for further cytokine analysis. Detailed description can be found in the methods description of Study IV.

This method was adapted from a previously described method for Flow cytometric Assay of Specific Cell-mediated Immune response in Activated whole blood (FASCIA), which has been published in several studies [145–147]. One difference with PBSC compared with whole blood is that it contains ACD as an anticoagulant, which binds calcium. Extracellular calcium is important for the activation of T cells and therefore extra calcium is added to the sample to neutralize any residual ACD before stimulation [148]. A further modification to this protocol compared with previous studies was the inclusion of PD-1 and HLA-DR markers. These markers were initially added to confirm the activation status of the blast population; however, their inclusion provided a separate readout following stimulation.

3.8 Cytokine analysis

Cytokines in the supernatant after stimulation were measured using the Luminex xMAP technology [149]. The method is a multiplexed, bead-based sandwich immunoassay in which each analyte is assigned to its own microscopic bead set [150]. The bead sets are internally labeled with two fluorophores in defined proportions, giving each set a unique spectral address — analogous to the distinct bead populations sorted in flow cytometry. Each bead set is coated with an antibody specific for one cytokine, allowing a single small-volume sample to be incubated with multiple different capture antibodies simultaneously [151]. Cytokine captured from the sample is detected by a biotinylated secondary

antibody and quantified using streptavidin-conjugated phycoerythrin (PE). The median fluorescent intensity per bead set was converted to concentration using a standard curve, and multiplex standard-curve analysis was applied accordingly.

3.9 Statistics

Descriptive results are presented as medians with ranges for continuous variables and as counts with proportions for categorical variables. Given the limited sample sizes and departures from normality in several continuous variables, non-parametric methods were used throughout. Continuous variables were compared between groups with the Mann–Whitney U test. Categorical variables were compared with the chi-square test, or with Fisher's exact test when expected cell counts were fewer than five. Non-parametric associations between graft variables were assessed with Spearman's rank correlation.

3.9.1.1 Survival analysis

Overall survival (OS) was defined as the time from transplantation to death from any cause, with patients alive at last follow-up censored. Survival probabilities were estimated with the Kaplan–Meier method and compared between groups with the log-rank test. Factors associated with OS were analyzed with the Cox proportional hazards model.

3.9.1.2 Competing risks

TRM and relapse were modeled as competing events, with death in remission competing with relapse and vice versa; the cumulative incidence of each was estimated while accounting for the competing risk. Acute and chronic GvHD were analyzed as time-to-event endpoints with death as a competing event [152]. In Studies I–III the associations between graft cell doses and TRM, relapse, and GvHD were analyzed using the proportional subdistribution hazard regression model of Fine and Gray.

Endpoint	Event of interest	Competing event
Relapse	First relapse	Death before relapse
Transplant-related mortality (TRM)	Death without prior relapse	Relapse
Grade II–IV acute GVHD	First attainment of grade II–IV acute GVHD	Death before that endpoint
Grade III–IV acute GVHD	First attainment of grade III–IV acute GVHD	Death before that endpoint
Chronic GVHD	First onset of the specified chronic-GVHD category	Death before that endpoint

***Table 2**. List of endpoints and competing events.*

In **Study IV**, relapse was instead analyzed with cause-specific Cox regression, in which competing events are censored and the hazard describes the event rate among patients still alive and event-free. This setup addresses the etiological question, whether a graft property changes the relapse rate, and the hazard ratio remains interpretable even when several risks are small-numbered.

3.9.1.3 Multivariable models

For multivariable models, factors with a p-value below 0.10 in the univariable analysis in Studies I–II, below 0.40 in Study III, and below 0.50 in Study IV were entered into backward-elimination multivariable analysis, with factors detailed in the studies. In Study IV, a series of sensitivity analysis were also performed to test the robustness of the models, adding clinically relevant factors into the models rather than a p-value-driven backward elimination. CMV reactivation within three months after transplantation was analyzed with logistic regression, and results are reported as odds ratios.

A p-value below 0.05 was considered statistically significant in all studies.

Analyses were performed in R (3.3.1 - 4.4.1, R Core Team, Vienna, Austria), and Stata version 14 (StataCorp, College Station, TX).

4 Results

The graft that reaches the patient is the product of a chain of small steps, including donor selection, mobilization, apheresis, transportation, and storage, each of which can affect its cellular composition and functional state before any clinical variable has come into play.

The following sections will highlight the major findings from the four studies and how they relate to the flow from donor to patient. The clinical implications and outcomes illustrate the complexity of allo-HSCT: no single factor in the graft determines the entire outcome, but small changes at the start of transplantation can sometimes increase the chances of a positive outcome after allo-HSCT.

4.1 The effect of collection method and association with graft cellular content

Study I was initiated by the observation that grafts collected using the Cobe and Optia devices differed in product volume and processed blood volume. This prompted us to examine additional graft data, which also revealed differences in lymphocyte yield. During the same period, the transplant unit reported a lower incidence of grade III–IV aGvHD, with no other major changes in clinical practice. Given these observations and the established role of lymphocyte dose in GvHD development [106,153–155], we hypothesized that the change in apheresis device might be associated with clinical outcome.

4.1.1 Graft composition in Cobe vs Optia

All 71 patients in **Study I** received PBSC grafts from HLA-identical sibling donors, collected by leukapheresis at Karolinska University Hospital between 2011 and 2015, without post-collection manipulation. During the study period, the apheresis device was changed from Cobe Spectra to Spectra Optia, creating a random comparison of collection methods in which donor and patient selection remained unchanged: 31 grafts were collected with Cobe Spectra and 40 with Spectra Optia. All the important graft changes are stated in Table 3. Note the roughly 30% difference in product volume, which consists of dilution based on flushing of the elutriation chamber. The TNC between Optia and Cobe remained comparable, as well as the product EVF.

	Cobe	Optia	p-value
Pre-collection peripheral blood			
CD34 (x 10^6/L)	**39.5** (7 – 174)	**37.7** (6 – 163)	n.s
WBC (x 10^9/L)	**41.8** (25.5 - 72.3)	**38.4** (12.3 - 65.4)	n.s
PLT (x 10^9/L)	**181.5** (52 – 303)	**182** (51 – 293)	n.s
EVF (%)	**39.4** (34.4 - 42.8)	**41.5** (32.4 - 46.1)	0.001
Efficacy (CE2 %)			
CD34	**50.4** (22.9 – 92.5)	**49.9** (24.9 – 117)	n.s
CD3	**59.2** (37.7 - 105.8)	**49.5** (16.1 - 134.5)	0.002
CD19	**68.0** (28.78 - 257.1)	**61.1** (21.62 - 170.6)	0.007
CD56	**59.8** (30.0 - 159.6)	**50.5** (14.6 - 116.8)	n.s
Processed blood volume	**18** (10.8 - 25)	**14.3** (7.3 - 19.5)	< 0.001
Graft composition			
Volume (ml)	**299** (205 – 447)	**408** (137 – 653)	< 0.001
WBC (x 10^9/L)	**243** (133 – 385)	**196** (107 – 311)	< 0.001
PLT (x 10^9/L)	**2177** (472 – 4820)	**1210** (240 – 2340)	< 0.001
EVF (%)	**2.05** (0.8 – 4.2)	**2.1** (0.8 – 5.2)	n.s
TNC (x 10^9)	**75** (33.7 – 152)	**77.8** (25.5 – 133)	n.s
MNC (%)	**76.5** (38 – 95)	**56.5** (27 – 89)	< 0.001
CD34 (x 10^6/L)	**1388** (242 – 3760)	**778** (162 – 2701)	< 0.001
CD3 (x 10^9/L)	**74.7** (35.7 – 142)	**38.1** (12.9 – 92.7)	< 0.001
CD19 (x 10^9/L)	**16.3** (7.3 - 40.1)	**9.2** (2.24 21.1)	< 0.001
CD56 (x 10^9/L)	**10.88** (3.2 - 28.0)	**4.74** (2.31 - 11.5)	< 0.001

Table 3: *Pre-collection values, collection efficiency and graft composition, a comparison between Cobe and Optia. CE2 – Collection efficiency 2, WBC – white blood cell count, PLT – platelets, EVF – Erythrocyte volume fraction, TNC – Total nucleated cells, MNC – Mononuclear cells.*

The lymphocyte collection efficiency (CE2) was higher in the Cobe compared with the Optia. Lymphocyte CE2 was on average around 10% higher on the Cobe compared with the Optia, whereas $CD34^+$ CE2 did not differ between the two machines. Previous validations and comparisons between these apheresis devices has mainly compared $CD34^+$ yield as the standard target fraction [156]. These lymphocyte CE2 values on the Optia fall within a lower range compared with steady-state DLI apheresis or CART apheresis, where CD3 CE2 usually are around 60-80% (unpublished data on cMNC).

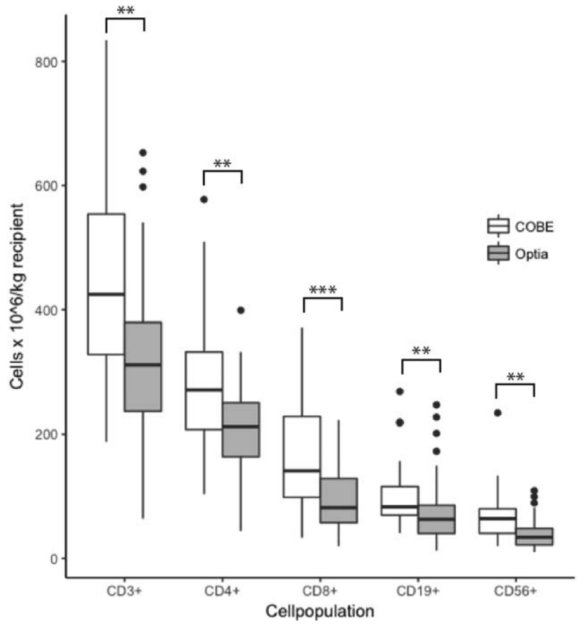


Figure 7. *Lymphocyte dose received by the patient (cells/kg recipient), comparing Cobe and Optia. * p < 0.05. ** p < 0.01. *** p < 0.001.*

However, the CE2 differences and processed blood volumes together resulted in a significantly lower lymphocyte dose administered to patients with Optia grafts compared with Cobe grafts (Fig. 7). The difference in $CD34^+$ dose was not large but still borderline significant, with Cobe at a median of 8.2 $x10^6$/kg and Optia at 7.3 x 10^6/kg ($p < 0.05$).

4.1.2 Possible reasons for the discrepancies in graft composition

Cell composition in the graft depends on how the buffy coat layer is separated in the centrifuge and which layer we collect the cells from. The Cobe and Optia differs in several important steps in the separation of cells and how to target the right cell type. The Cobe and Optia differ in how densely the buffy coat layers are packed and in what the operator can control. See section 3.4 for methods.

As noted above, elutriation selects cells based on size. However, the literature provides limited evidence for distinguishing $CD34^+$ cells from normal

lymphocytes by size alone. Smaller lymphocytes could theoretically pass through the CFC system without being collected and returned to the donor; however, this possibility remains speculative in the absence of measurements from the elutriation kit.

Another important factor, difficult to account for, is the individual adjustments made by the apheresis nurse. The apheresis nurse makes multiple important calls during the collection, based on experience, to best optimize the collection. A clean venous access, a relaxed patient, and a stable and optimal collection interface are crucial for a successful apheresis collection. Complications during collection, such as clotting in the machine, could greatly impact graft composition; however, these factors were not recorded between machines and were assumed to be randomly distributed.

A previous study has shown a difference in graft composition between unmobilized donors using Cobe and Optia [157]. Two major weaknesses with that study was that one procedure was set on a standard time, while the other endpoint was set as a set number of processed blood volume. This could be because the Optia needs to pause when emptying the elutriation chamber, resulting in lower processed blood volume over the same unit of time. At the same time, the rinsing step to empty the elutriation chamber creates a larger product volume because it requires fluids to flush the chamber. The final PBSC product consists of a larger volume and lower cell concentration for the Optia. This could in theory be beneficial when transporting the PBSC product with sufficient plasma and nutrients, which has been described in previous studies, but not evaluated in **Study I** [134].

4.1.3 Differences in clinical outcome

Comparison between the two apheresis devices showed a difference in aGvHD grades II–IV, with an incidence of 23% for the Optia recipients versus 45% for the Cobe recipients. This finding was even stronger when comparing severe aGvHD grade III–IV between the groups (5% vs 23%, $p = 0.03$) (Fig. 8).

Considering the relatively large difference in aGvHD grade III–IV, it was no surprise that TRM also differed between the groups (2.5% versus 16%, $p < 0.05$). However, we did not further analyze the cause of TRM in these cases. For some reason, patients receiving Optia grafts had a significantly higher risk of cGvHD. The data clearly showed a higher incidence of cGvHD among Optia patients. We

did not stratify cGvHD based on severity which would have been useful to determine the characteristics of the complication.

In the multivariable analysis, the $CD3^+$ dose was the strongest predictor of aGvHD grade II-IV and III-IV. The apheresis machines were not significant in this analysis. A possible cause could be the strong collinearity between the use of Cobe and $CD3^+$ dose in the product. For cGvHD, patients receiving grafts from the Optia was the strongest predictor. There was also a small but significant correlation between $CD19^+$ dose and cGvHD. Relapse and OS did not differ between the devices.

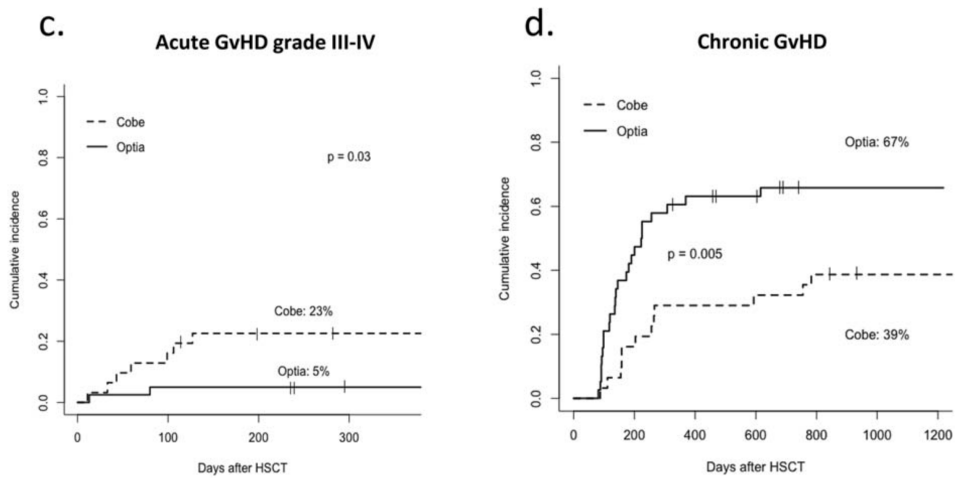


Figure 8. *Acute and chronic GvHD, cumulative incidence comparison between Cobe and Optia.*

These findings are in line with previous findings, as high $CD3^+$, especially $CD8^+$ are associated with aGvHD pathophysiology [158]. It is still uncertain whether the difference in cell dose, roughly 30% difference between apheresis devices, would be clinically significant, given that PBSC grafts contain 3-10 times more lymphocytes than BM grafts [159]. BM and PBSC are not directly comparable because of several differences between these grafts, including G-CSF-driven T cell skewing, possible co-contaminating cells in BM, and other factors that influence graft function. This study standardizes the comparison across several confounders by using both apheresis devices at the same center, with the same graft handling and no transportation time. All subsequent clinical treatments of the patients were the same, largely because the transplant unit was effectively blind to which apheresis machine was used for which patient.

The direction of the effect is unfortunately not uniformly beneficial. One possible explanation is that the two devices collect a different cellular product. Interface-based two-stage separation collects a more defined mononuclear fraction, and the reduced lymphocyte co-collection reduces acute GvHD, which is associate with graft $CD3^+$ content. However, the higher cGvHD incidence in this group is difficult to understand. A larger cohort from our transplant center, proposed several possible explanations [160]: one was that no ATG was given to HLA-matched sibling donors. The second reason could be that patients with URDs receive longer and higher doses of immunosuppression compared with patients receiving grafts from related donors. This still does not explain why cGvHD differed between apheresis machines, given that aGvHD was associated in the opposite direction. It would have been interesting to examine the lymphocyte profiles in more detail based on cell size.

In conclusion, **Study I** compared two apheresis devices in terms of cellular composition and clinical outcomes. It has been demonstrated that a purely technical change, not a change in donors, patients, or transplant indication, shifted the clinical risk profile, establishing collection methodology as a modifiable determinant of graft composition with measurable clinical consequences. Several other apheresis systems are available, and new programs have been introduced for existing devices. Further standardized comparisons among apheresis devices are warranted.

4.2 Graft lymphocyte composition – the current perspective

The lymphocyte composition of grafts is monitored at our clinic to assess and ensure the quality of all cellular products processed by the stem cell laboratory. **Study II** used these data to examine the association between graft lymphocyte content and clinical outcomes. The findings should be interpreted in light of the dataset's age, as current clinical practices have improved the outcome. The following section relates each major finding of **Study II** to recent evidence on graft lymphocyte content and clinical outcomes.

4.2.1 Cohorts and clinical outcome

In Study II, 299 patients with malignant hematological diseases (97 HLA-identical related donors, 202 unrelated donors; transplanted 2006–2013) were analyzed,

correlating the flow-cytometric content of the graft, $CD34^+$ cells, $CD3^+/CD4^+/CD8^+$ T cells, $CD19^+$ B cells, NK cells and regulatory T cells ($CD4^+$ $CD127^{low}$ $CD25^{high}$), with clinical outcomes. The cohorts were analyzed separately, due to ATG usage in the URD group compared to no ATG for the sibling group. Five-year overall survival was similar between cohorts, but relapse incidence was lower with sibling donors (24% vs 35%, p = 0.04). (Fig. 9)

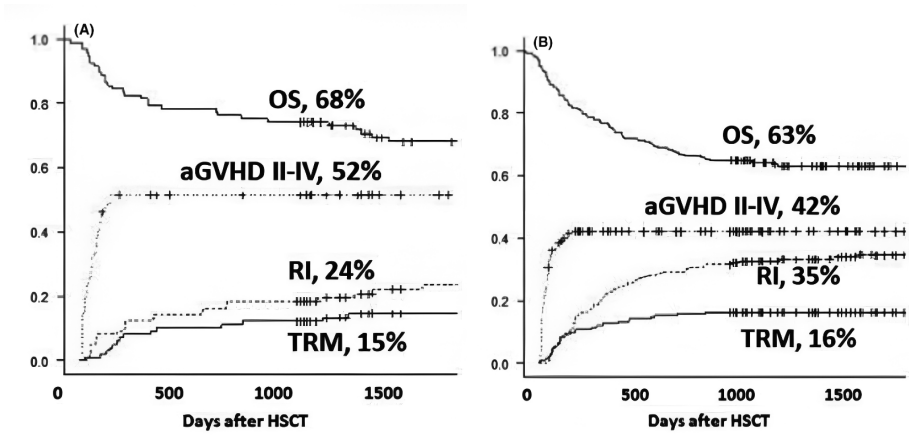


Figure 9. *Cumulative incidence in patients with a) sibling donors b) unrelated donors. aGVHD, acute graft-versus-host disease grade II to IV; OS, Overall survival; RI, relapse incidence; TRM, transplant-related mortality*

4.2.2 $CD8^+$ lymphocyte dose and relapse

In the URD cohort, a higher $CD8^+$ T cell dose was associated with significantly lower relapse (HR 0.94 per 10^8/kg, 95% CI 0.90–0.98, p = 0.006), indicating a dose-dependent GvL signal that can be measured in the graft before infusion. Although this finding is robust, it remains unclear which $CD8^+$ subsets contribute to the lower relapse risk. Further analysis of the specific subsets involved would be valuable, given that naïve, memory, and effector T cells have different GvL effects. Clinical studies of naïve-T cell-depleted PBSC transplantations suggest that substantial GvL can be retained in memory-containing grafts, while experimental studies demonstrate GvL activity in both central- and effector-memory populations. [161,162]. These findings do not establish an optimal naïve-to-memory composition or subset-specific dose for relapse prevention. A more recent study evaluated graft $CD8^+$ doses in the haplotransplantation setting with ATG and found improved survival and disease-free survival among patients receiving grafts with CD8+ doses above 0.85 × 10^8/kg [163]. There was also a negative correlation between age and $CD8^+$ dose in the graft, where younger

donors with high lymphocyte dose resulted in better survival [164]. A large study (2736 patients) from Center of International Blood and Marrow Transplant Research (CIBMTR) from 2019 had a similar setup as **Study II** with both related and unrelated donors. They found no correlation between $CD3^+$ doses and any major clinical outcome. The authors commented on the CD4/CD8 ratio and further analysis, but concluded the data on $CD3^+$ subsets were too small for more detailed analysis [165]. This will also be further discussed in section 4.2.4.

Taken together, the findings are not contradictory, they may simply reflect differences in resolution. The CIBMTR analysis tested total $CD3^+$ counts, whereas the relapse signal in **Study II** emerged only when $CD8^+$ cells were analyzed as a separate subset. Even $CD8^+$ cells, however, are a crude representation of a multitude of subsets. The haploidentical data support the same interpretation: a survival advantage appears at a certain $CD8^+$ graft dose and persists in the haploidentical setting despite differences in conditioning and HLA matching. Although selecting donors based on $CD8^+$ cells remains impractical, donor age appears to correlate well with $CD8^+$ graft content and could therefore be taken into consideration.

4.2.3 High $CD34^+$ dose and negative outcome

Conversely, a higher $CD34^+$ dose was associated with both increased TRM (HR 1.09, p = 0.02) and increased relapse (HR 1.09, p = 0.025); in quartile analysis, the two highest $CD34^+$ quartiles carried significantly more TRM (p = 0.029). This finding is in line with previous publications that high $CD34^+$ dose can be associated with poor outcome [153,166,167]. However, several other more recent studies did not show any correlation between $CD34^+$ doses in the grafts and outcomes such as TRM or relapse [135,168,169]. Very low $CD34^+$/kg cell dose has been associated with poor outcome, as well as higher doses. The idea of an optimal $CD34^+$ dose exists and there are several proposed doses ranging from 2.5 -11 x 10^6/kg to 4 - 8 x10^6/kg [167,170]. These data have prompted changes in clinical practice by limiting the $CD34^+$ doses for URD to < 10 million $CD34^+$/kg. We have not evaluated data from the current era, in which plerixafor, a CXCR4 inhibitor, is used to boost poor mobilizers to reach their target. Improved mobilization would probably significantly change graft composition relative to $CD34^+$ doses, with several subsets changes compared to only G-CSF mobilized grafts [123].

4.2.4 CD4/CD8 and CD19⁺ in the graft correlates with GvHD

In the sibling cohort, a higher CD19⁺ B-cell dose was associated with aGvHD (HR 1.09, p = 0.003) and TRM (HR 1.09, p = 0.036). This finding is in line with some previously published data surrounding the association between B cells and aGvHD and TRM [106]. High numbers of B cells in the graft have previously been linked with both aGvHD and cGvHD while there are multiple suggested mechanisms of action [107]. It has been shown that B cell presence is required for some T cell priming against minor antigens in animal models for aGvHD could be one of the reasons [171]. Other mechanisms especially BAFF signaling has been shown to play an important role in cGvHD [172]. B cell depletion with Rituximab administered as part of the conditioning regimen has shown positive effects, including a lower incidence of aGvHD [173]. However, there are no current recommendations to use Rituximab or other B cell depletion as treatment of aGvHD. More recent publications on regulatory B cells, TIM-1+ or CD1c+, have shown immunosuppressive effects in mouse models and GvHD models [174,175]. Again, this highlights the different subsets within B cell population that could have opposite effects in the regulation and pathogenesis of GvHD. The role of B cells in aGvHD remains controversial.

A higher CD4/CD8 ratio was also associated with aGvHD (HR 1.51, p = 0.02), and a higher CD4⁺ dose showed a trend toward cGvHD (HR 1.02, p = 0.06) in our study. The graft CD4/CD8 ratio has been associated with adverse outcomes in the haploidentical setting. In G-CSF-primed BM grafts, a high ratio was associated with aGvHD and inferior survival [176,177]. The ratio has also been translated into clinical practice: in a randomized trial of 228 haploidentical transplantations, low-dose corticosteroid prophylaxis directed by the graft CD4/CD8 ratio reduced grade II–IV aGvHD from 48% to 21% [178]. In PBSC grafts, however, the evidence base is limited. The largest registry study of 2,736 PBSC recipients analyzed CD4⁺ and CD8⁺ doses and their ratio, but the cohort was underpowered for subset and ratio analyses [165]. Ratios measured after transplantation point in the same direction: in pediatric recipients, a higher and earlier post-transplant CD4/CD8 ratio was associated with subsequent grade II–IV aGvHD [179]. Whether the pre-infusion graft CD4/CD8 ratio carries comparable information in HLA-identical sibling PBSC transplantation had not been established before **Study II**.

Study II showed that lymphocyte subset composition carries information independent of the standardized $CD34^+$ count: the graft is an immune system in miniature, and its T cell, B-cell and regulatory compartments are measurable and clinically meaningful. This is in line with for example a growing literature in which graft $CD8^+$ and γδ T cell frequencies predict relapse and CMV reactivation [180,181], and in which T_{reg} content has been associated with improved outcome in a systematic review [182]. The most counterintuitive finding of this study is that $CD34^+$ dose as the parameter most routinely optimized, reported, and validated, was associated with poor outcomes at high doses in the URD cohort, for both TRM and relapse. It might be that $CD34^+$ count serves as a surrogate for the total co-collected cellular product rather than being detrimental in itself. This finding is consistent with the complex picture in the broader $CD34^+$-dose literature, in which the optimal dose depends on graft source, T cell depletion strategy, and donor type [183].

4.3 Viability and transportation

Unrelated-donor grafts must often travel from collection at a donor center abroad or elsewhere in Sweden to infusion at our transplant center. The graft spends one to two days in validated cooled transport boxes at 2–8 °C, under the care of a courier responsible for the graft's safety. Previous publications have described a decline in graft viability over time, with viability varying across cell subsets [131,184]. In **Study III**, we analyzed PBSC graft viability in relation to transportation time and to evaluate the effect of viability on clinical outcome.

4.3.1 Graft viability – Apoptosis and necrosis

We analyzed 91 unrelated-donor PBSC recipients (2014–2017), assessing graft viability by Annexin V/7-AAD flow cytometry, which distinguishes early apoptosis (phosphatidylserine externalization, intact membrane) from late apoptotic and necrotic cells (membrane loss). Grafts transported for two days had lower viability than grafts transported for one day (median 83.2% vs 86.4%, $p = 0.003$, Fig. 10).

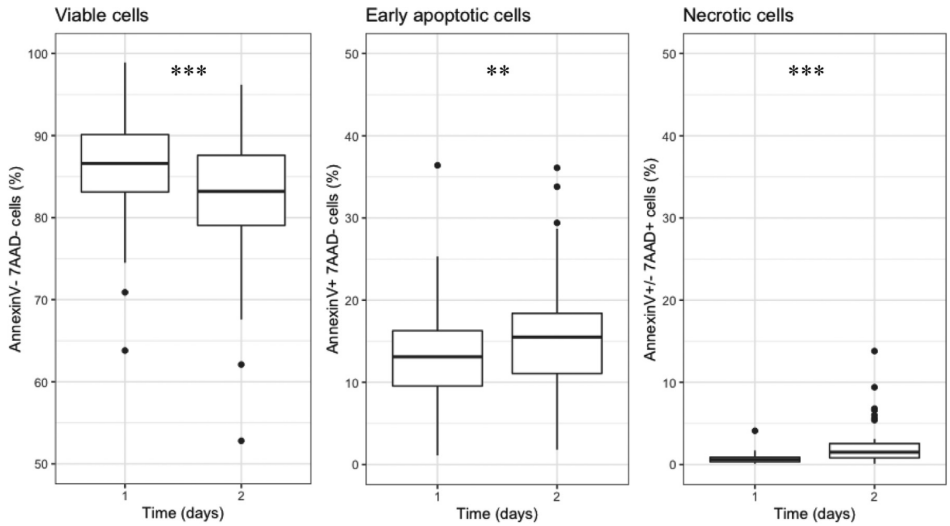


Figure 10. *Percentage of graft cells positive for apoptotic or necrotic markers. The box plots are divided based on 1 or 2 days of transportation. Plots from left to right: (a) Viable cells: Annexin V and 7-AAD-negative cells. (b) Early apoptotic cells: Annexin V positive and 7-AAD-negative. (c) Necrotic cells: 7-AAD-positive and Annexin V positive or negative cells. *P < .05, ** P < .01, *** P < .001.*

The decrease in graft viability was not evenly distributed between necrotic and apoptotic cells. Grafts contained significantly more Annexin V–positive cells than 7-AAD–positive cells, suggesting that transport-related damage was insufficient to cause complete loss of membrane integrity, although phosphatidylserine (PS) exposure had begun. It has been shown that apoptotic cells expressing PS can be reversed in animal models [118], but cells expressing the PS signaling as a 'eat-me' signal are highly likely to be cleared out of circulation after infusion [185].

The cellular composition of the graft was not subdivided into lymphocyte or granulocyte subsets. It would have been interesting to analyze the viability of $CD34^+$ cells separately and to see if the viability was comparable to other important lymphocyte subsets. A small unpublished analysis of viability of $CD3^+$ cells in **Study III**, showed higher viability in the $CD3^+$ population, compared with overall graft viability, in line with previous observations that different cell types are more or less susceptible to storage damage [131,186].

4.3.2 Survival based on graft viability

When the cohort was divided at the median viability of 85.1%, recipients of low-viability grafts had inferior one-year survival compared with recipients of high-viability grafts, and high graft viability remained an independent predictor of survival in the multivariable analysis (HR 0.26, 95% CI 0.09–0.77, p = 0.015) with a corresponding effect on TRM (HR 0.16, p = 0.027). Acute and chronic GvHD and relapse did not differ between the viability groups.

Detailed analysis suggested that the decreased one-year survival was driven by early relapses and infectious complications. The low-viability group had five TRM cases attributed to infectious complications and PTLD, whereas none were observed in the high-viability group. Even if this was not statistically significant, it is still worth noting (Fig. 11). The graft-derived early immune constitution is crucial for protection against early infections and, in high-viability grafts, could benefit the patient.

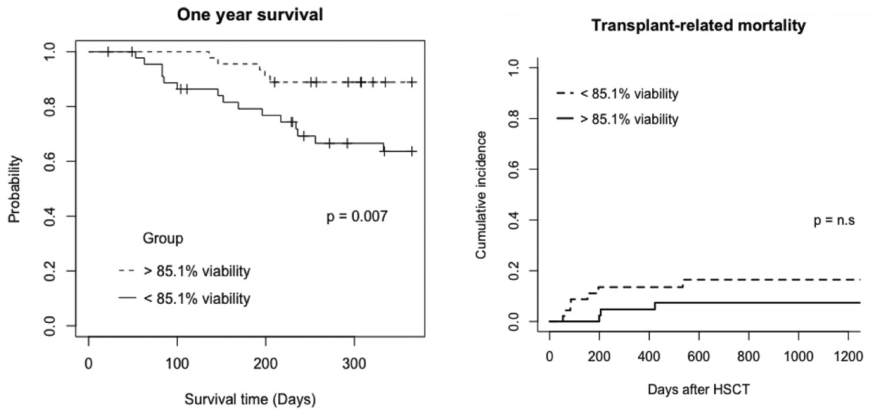


***Figure 11**. Left panel: One-year survival comparing low- and high-viability grafts. Right panel: Transplant-related mortality comparing low- and high-viability grafts. n.s. = Not significant.*

This point is countered by the fact that ATG was administered to 96% of the patients, removing much of the graft derived lymphocytes. Whether transportation-related injury alters ATG binding or susceptibility to ATG-mediated depletion remains unclear. Although ATG-mediated cytotoxicity depends on cellular activation state [187], preferential depletion of storage-damaged PBSC cells has not been demonstrated to our knowledge. However, some graft-derived $CD8^+$ memory-phenotype cells remain and may support early reconstitution [46]. Other subsets, such as $\gamma\delta$ T cells, are also important for early infection control but have not been characterized in these grafts.

Graft quality has previously been shown to affect clinical outcome. An earlier study from our center showed that 7-AAD viability measured in the frozen-thawed control vial could be regarded as a surrogate marker for the fitness of the graft. Decreased viability in the control vials was associated with increased risk of aGvHD grades I–IV and with TRM [188]. On this basis, we hypothesized that the apoptotic content of the graft itself, measured before infusion, would influence the risk of GvHD, TRM and possibly relapse, given the well-described immunomodulatory role of apoptotic cells in transplantation and autoimmune disease [113,189,190]. However, in this cohort neither GvHD incidence nor relapse risk correlated with graft viability, while the effect on outcome was confined to TRM in a multivariable analysis.

To summarize the findings in **Study III**. Transportation decreased graft viability. Second, the survival penalty operated through TRM, consistent with impaired early immune system and engraftment-associated toxicity, rather than through any measurable rebalancing of GvHD or relapse. Notably, the pre-study hypothesis that apoptotic cells would reduce the risk of GvHD, was not confirmed in this study [114,191]. Viability loss did not protect against GvHD, but cost survival. Transportation of PBSC is not just a logistical detail, but a step in the allo-HSCT process that should be optimized.

4.4 The functional analysis of the graft

In **Study IV** we wanted to evaluate the immunological function of the graft before infusion to the patient. To the best of our knowledge, there is no standardized method for measuring graft function other than CFU assays. The CFU assay reflects more the engraftment potential rather than immunological function. For **Study IV**, we measured activation after mitogen or antigen stimulation, based on a previous study that found a correlation between infectious complications and the graft's response to antigen or mitogen stimulation [147]. We aimed to explore graft activation in more depth and hypothesized that graft function was not limited to infectious complications, but that graft fitness, measured by the ability to proliferate after stimulation, could also be associated with GvHD, relapse risk, and overall survival.

Fifty-three unrelated-donor PBSC grafts, between 2020–2022, were analyzed using the ex-vivo proliferation assay described in the methods. Two main findings emerged.

1. Recipients whose grafts showed low proliferative responses to PWM and SEA had a significantly higher risk of CMV reactivation ($p = 0.013$, $p = 0.003$).
2. Recipients of grafts with above-median PD-1 expression on activated $CD8^+$ blasts had a higher relapse incidence (18% vs 50%, $p = 0.02$) and lower overall survival.

4.4.1 CMV reactivation and graft function

For robustness testing we compared the lymphocyte response of known sero positive and negative donors. Seropositive donors exhibited higher responses than seronegative donors. Although all seronegative donors showed very low IFN-γ production in response to the CMV-specific antigen, responses among seropositive donors were heterogeneous (Fig. 12). Interestingly, lower reactivity to CMV stimulation did not correlate with CMV reactivation after transplantation.

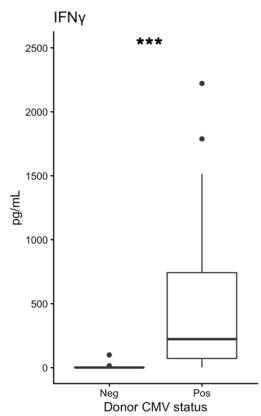


***Figure 12**. Donor CMV status and IFNy production after stimulation. p < 0.001*

Instead, we found that blast formation after PWM and SEA stimulation correlated with CMV reactivation. We also assessed other common post-transplant infections, including bacterial and fungal infections, as well as BK and EBV infections, and found no significant associations with activation after mitogen stimulation. (Fig. 13)

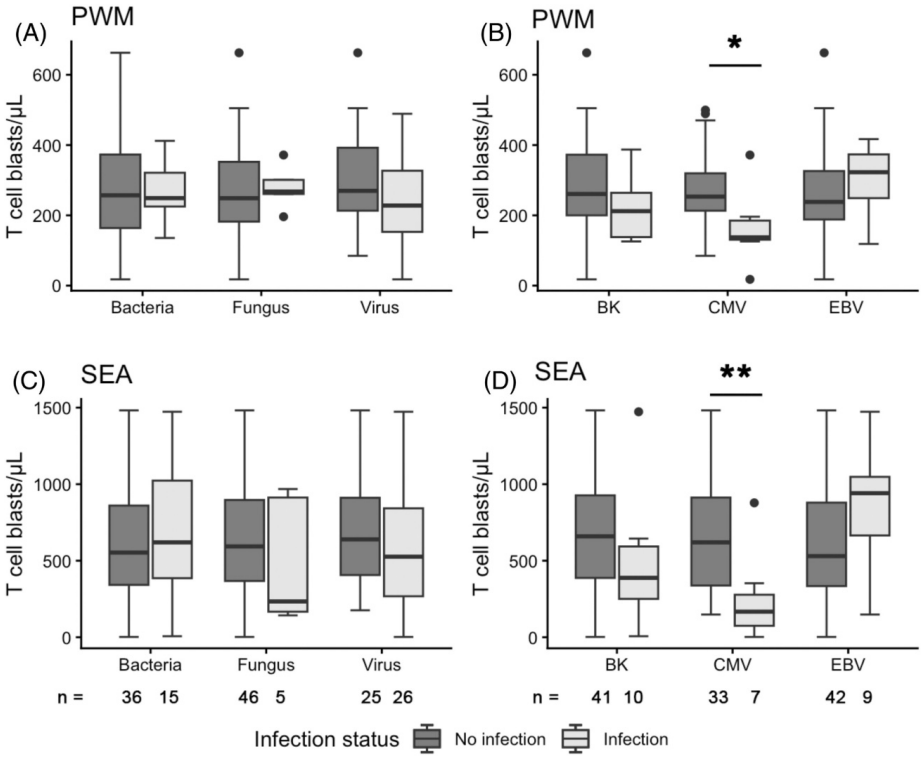


Figure 13. *CD3⁺ blast formation after pokeweed mitogen (PWM) and Staphylococcal enterotoxin A (SEA) stimulation. (a and c) Differences in the concentration of T cell blasts after stimulation with SEA/PWM between patients with or without bacterial, fungal, or viral infections/reactivations within 3 months post-transplantation. (b and d) Differences in the concentration of T cell blasts after stimulation with SEA/PWM between patients with or without the most common viral reactivations within 3 months post-transplantation (human polyomavirus 1 BK, cytomegalovirus [CMV], Epstein Barr virus [EBV]). T cell blast concentration after PWM and SEA stimulation correlated with CMV reactivation within 3 months post-transplantation. *p < .05, **p < .01.*

4.4.2 CMV-specific response vs overall graft response

An interesting aspect of the **Study IV** findings deserves discussion: the response to CMV-specific stimulation of the graft did not predict CMV reactivation, whereas the responses to the broadly acting stimuli PWM and SEA did. Several, non-exclusive explanations are possible. First, the two classes of stimuli engage two different layers of graft function. PWM and SEA bypass antigen processing and HLA-restricted presentation and thereby measure the overall activation and proliferation capacity of the entire graft. In contrast, CMV-specific stimulation stimulates a narrow memory population whose in vitro response also depends

on HLA restriction, the peptide mix used and the antigen-presenting content of the product. Second, all recipients in the cohort received ATG for in vivo T cell depletion. ATG removes the infused T cells, including the CMV-specific memory population measured before infusion. The antigen-specific response is thus functionally dampened in vivo, whereas the general fitness and proliferative robustness of the graft cells remain properties of the new immune system during reconstitution. Third, CMV viral control is not purely αβ T cell driven [192–195]. The reduced γδ T cell response in grafts to reactivating recipients, together with the known contribution of γδ T cells and NK cells to early CMV control, supports the interpretation that the HLA-independent arms of the graft are important in the first months. Fourth, statistical power in this small cohort is limited: with only seven reactivations, the finding should be interpreted with caution and validated in a larger cohort. Taken together, the finding should not be read as antigen-specific immunity being irrelevant, but as evidence that overall graft fitness may carry important information.

4.4.3 T cell PD-1 expression and relapse risk

PD-1 and HLA-DR were added to the previous lymphocyte proliferation assay to assess activation in combination with proliferation as indicated by blast formation. Above median PD-1 expression on $CD8^+$ cells after PWM stimulation was associated with higher relapse risk and lower overall survival (Fig. 14).

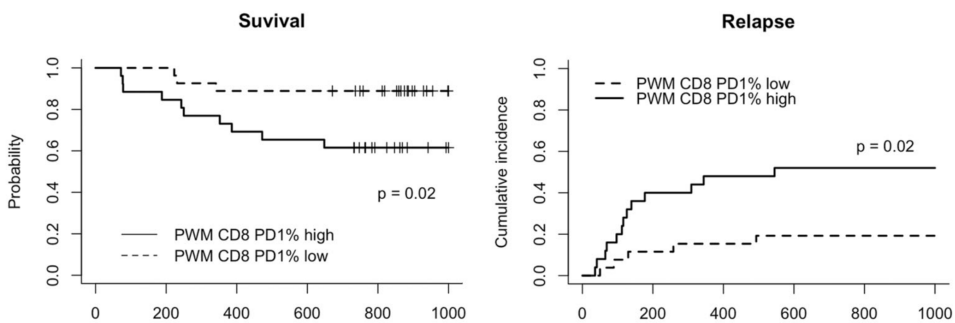


Figure 14. *Clinical outcome and CD8+ T cell programmed death-1 (PD-1) expression upon pokeweed mitogen (PWM) stimulation. The relapse graph shows patients with malignant disease only (n = 51). HSCT, hematopoietic stem cell transplantation. n.s, not significant.*

Activation and exhaustion signals in T cells constitute functional components that offer a new perspective on graft function. The graft's PD-1 state captured

part of the exhaustion axis of donor T cell biology, the same axis discussed in the T cell activation subsection as a ceiling on GvL activity, and translated it into an individual relapse-risk estimate at the time of graft release, several days before any clinical event. Neither the $CD34^+$ dose, nor the lymphocyte subset doses of **Study II**, nor the viability of **Study III** would have separated these patients.

The lymphocyte proliferation itself was not associated with relapse or survival, while the PD-1 expression did. However, other checkpoint markers such as TIM3, LAG3, or CTLA-4 were not measured in our functional analysis, making it difficult to determine whether PD-1 expression was a signal of activation or possible exhaustion. However, another recent study evaluating PD-1 in allo-HSCT donor grafts without stimulation and found that PD-1+ CD8+Tscm-like regulatory subset lowered the incidence of aGvHD without any survival penalty [196]. Future studies with more robust activation/exhaustion markers could provide more accurate information to better understand the underlying biology.

Conceptually, graft function is highly individual and depends on the donor's overall immune status and the fitness of the transplanted cells. Ideally, we would identify a functional aspect of the cells that we can target—either before transplantation by selecting the right donor or after transplantation, for example, with checkpoint inhibitors. Checkpoint inhibitors such as nivolumab and pembrolizumab have been studied in phase I and II trials for relapse after allo-HSCT. Reports describe their use in lymphomas with altered PD-L1 expression, as well as in AML and other malignancies, where they may inhibit the GvL effect against leukemia [197–199].

Overall, Study IV offered an interesting perspective on the graft through functional analysis that could be explored further. Although the lymphocyte proliferation assay uses an artificial ex-vivo stimulus, the findings suggest that graft activation and subsequent PD-1 expression were associated with relapse in patients. Similarly, low lymphocyte activation following PWM and SEA stimulation, rather than in CMV-specific cells, appeared to reflect CMV reactivation. This may contribute to reduced viral reactivation after transplantation. A major limitation of this study is its small sample size, particularly the limited number of viral reactivation cases. However, it demonstrates that functional analysis can help identify graft functions and may ultimately offer an easier way to quantify and apply this information in clinical practice.

4.5 Considerations and limitations

These conclusions must be interpreted in light of the limitations of the individual studies. All four studies are retrospective and of single-center character; diagnoses, conditioning intensities, and prophylaxis platforms vary across cohorts and study years. Multiple confounding factors and, in some cases, few events in the subgroup analyses increase the risk of type I and type II errors across all studies in the thesis**.** There is a substantial risk of overfitting our models when dealing with very few cases in some cohorts.

The viability cutoff in **Study III** was a statistical median rather than a clinically validated threshold. Even though we used continuous variables for most of the analysis, some data grouping and cutoffs had to be set. We used the median or mean, and sometimes quartiles, to divide the data into comparable groups. In one instance, AUC was used to determine whether a better cutoff could be identified, but we failed to find any suitable cutoff for the cytokine analysis in **Study IV**.

The functional cohort of **Study IV** provides proof of concept rather than a generalizable finding. To be more certain about these findings, more robust preclinical experimental models should have been conducted in parallel with the clinical studies. Without these experimental studies, we can only claim correlations, not established causality, to guide better diagnosis or treatment using PBSC grafts. Within these limits, however, there is a common theme across independent cohorts, time periods, and assay types. Each study, using different methods of observation, concludes that graft characteristics are not an insignificant variable.

A future improvement would be to collect follow-up blood samples after transplantation to assess patient function and immune reconstitution in relation to clinical outcomes. It would be interesting to evaluate engraftment dynamics, including chimerism and peripheral cell composition, in relation to graft composition and the patient's clinical status during different phases post-transplantation. Some of the above-mentioned data exist in different registry datasets, such as the EBMT registry, but were not included in the original study design. In-depth TCR analysis, better exhaustion markers, evaluation of effector or memory phenotypes, and many other factors would be important to assess when correlating graft composition and function with the clinical picture.

A key challenge in analyzing complex clinical data is limited sample size. The available data may be insufficient to account for patient heterogeneity and the number of relevant covariates. Larger, multicenter studies are therefore needed to provide sufficient data to test hypotheses with adequate confidence. Comparisons across transplant centers may be further complicated by center- or platform-specific factors. However, these difficulties should not discourage collaboration through European quality registries such as EBMT or international registries. Sharing data between research sites should be encouraged to support more research on different aspects of allo-HSCT.

5 Conclusions

This thesis summarizes four studies on the collection, composition, viability, and function of PBSC grafts. These studies indicate that the apheresis process, transportation, and graft function may influence the clinical outcome of allo-HSCT. Below are the main findings from Studies I-IV:

- Apheresis collection
    - The apheresis device and its collection protocol influenced graft composition: lymphocyte doses were significantly higher in grafts collected with COBE Spectra than with Optia MNC program, a purely technical change that altered the immune composition of the graft.
    - The risk of aGvHD (grade II–IV) was higher in patients receiving COBE-collected grafts, indicating that the collection method was associated with clinical outcome through the co-collected lymphocyte content.
- Graft composition
    - A higher graft $CD34^+$ cell dose was associated with increased TRM and relapse, questioning the assumption that more $CD34^+$ cells are always better.
    - In the URD cohort, a higher $CD8^+$ T cell dose was associated with lower relapse, a dose-dependent GvL signal measurable in the graft before infusion.
    - In the sibling cohort, a higher $CD19^+$ B-cell dose correlated with aGvHD and TRM, and a higher CD4/CD8 ratio correlated with aGvHD, implicating graft B cells and the CD4/CD8 balance in alloreactivity.
- Graft viability
    - PBSC graft viability measured with 7-AAD and Annexin V declined with transport time: grafts transported for one day had a median viability of 86.4% versus 83.2% after two days.
    - Recipients of low-viability grafts had a 1-year survival of 63.7% versus 88.9%, and low viability was an independent predictor of TRM in multivariate analysis.
- Graft function
    - Graft fitness, measured by proliferation in response to PWM and SEA, was associated with CMV reactivation, whereas responses to CMV-specific stimuli showed no association, indicating that overall

graft function, not only virus-specific T cells, is important for virus control after HSCT.

- Higher PD-1 expression on PWM-activated $CD8^+$ T cells was associated with increased relapse risk and reduced survival. Further studies are needed to determine how functional analysis of the graft translates into post-transplantation immune functions.

6 Points of perspective

The art of cell collection and processing has evolved over the past 50 years alongside autologous and allo-HSCT. Studies I-IV contribute to the extensive body of research examining graft composition and its effects on transplantation outcomes. Stem cell grafts merit study because they provide the foundation for the host's new immune system, and their composition can influence outcomes. Graft properties do not determine clinical outcomes in isolation, given confounding factors such as diagnosis, conditioning, age, medications, and infections; however, they play a central role in many outcomes. Grafts can be viewed as donor-derived traits with specific potentials, including GvL/GvHD and responses to infections, which are triggered only under the appropriate post-transplantation conditions. This complexity complicates comparisons between transplantation programs and contributes to conflicting findings. Graft attributes may be protective in one setting yet increase mortality in another, depending on the above-mentioned factors. Although this thesis focused primarily on PBSC grafts, optimizing apheresis yields, maintaining cell viability during transport, and assessing cellular composition are also important considerations for ATMP starting materials.

We can influence graft composition and viability through modest changes in transportation time and apheresis device selection, as demonstrated in Studies I and III. Some believe that stem cell grafts arrive as they are, and that we have no influence over donor T cell function or quantity in peripheral blood and, consequently, in the PBSC graft. I would argue the opposite: our ability to influence the starting material is precisely what makes this field interesting to study. Different collection preferences can increase or decrease the number of co-contaminating granulocytes, platelets, or erythrocytes in the graft [200]. Changes in transportation time and graft cell concentration during storage have significantly affected graft viability in previous studies and in Study III [136,188].

Another approach is to find better ways to mobilize the target cell population. Recently, Plerixafor has increased CD34+ yield in autologous patients and is also approved for donors. By increasing CD34+ concentration in the peripheral blood, target doses of around 5 million CD34+/kg recipient can be reached faster than with G-CSF-stimulated donors alone, resulting in less processed blood volume and fewer lymphocytes to the patient. For donors, plerixafor mobilization might increase the chances of one day apheresis to reach target CD34+ doses,

shortening the storage time of the PBSC graft, at the cost of possible gastrointestinal side effects of Plerixafor. To our knowledge, no mobilizing agents for T cells or NK cells are approved for donors, even though these cell therapy products have grown exponentially in recent years.

Looking ahead, the landscape of transplantation and cellular therapies is changing rapidly. New cell therapies based on T cells, NK cells, DCs, MSCs, TILs, and many other cell types are under development; some have already received market authorization and are reaching patients around the world. Cell collection through apheresis will be a crucial step for most of the current generation of cell and gene therapies, as long as ex vivo manipulation is still required. When targeting low-frequency cell populations or manufacturing steps with high cell losses, a high-yield apheresis product would be a great start. Studies have shown that the starting material matters for CART products: the T cell phenotype in the apheresis product is reflected in the final product, which is, in turn, associated with the efficacy and toxicity of the specific drug [201]. Considering that most ATMPs are currently manufactured in centralized GMP facilities, transportation time and delays in the chain of custody will likely play a role. Point-of-care production of ATMPs closer to the patient would eliminate transportation time, but it may introduce new challenges.

We have successfully transplanted hundreds of thousands of patients, which raises the question: is all this really necessary? There are still many challenges in allo-HSCT, and poor graft function has been linked to several complications and poor prognosis [202]. In my opinion, we still have a long way to go to fully understand how graft fitness affects transplantation outcomes. By studying PBSC graft quality, we can continue to optimize apheresis and transportation strategies that may carry benefits not only for allo-HSCT but also for other cell therapies in the future. We identified the lack of functional graft analysis as an area for future improvement. Study IV begins to address this gap, but further experimental studies are needed to verify many of this thesis's main findings.

Although some data from Studies I-IV are now more than a decade old, the topics of apheresis procedures, transportation, composition, and functional analysis remain highly relevant. PBSC collection and leukapheresis are not equivalent, but they share many similarities. Understanding the characteristics of the initial graft and how the starting material can be manipulated can improve

awareness of the risks, potential effects, and side effects associated with cell therapy.

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8 Declaration about the use of generative AI

Perplexity AI tools have been used to support literature identification. References suggested by the AI were verified against the original publications.

I have critically evaluated all the information provided by AI and take full responsibility for the content of this thesis.

9 References

1. Appelbaum FR. Hematopoietic-Cell Transplantation at 50. N Engl J Med. 2007 Oct 11;357(15):1472–5. doi:10.1056/NEJMp078166

2. for the European Society for Blood and Marrow Transplantation (EBMT), Duarte RF, Labopin M, Bader P, Basak GW, Bonini C, et al. Indications for haematopoietic stem cell transplantation for haematological diseases, solid tumours and immune disorders: current practice in Europe, 2019. Bone Marrow Transplant. 2019 Oct;54(10):1525–52. doi:10.1038/s41409-019-0516-2

3. Horgan C, Valcárcel D. Selection of Stem Cell Source. In: Sureda A, Corbacioglu S, Greco R, Kröger N, Carreras E, editors. The EBMT Handbook [Internet]. Cham: Springer International Publishing; 2024 [cited 2026 Sep 29]. p. 135–41. Available from: https://link.springer.com/10.1007/978-3-031-44080-9_14 doi:10.1007/978-3-031-44080-9_14

4. Kiene S, Albrecht M, Theurich S, Scheid C, Skoetz N, Holtick U. Bone marrow versus peripheral blood allogeneic haematopoietic stem cell transplantation for haematological malignancies in adults. Cochrane Database of Systematic Reviews. 2024 Nov 7;2024(11). doi:10.1002/14651858.CD010189.pub3 PubMed PMID: 39508306.

5. Nikiforow S, Kim HT, Daley H, Reynolds C, Jones KT, Armand P, et al. A phase I study of CD25/regulatory T-cell-depleted donor lymphocyte infusion for relapse after allogeneic stem cell transplantation. Haematologica. 2016;101(10):1251–9. doi:10.3324/haematol.2015.141176 PubMed PMID: 27354021.

6. Cantilena CR, Ito S, Tian X, Jain P, Chinian F, Anandi P, et al. Distinct Biomarker Profiles in Ex Vivo T Cell Depletion Graft Manipulation Strategies: CD34+ Selection versus CD3+/19+ Depletion in Matched Sibling Allogeneic Peripheral Blood Stem Cell Transplantation. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2018 Mar;24(3):460–6. doi:10.1016/j.bbmt.2017.11.028 PubMed PMID: 29197677.

7. Abdelhakim H, Abdel-Azim H, Saad A. Role of αβ T Cell Depletion in Prevention of Graft versus Host Disease. Biomedicines. 2017;5(4):35. doi:10.3390/biomedicines5030035

8. Passweg JR, Baldomero H, Atlija M, Kleovoulou I, Witaszek A, Alexander T, et al. The 2023 EBMT report on hematopoietic cell transplantation and cellular therapies. Increased use of allogeneic HCT for myeloid malignancies and of CAR-T at the expense of autologous HCT. Bone Marrow Transplantation.

2025 Apr 1;60(4):519–28. doi:10.1038/s41409-025-02524-2 PubMed PMID: 39939433.

9. Armand P, Kim HT, Logan BR, Wang Z, Alyea EP, Kalaycio ME, et al. Validation and refinement of the Disease Risk Index for allogeneic stem cell transplantation. Blood. 2014 Jun 5;123(23):3664–71. doi:10.1182/blood-2014-01-552984 PubMed PMID: 24744269; PubMed Central PMCID: PMC4047501.

10. Gratwohl A, Stern M, Brand R, Apperley J, Baldomero H, de Witte T, et al. Risk score for outcome after allogeneic hematopoietic stem cell transplantation: a retrospective analysis. Cancer. 2009 Oct 15;115(20):4715–26. doi:10.1002/cncr.24531 PubMed PMID: 19642176.

11. Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood. 2005 Oct 15;106(8):2912–9. doi:10.1182/blood-2005-05-2004 PubMed PMID: 15994282; PubMed Central PMCID: PMC1895304.

12. Shahzad M, Iqbal Q, Amin MK, Kasaiean A, Oskouie IM, Warraich SZ, et al. Outcomes of Allogeneic Hematopoietic Stem Cell Transplantation in Patients Aged 70 Years and Older: A Systematic Review and Meta-Analysis. Transplantation and Cellular Therapy. 2025 Mar;31(3):172.e1-172.e13. doi:10.1016/j.jtct.2024.12.022

13. Greco R, Sánchez-Ortega I, Risitano AM, Kalwak K, Ruggeri A, Kleovoulou I, et al. The 2024 EBMT activity report: crossing one million HCTs and 20,000 CAR-T. A landmark in cellular therapy. Bone Marrow Transplant. 2026 Jul;61(7):899–912. doi:10.1038/s41409-026-02919-9 PubMed PMID: 42203900; PubMed Central PMCID: PMC13349854.

14. Kantarjian HM, DiNardo CD, Kadia TM, Daver NG, Altman JK, Stein EM, et al. Acute myeloid leukemia management and research in 2025. CA Cancer J Clin. 2025;75(1):46–67. doi:10.3322/caac.21873 PubMed PMID: 39656142; PubMed Central PMCID: PMC11745214.

15. Döhner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022 Sep 22;140(12):1345–77. doi:10.1182/blood.2022016867 PubMed PMID: 35797463.

16. Foeken LM, Feinberg JA, Dijkman JHM, Eberhard HPR, Van Eerden E, Healey GS, et al. "30 years of global collaboration in transplantation and cellular therapy: WMDA's commitment to donor safety, ethical standards, and equitable access." Bone Marrow Transplant. 2025 Dec;60(12):1559–64. doi:10.1038/s41409-025-02704-0

17. Gragert L, Eapen M, Williams E, Freeman J, Spellman S, Baitty R, et al. HLA Match Likelihoods for Hematopoietic Stem-Cell Grafts in the U.S. Registry. N Engl J Med. 2014 Jul 24;371(4):339–48. doi:10.1056/NEJMsa1311707

18. Schmidt AH. Unrelated hematopoietic stem cell donor registries: present reality and future prospects. Current Opinion in Hematology. 2024 Nov;31(6):251–60. doi:10.1097/MOH.0000000000000835

19. Region Stockholm [Internet]. 2026 [cited 2026 Oct 10]. Ökning av givare till Tobiasregistret men behovet kvarstår. Available from: https://www.regionstockholm.se/nyheter/2026/05/okning-av-givare-till-tobiasregistret-men-behovet-kvarstar/

20. Pulsipher MA, Chitphakdithai P, Miller JP, Logan BR, King RJ, Rizzo JD, et al. Adverse events among 2408 unrelated donors of peripheral blood stem cells: results of a prospective trial from the National Marrow Donor Program. Blood. 2009 Apr 9;113(15):3604–11. doi:10.1182/blood-2008-08-175323

21. Tabilio A, Falzetti F, Aversa F, Minelli O. Spontaneous rupture of spleen during peripheral blood stem-cell mobilisation in a healthy donor. The Lancet. 1999 Feb;353(9152):555. doi:10.1016/S0140-6736(99)00268-8

22. Halter J, Kodera Y, Ispizua AU, Greinix HT, Schmitz N, Favre G, et al. Severe events in donors after allogeneic hematopoietic stem cell donation. Haematologica. 2009 Jan 1;94(1):94–101. doi:10.3324/haematol.13668

23. Gatti RA, Meuwissen HJ, Allen HD, Hong R, Good RA. Immunological reconstitution of sex-linked lymphopenic immunological deficiency. Lancet. 1968 Dec 28;2(7583):1366–9. doi:10.1016/s0140-6736(68)92673-1 PubMed PMID: 4177932.

24. Flomenberg N. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. Blood. 2004 Oct 1;104(7):1923–30. doi:10.1182/blood-2004-03-0803

25. Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, Confer DL, Eapen M, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. Blood. 2007 Dec 15;110(13):4576–83. doi:10.1182/blood-2007-06-097386

26. Sasazuki T, Juji T, Morishima Y, Kinukawa N, Kashiwabara H, Inoko H, et al. Effect of Matching of Class I HLA Alleles on Clinical Outcome after Transplantation of Hematopoietic Stem Cells from an Unrelated Donor. N Engl J Med. 1998 Oct 22;339(17):1177–85. doi:10.1056/NEJM199810223391701

27. Graw RG, Rogentine GN, Leventhal BG, Halterman RH, Berard C, Herzig GP, et al. Graft-versus-host reaction complicating HL-A matched bone-marrow transplantation. Lancet. 1970 Nov 21;2(7682):1053–5. doi:10.1016/s0140-6736(70)90285-0 PubMed PMID: 4098350.

28. Jimenez Jimenez AM, Stanek J, Logan BR, Leckrone E, Stefanski HE, Auletta JJ, et al. Outcomes after 7/8 and <7/8 mismatched unrelated donor PBSC transplantation with PTCy: results from the ACCESS trial. Blood Advances. 2026 Aug 19;bloodadvances.2026021081. doi:10.1182/bloodadvances.2026021081

29. Sahasrabudhe K, Otto M, Hematti P, Kenkre V. TCR αβ+/CD19+ cell depletion in haploidentical hematopoietic allogeneic stem cell transplantation: a review of current data. Leukemia & Lymphoma. 2019 Feb 23;60(3):598–609. doi:10.1080/10428194.2018.1485905

30. Ljungman P, Brand R, Hoek J, de la Camara R, Cordonnier C, Einsele H, et al. Donor cytomegalovirus status influences the outcome of allogeneic stem cell transplant: a study by the European group for blood and marrow transplantation. Clin Infect Dis. 2014 Aug 15;59(4):473–81. doi:10.1093/cid/ciu364 PubMed PMID: 24850801.

31. Mehta RS, Choe H, Saultz J, Gong Z, Sharma P, Al-Juhaishi T, et al. Impact of Donor Age and Donor Cytomegalovirus Serostatus on Outcomes After Related Donor Allogeneic Hematopoietic Stem Cell Transplantation. American J Hematol. 2025 Jun;100(6):987–97. doi:10.1002/ajh.27662

32. Mendoza MA, Bhaimia E, Alkhateeb HB, Razonable RR, Thoendel M. Which allogeneic hematopoietic cell transplant recipients have an increased risk for delayed-onset clinically significant cytomegalovirus infection after letermovir prophylaxis? Transplant Infectious Dis. 2024 Dec;26(6):e14377. doi:10.1111/tid.14377

33. Shaw BE, Logan BR, Spellman SR, Marsh SGE, Robinson J, Pidala J, et al. Development of an Unrelated Donor Selection Score Predictive of Survival after HCT: Donor Age Matters Most. Biology of Blood and Marrow Transplantation. 2018 May;24(5):1049–56. doi:10.1016/j.bbmt.2018.02.006

34. Kollman C, Spellman SR, Zhang MJ, Hassebroek A, Anasetti C, Antin JH, et al. The effect of donor characteristics on survival after unrelated donor transplantation for hematologic malignancy. Blood. 2016 Jan 14;127(2):260–7. doi:10.1182/blood-2015-08-663823 PubMed PMID: 26527675; PubMed Central PMCID: PMC4713163.

35. Rezvani AR, Storer BE, Guthrie KA, Schoch HG, Maloney DG, Sandmaier BM, et al. Impact of donor age on outcome after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2015 Jan;21(1):105–12.

doi:10.1016/j.bbmt.2014.09.021 PubMed PMID: 25278458; PubMed Central PMCID: PMC4286386.

36. Nivison-Smith I, Bajel A, Dodds AJ, Gottlieb D, Hamad N, Kennedy G, et al. Effect of donor age on adult unrelated donor haemopoietic cell transplant outcome: the Australian experience. Intern Med J. 2022 Jan;52(1):57–62. doi:10.1111/imj.15128 PubMed PMID: 33131163.

37. Kim HT, Zhang MJ, Woolfrey AE, St. Martin A, Chen J, Saber W, et al. Donor and recipient sex in allogeneic stem cell transplantation: what really matters. Haematologica. 2016 Oct 1;101(10):1260–6. doi:10.3324/haematol.2016.147645

38. Chakraverty R, Sykes M. The role of antigen-presenting cells in triggering graft-versus-host disease and graft-versus-leukemia. Blood. 2007 Jul 1;110(1):9–17. doi:10.1182/blood-2006-12-022038

39. Gyurkocza B, Sandmaier BM. Conditioning regimens for hematopoietic cell transplantation: one size does not fit all. Blood. 2014 Jul 17;124(3):344–53. doi:10.1182/blood-2014-02-514778

40. Slavin S, Nagler A, Naparstek E, Kapelushnik Y, Aker M, Cividalli G, et al. Nonmyeloablative Stem Cell Transplantation and Cell Therapy as an Alternative to Conventional Bone Marrow Transplantation With Lethal Cytoreduction for the Treatment of Malignant and Nonmalignant Hematologic Diseases. Blood. 1998 Feb 1;91(3):756–63. doi:10.1182/blood.V91.3.756

41. McClune BL, Weisdorf DJ, Pedersen TL, Tunes Da Silva G, Tallman MS, Sierra J, et al. Effect of Age on Outcome of Reduced-Intensity Hematopoietic Cell Transplantation for Older Patients With Acute Myeloid Leukemia in First Complete Remission or With Myelodysplastic Syndrome. JCO. 2010 Apr 10;28(11):1878–87. doi:10.1200/JCO.2009.25.4821

42. Orio F, Muscogiuri G, Palomba S, Serio B, Sessa M, Giudice V, et al. Endocrinopathies after Allogeneic and Autologous Transplantation of Hematopoietic Stem Cells. The Scientific World Journal. 2014;2014:1–13. doi:10.1155/2014/282147

43. Scott BL, Pasquini MC, Fei M, Fraser R, Wu J, Devine SM, et al. Myeloablative versus Reduced-Intensity Conditioning for Hematopoietic Cell Transplantation in Acute Myelogenous Leukemia and Myelodysplastic Syndromes—Long-Term Follow-Up of the BMT CTN 0901 Clinical Trial. Transplantation and Cellular Therapy. 2021 Jun;27(6):483.e1-483.e6. doi:10.1016/j.jtct.2021.02.031

44. Ogonek J, Kralj Juric M, Ghimire S, Varanasi PR, Holler E, Greinix H, et al. Immune Reconstitution after Allogeneic Hematopoietic Stem Cell

Transplantation. Front Immunol. 2016 Nov 17;7. doi:10.3389/fimmu.2016.00507

45. Bosch M, Khan FM, Storek J. Immune reconstitution after hematopoietic cell transplantation: Current Opinion in Hematology. 2012 Jul;19(4):324–35. doi:10.1097/MOH.0b013e328353bc7d

46. Dai W, Dong F, Cheng T, Hao S. T Cell immune reconstitution after allo-HSCT: key factors and implications. Blood Sci. 2025 Dec;7(4):e00261. doi:10.1097/BS9.0000000000000261 PubMed PMID: 41347010; PubMed Central PMCID: PMC12672197.

47. Simons L, Cavazzana M, André I. Concise Review: Boosting T-Cell Reconstitution Following Allogeneic Transplantation-Current Concepts and Future Perspectives. Stem Cells Transl Med. 2019 Jul;8(7):650–7. doi:10.1002/sctm.18-0248 PubMed PMID: 30887712; PubMed Central PMCID: PMC6591542.

48. Yanir A, Schulz A, Lawitschka A, Nierkens S, Eyrich M. Immune Reconstitution After Allogeneic Haematopoietic Cell Transplantation: From Observational Studies to Targeted Interventions. Front Pediatr. 2021;9:786017. doi:10.3389/fped.2021.786017 PubMed PMID: 35087775; PubMed Central PMCID: PMC8789272.

49. Perko R, Kang G, Sunkara A, Leung W, Thomas PG, Dallas MH. Gamma Delta T Cell Reconstitution Is Associated with Fewer Infections and Improved Event-Free Survival after Hematopoietic Stem Cell Transplantation for Pediatric Leukemia. Biology of Blood and Marrow Transplantation. 2015 Jan;21(1):130–6. doi:10.1016/j.bbmt.2014.09.027

50. Arruda LCM, Gaballa A, Uhlin M. Impact of γδ T cells on clinical outcome of hematopoietic stem cell transplantation: systematic review and meta-analysis. Blood Advances. 2019 Nov 12;3(21):3436–48. doi:10.1182/bloodadvances.2019000682

51. Matsuoka KI. Designing immune reconstitution to prevent graft-versus-host disease: a novel therapeutic paradigm beyond T cell suppression. Int J Hematol. 2026 Jun;123(6):802–10. doi:10.1007/s12185-026-04176-4 PubMed PMID: 41655162.

52. Admiraal R, Nierkens S, Bierings MB, Belderbos ME, Huitema AD, Bredius RGM, et al. Improved survival with model-based dosing of antithymocyte globulin in pediatric hematopoietic cell transplantation. Blood Adv. 2025 May 13;9(9):2344–53. doi:10.1182/bloodadvances.2024014836 PubMed PMID: 39983052; PubMed Central PMCID: PMC12142804.

53. Gooptu M, Kim HT, Chen YB, Rybka W, Artz A, Boyer M, et al. Effect of Antihuman T Lymphocyte Globulin on Immune Recovery after Myeloablative

Allogeneic Stem Cell Transplantation with Matched Unrelated Donors: Analysis of Immune Reconstitution in a Double-Blind Randomized Controlled Trial. Biol Blood Marrow Transplant. 2018 Nov;24(11):2216–23. doi:10.1016/j.bbmt.2018.07.002 PubMed PMID: 30006305.

54. Jiang P, Zhou X, Cai Y, Yang J, Zhou K, Tong Y, et al. Lymphocyte subset reconstitution and clinical outcomes following haploidentical hematopoietic stem cell transplantation. Br J Cancer. 2026 May;134(9):1289–99. doi:10.1038/s41416-026-03345-w PubMed PMID: 41772275; PubMed Central PMCID: PMC13079791.

55. Qaiser M, Zhou H, Zhou G, Ren G, Dou X, Wang X, et al. Immune reconstitution and viral reactivation after allogeneic hematopoietic stem cell transplantation in hematologic malignancies. Hematology. 2026 Dec 31;31(1):2707676. doi:10.1080/16078454.2026.2707676 PubMed PMID: 42535760.

56. Basso S, Compagno F, Zelini P, Giorgiani G, Boghen S, Bergami E, et al. Harnessing T Cells to Control Infections After Allogeneic Hematopoietic Stem Cell Transplantation. Front Immunol. 2020;11:567531. doi:10.3389/fimmu.2020.567531 PubMed PMID: 33178192; PubMed Central PMCID: PMC7593558.

57. Gill J, Busca A, Cinatti N, Passera R, Dellacasa CM, Giaccone L, et al. Bacterial Bloodstream Infections after Allogeneic Hematopoietic Stem Cell Transplantation: Etiology, Risk Factors and Outcome in a Single-Center Study. Microorganisms. 2023 Mar 14;11(3):742. doi:10.3390/microorganisms11030742

58. Biyun L, Yahui H, Yuanfang L, Xifeng G, Dao W. Risk factors for invasive fungal infections after haematopoietic stem cell transplantation: a systematic review and meta-analysis. Clin Microbiol Infect. 2024 May;30(5):601–10. doi:10.1016/j.cmi.2024.01.005 PubMed PMID: 38280518.

59. Chan ST, Logan AC. The clinical impact of cytomegalovirus infection following allogeneic hematopoietic cell transplantation: Why the quest for meaningful prophylaxis still matters. Blood Reviews. 2017 May 1;31(3):173–83. doi:10.1016/j.blre.2017.01.002 PubMed PMID: 28173959.

60. Norkin M, Shaw BE, Brazauskas R, Tecca HR, Leather HL, Gea-Banacloche J, et al. Characteristics of Late Fatal Infections after Allogeneic Hematopoietic Cell Transplantation. Biology of Blood and Marrow Transplantation. 2019 Feb;25(2):362–8. doi:10.1016/j.bbmt.2018.09.031

61. Ljungman P, Brand R, Einsele H, Frassoni F, Niederwieser D, Cordonnier C. Donor CMV serologic status and outcome of CMV-seropositive recipients after unrelated donor stem cell transplantation: an EBMT megafile analysis.

Blood. 2003 Dec 15;102(13):4255–60. doi:10.1182/blood-2002-10-3263 PubMed PMID: 12933590.

62. Su Y, Stern A, Karantoni E, Nawar T, Han G, Zavras P, et al. Impact of Letermovir Primary Cytomegalovirus Prophylaxis on 1-Year Mortality After Allogeneic Hematopoietic Cell Transplantation: A Retrospective Cohort Study. Clin Infect Dis. 2022 Sep 14;75(5):795–804. doi:10.1093/cid/ciab1064 PubMed PMID: 34979021; PubMed Central PMCID: PMC9477449.

63. Marty FM, Ljungman P, Chemaly RF, Maertens J, Dadwal SS, Duarte RF, et al. Letermovir Prophylaxis for Cytomegalovirus in Hematopoietic-Cell Transplantation. N Engl J Med. 2017 Dec 21;377(25):2433–44. doi:10.1056/NEJMoa1706640 PubMed PMID: 29211658.

64. Mori Y, Harada T, Yoshimoto G, Shima T, Numata A, Jinnouchi F, et al. Risk factors for late cytomegalovirus infection after completing letermovir prophylaxis. Int J Hematol. 2022 Aug;116(2):258–65. doi:10.1007/s12185-022-03348-2 PubMed PMID: 35524024.

65. Gabanti E, Borsani O, Colombo AA, Zavaglio F, Binaschi L, Caldera D, et al. Human Cytomegalovirus-Specific T-Cell Reconstitution and Late-Onset Cytomegalovirus Infection in Hematopoietic Stem Cell Transplantation Recipients following Letermovir Prophylaxis. Transplant Cell Ther. 2022 Apr;28(4):211.e1-211.e9. doi:10.1016/j.jtct.2022.01.008 PubMed PMID: 35042012.

66. Derigs P, Radujkovic A, Schubert ML, Schnitzler P, Schöning T, Müller-Tidow C, et al. Letermovir prophylaxis is effective in preventing cytomegalovirus reactivation after allogeneic hematopoietic cell transplantation: single-center real-world data. Ann Hematol. 2021 Aug;100(8):2087–93. doi:10.1007/s00277-020-04362-2 PubMed PMID: 33270162; PubMed Central PMCID: PMC8285358.

67. Zeiser R, Blazar BR. Acute Graft-versus-Host Disease — Biologic Process, Prevention, and Therapy. Longo DL, editor. N Engl J Med. 2017 Nov 30;377(22):2167–79. doi:10.1056/NEJMra1609337

68. Ferrara JLM, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet. 2009 May 2;373(9674):1550–61. doi:10.1016/S0140-6736(09)60237-3 PubMed PMID: 19282026.

69. Socie G. Challenges in GVHD and GVL after hematopoietic stem cell transplantation for myeloid malignancies. Blood. 2025 Aug 21;146(8):926–37. doi:10.1182/blood.2025028617

70. Rafei H, Jenq RR. Microbiome-intestine cross talk during acute graft-versus-host disease. Blood. 2020 Jul 23;136(4):401–9. doi:10.1182/blood.2019000950

71. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group Report. Biology of Blood and Marrow Transplantation. 2015;21(3):389–401. doi:10.1016/j.bbmt.2014.12.001 PubMed PMID: 25529383.

72. Schoemans HM, Lee SJ, Ferrara JL, Wolff D, Levine JE, Schultz KR, et al. EBMT-NIH-CIBMTR Task Force position statement on standardized terminology & guidance for graft-versus-host disease assessment. Bone Marrow Transplant. 2018 Nov;53(11):1401–15. doi:10.1038/s41409-018-0204-7 PubMed PMID: PMC6786777.

73. MacMillan ML, Robin M, Harris AC, DeFor TE, Martin PJ, Alousi A, et al. A refined risk score for acute graft-versus-host disease that predicts response to initial therapy, survival, and transplant-related mortality. Biol Blood Marrow Transplant. 2015 Apr;21(4):761–7. doi:10.1016/j.bbmt.2015.01.001 PubMed PMID: 25585275; PubMed Central PMCID: PMC4359643.

74. Glucksberg H, Storb R, Fefer a, Buckner CD, Neiman PE, Clift R a, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974;18(4):295–304. doi:10.1097/00007890-197410000-00001 PubMed PMID: 4153799.

75. Flowers MED, Martin PJ. How we treat chronic graft-versus-host disease. Blood. 2015 Jan 22;125(4):606–15. doi:10.1182/blood-2014-08-551994 PubMed PMID: 25398933; PubMed Central PMCID: PMC4304105.

76. Zeiser R, von Bubnoff N, Butler J, Mohty M, Niederwieser D, Or R, et al. Ruxolitinib for Glucocorticoid-Refractory Acute Graft-versus-Host Disease. N Engl J Med. 2020 May 7;382(19):1800–10. doi:10.1056/NEJMoa1917635 PubMed PMID: 32320566.

77. Flowers MED, Apperley JF, Van Besien K, Elmaagacli A, Grigg A, Reddy V, et al. A multicenter prospective phase 2 randomized study of extracorporeal photopheresis for treatment of chronic graft-versus-host disease. Blood. 2008 Oct 1;112(7):2667–74. doi:10.1182/blood-2008-03-141481

78. Kröger N. Preventing Graft-Versus-Host Disease Without Losing Graft-Versus-Leukemia Effect After Allogeneic Stem-Cell Transplantation. J Clin Oncol. 2020 Oct 10;38(29):3357–60. doi:10.1200/JCO.20.01756 PubMed PMID: 32706637.

79. Storb R, Deeg HJ, Whitehead J, Appelbaum F, Beatty P, Bensinger W, et al. Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation

for leukemia. N Engl J Med. 1986 Mar 20;314(12):729–35. doi:10.1056/NEJM198603203141201 PubMed PMID: 3513012.

80. Finke J, Bethge WA, Schmoor C, Ottinger HD, Stelljes M, Zander AR, et al. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. Lancet Oncol. 2009 Sep;10(9):855–64. doi:10.1016/S1470-2045(09)70225-6 PubMed PMID: 19695955.

81. Cibich A, Wechalekar G, Shanmuganathan N, Singhal D, Beligaswatte A, Wayte R, et al. Long-Term Survival Gains after Allogeneic Hematopoietic Stem Cell Transplant are Driven by Reductions in Non-Relapse Mortality: A 35-Year Statewide Australian Cohort Study. Transplantation and Cellular Therapy. 2026 Jun;32(6):689.e1-689.e13. doi:10.1016/j.jtct.2026.01.024

82. Spellman SR, Xu K, Oloyede T, Ahn KW, Akhtar O, Bolon YT, et al. Current Activity Trends and Outcomes in Hematopoietic Cell Transplantation and Cellular Therapy – A Report from the CIBMTR. Transplantation and Cellular Therapy. 2025 Aug;31(8):505–32. doi:10.1016/j.jtct.2025.05.014

83. Christopher MJ, Petti AA, Rettig MP, Miller CA, Chendamarai E, Duncavage EJ, et al. Immune Escape of Relapsed AML Cells after Allogeneic Transplantation. N Engl J Med. 2018 Dec 13;379(24):2330–41. doi:10.1056/NEJMoa1808777

84. Sauerer T, Velázquez GF, Schmid C. Relapse of acute myeloid leukemia after allogeneic stem cell transplantation: immune escape mechanisms and current implications for therapy. Mol Cancer. 2023 Nov 11;22(1):180. doi:10.1186/s12943-023-01889-6

85. Heuser M, Freeman SD, Ossenkoppele GJ, Buccisano F, Hourigan CS, Ngai LL, et al. 2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party. Blood. 2021 Dec 30;138(26):2753–67. doi:10.1182/blood.2021013626 PubMed PMID: 34724563; PubMed Central PMCID: PMC8718623.

86. Georgi JA, Stasik S, Bornhäuser M, Platzbecker U, Thiede C. Analysis of Subset Chimerism for MRD-Detection and Pre-Emptive Treatment in AML. Front Oncol. 2022 Feb 17;12:841608. doi:10.3389/fonc.2022.841608

87. Kolb H, Schattenberg A, Goldman J, Hertenstein B, Jacobsen N, Arcese W, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. European Group for Blood and Marrow Transplantation Working Party Chronic Leukemia [see comments]. Blood. 1995 Sep 1;86(5):2041–50. doi:10.1182/blood.V86.5.2041.bloodjournal8652041

88. Mackinnon S, Papadopoulos E, Carabasi M, Reich L, Collins N, Boulad F, et al. Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: separation of graft-versus-leukemia responses from graft-versus-host disease. Blood. 1995 Aug 15;86(4):1261–8. doi:10.1182/blood.V86.4.1261.bloodjournal8641261

89. Nikiforow S, Alyea EP. Maximizing GVL in allogeneic transplantation: Role of donor lymphocyte infusions. Hematology. 2014;2014(1):570–5. doi:10.1182/asheducation-2014.1.570

90. Santoro N, Schmid C, de Witte M, Roeven MWH, Potter V, Richardson D, et al. Current use of donor lymphocyte infusions after allogenic stem cell transplantation in Europe: a survey on behalf of the cellular therapy and immunobiology working party of the EBMT. Bone Marrow Transplantation. 2025 May 1;60(5):697–701. doi:10.1038/s41409-025-02555-9 PubMed PMID: 40123004.

91. Thorsby E. A short history of HLA. Tissue Antigens. 2009 Aug;74(2):101–16. doi:10.1111/j.1399-0039.2009.01291.x

92. Mandal S, Deb J, Mohan AK, Sil S, Maji M, Hasan S, et al. Beyond histocompatibility: human leukocyte antigen as a cross-disciplinary platform for immune recognition and clinical decision-making. Front Immunol. 2026 Sep 8;17:1931212. doi:10.3389/fimmu.2026.1931212

93. Kulski JK, Shiina T, Dijkstra JM. Genomic diversity of the major histocompatibility complex in health and disease. Cells. 2019 Oct 1;8(10). doi:10.3390/cells8101270 PubMed PMID: 31627481.

94. Neefjes JJ, Hämmerling GJ, Momburg F. Folding and assembly of major histocompatibility complex class I heterodimers in the endoplasmic reticulum of intact cells precedes the binding of peptide. The Journal of experimental medicine. 1993 Dec 1;178(6):1971–80. doi:10.1084/jem.178.6.1971

95. Nguyen AT, Szeto C, Gras S. The pockets guide to HLA class I molecules. Biochemical Society Transactions. 2021 Nov 1;49(5):2319–31. doi:10.1042/BST20210410

96. Roche PA, Furuta K. The ins and outs of MHC class II-mediated antigen processing and presentation. Nat Rev Immunol. 2015 Apr;15(4):203–16. doi:10.1038/nri3818

97. Falkenburg JHF, Heslop HE, Barrett AJ. T Cell Therapy in Allogeneic Stem Cell Transplantation. Biology of Blood and Marrow Transplantation. 2008 Jan;14(1):136–41. doi:10.1016/j.bbmt.2007.10.022

98. Zhu X, Zhu J. CD4 T Helper Cell Subsets and Related Human Immunological Disorders. IJMS. 2020 Oct 28;21(21):8011. doi:10.3390/ijms21218011

99. Rao A, Agrawal A, Borthakur G, Battula VL, Maiti A. Gamma delta T cells in acute myeloid leukemia: biology and emerging therapeutic strategies. J Immunother Cancer. 2024 Feb 27;12(2):e007981. doi:10.1136/jitc-2023-007981 PubMed PMID: 38417915; PubMed Central PMCID: PMC10900322.

100. Janssen A, van Diest E, Vyborova A, Schrier L, Bruns A, Sebestyen Z, et al. The Role of γδ T Cells as a Line of Defense in Viral Infections after Allogeneic Stem Cell Transplantation: Opportunities and Challenges. Viruses. 2022 Jan 10;14(1):117. doi:10.3390/v14010117 PubMed PMID: 35062321; PubMed Central PMCID: PMC8779492.

101. Sallusto F, Lenig D, Förster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature. 1999 Oct 14;401(6754):708–12. doi:10.1038/44385 PubMed PMID: 10537110.

102. Appay V, Dunbar PR, Callan M, Klenerman P, Gillespie GMA, Papagno L, et al. Memory CD8+ T cells vary in differentiation phenotype in different persistent virus infections. Nat Med. 2002 Apr;8(4):379–85. doi:10.1038/nm0402-379 PubMed PMID: 11927944.

103. Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol. 2012;30:531–64. doi:10.1146/annurev.immunol.25.022106.141623 PubMed PMID: 22224781; PubMed Central PMCID: PMC6066374.

104. Pieper K, Grimbacher B, Eibel H. B-cell biology and development. J Allergy Clin Immunol. 2013 Apr;131(4):959–71. doi:10.1016/j.jaci.2013.01.046 PubMed PMID: 23465663.

105. Gómez-Manríquez J, Hernández-Bello J, Muñoz-Valle JF, Sifuentes-Franco S, Graciano-Machuca O, Morales-Núñez JJ. B cell development: transcriptional regulation and immunological mechanisms in homeostasis. Front Immunol. 2025 Aug 8;16:1593338. doi:10.3389/fimmu.2025.1593338

106. Iori AP, Torelli GF, De Propris MS, Milano F, Pupella S, Gozzer M, et al. B-cell concentration in the apheretic product predicts acute graft-versus-host disease and treatment-related mortality of allogeneic peripheral blood stem cell transplantation. Transplantation. 2008 Feb;85(3):386–90. doi:10.1097/TP.0b013e3181622e36 PubMed PMID: 18322430.

107. Shimabukuro-Vornhagen A, Hallek MJ, Storb RF, Von Bergwelt-Baildon MS. The role of B cells in the pathogenesis of graft-versus-host disease. Blood. 2009 Dec 3;114(24):4919–27. doi:10.1182/blood-2008-10-161638

108. Pauken KE, Wherry EJ. Overcoming T cell exhaustion in infection and cancer. Trends in Immunology. 2015 Apr;36(4):265–76. doi:10.1016/j.it.2015.02.008

109. Li Y, Guo H, Zhang Y, Chen J, Zhong L, Huang X, et al. The impact of T cell exhaustion in hematopoietic stem cell transplantation. Stem Cell Res Ther. 2026 Jun 28;17(1):315. doi:10.1186/s13287-026-05141-y PubMed PMID: 42366382; PubMed Central PMCID: PMC13579798.

110. Noviello M, Manfredi F, Ruggiero E, Perini T, Oliveira G, Cortesi F, et al. Bone marrow central memory and memory stem T-cell exhaustion in AML patients relapsing after HSCT. Nat Commun. 2019 Mar 25;10(1):1065. doi:10.1038/s41467-019-08871-1 PubMed PMID: 30911002; PubMed Central PMCID: PMC6434052.

111. Liu L, Chang YJ, Xu LP, Zhang XH, Wang Y, Liu KY, et al. Reversal of T Cell Exhaustion by the First Donor Lymphocyte Infusion Is Associated with the Persistently Effective Antileukemic Responses in Patients with Relapsed AML after Allo-HSCT. Biology of Blood and Marrow Transplantation. 2018 Jul;24(7):1350–9. doi:10.1016/j.bbmt.2018.03.030

112. Liu L, Chang YJ, Xu LP, Zhang XH, Wang Y, Liu KY, et al. T cell exhaustion characterized by compromised MHC class I and II restricted cytotoxic activity associates with acute B lymphoblastic leukemia relapse after allogeneic hematopoietic stem cell transplantation. Clin Immunol. 2018 May;190:32–40. doi:10.1016/j.clim.2018.02.009 PubMed PMID: 29477343.

113. Saas P, Daguindau E, Perruche S. Concise Review: Apoptotic Cell-Based Therapies-Rationale, Preclinical Results and Future Clinical Developments. Stem Cells. 2016;34(6):1464–73. doi:10.1002/stem.2361 PubMed PMID: 27018198.

114. Mevorach D, Zuckerman T, Reiner I, Shimoni A, Samuel S, Nagler A, et al. Single Infusion of Donor Mononuclear Early Apoptotic Cells as Prophylaxis for Graft-versus-Host Disease in Myeloablative HLA-Matched Allogeneic Bone Marrow Transplantation: A Phase I/IIa Clinical Trial. Biology of Blood and Marrow Transplantation. 2014;20(1):58–65. doi:10.1016/j.bbmt.2013.10.010 PubMed PMID: 24140121.

115. Poon IKH, Lucas CD, Rossi AG, Ravichandran KS. Apoptotic cell clearance: basic biology and therapeutic potential. Nat Rev Immunol. 2014 Mar;14(3):166–80. doi:10.1038/nri3607 PubMed PMID: 24481336; PubMed Central PMCID: PMC4040260.

116. Land WG. How evolution tells us to induce allotolerance. Exp Clin Transplant. 2015 Apr;13 Suppl 1:46–54. PubMed PMID: 25894127.

117. Pugel A, Yu XZ, Wu Y. Cell death pathways in graft-versus-host disease. Curr Opin Immunol. 2026 Apr;99:102740. doi:10.1016/j.coi.2026.102740 PubMed PMID: 41689939.

118. Tang HM, Tang HL. Anastasis: recovery from the brink of cell death. R Soc open sci. 2018 Sep;5(9):180442. doi:10.1098/rsos.180442

119. Melve GK, Ersvaer E, Eide GE, Kristoffersen EK, Bruserud Ø. Peripheral Blood Stem Cell Mobilization in Healthy Donors by Granulocyte Colony-Stimulating Factor Causes Preferential Mobilization of Lymphocyte Subsets. Front Immunol. 2018 May 2;9:845. doi:10.3389/fimmu.2018.00845

120. Collins NH, Gee AP, Durett AG, Kan F, Zhang MJ, Champlin RE, et al. The Effect of the Composition of Unrelated Donor Bone Marrow and Peripheral Blood Progenitor Cell Grafts on Transplantation Outcomes. Biology of Blood and Marrow Transplantation. 2010 Feb;16(2):253–62. doi:10.1016/j.bbmt.2009.10.004

121. Siena S, Bregni M, Brando B, Ravagnani F, Bonadonna G, Gianni A. Circulation of CD34+ hematopoietic stem cells in the peripheral blood of high-dose cyclophosphamide-treated patients: enhancement by intravenous recombinant human granulocyte-macrophage colony-stimulating factor. Blood. 1989 Nov 1;74(6):1905–14. doi:10.1182/blood.V74.6.1905.1905

122. Weaver C, Hazelton B, Birch R, Palmer P, Allen C, Schwartzberg L, et al. An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy. Blood. 1995 Nov 15;86(10):3961–9. doi:10.1182/blood.V86.10.3961.bloodjournal86103961

123. Teipel R, Oelschlägel U, Wetzko K, Schmiedgen M, Kramer M, Rücker-Braun E, et al. Differences in Cellular Composition of Peripheral Blood Stem Cell Grafts from Healthy Stem Cell Donors Mobilized with Either Granulocyte Colony-Stimulating Factor (G-CSF) Alone or G-CSF and Plerixafor. Biology of Blood and Marrow Transplantation. 2018 Nov;24(11):2171–7. doi:10.1016/j.bbmt.2018.06.023

124. Maung KK, Chen BJ, Barak I, Li Z, Rizzieri DA, Gasparetto C, et al. Phase I dose escalation study of naive T-cell depleted donor lymphocyte infusion following allogeneic stem cell transplantation. Bone Marrow Transplant. 2021 Jan;56(1):137–43. doi:10.1038/s41409-020-0991-5

125. Martino M, Gori M, Pitino A, Gentile M, Dattola A, Pontari A, et al. Basal CD34+ Cell Count Predicts Peripheral Blood Stem Cell Mobilization in Healthy Donors after Administration of Granulocyte Colony–Stimulating Factor: A Longitudinal, Prospective, Observational, Single-Center, Cohort Study. Biology of Blood and Marrow Transplantation. 2017 Jul 1;23(7):1215–20. doi:10.1016/j.bbmt.2017.03.024 PubMed PMID: 28344060.

126. Lawlor KE, Campbell IK, Metcalf D, O'Donnell K, van Nieuwenhuijze A, Roberts AW, et al. Critical role for granulocyte colony-stimulating factor in inflammatory arthritis. Proceedings of the National Academy of Sciences of the United States of America. 2004 Aug 3;101(31):11398–403. doi:10.1073/pnas.0404328101 PubMed PMID: 15272075.

127. Dexter T, Lozano S, Leather A, Slatter M, Perera K, Griffin J, et al. UK consensus statement on the use of plerixafor in healthy stem cell donors. British Journal of Haematology. 2025 Mar 1;206(3):992–5. doi:10.1111/bjh.19963 PubMed PMID: 39746842.

128. Kuan JW, Su AT, Leong CF. Pegylated granulocyte-colony stimulating factor versus non-pegylated granulocyte-colony stimulating factor for peripheral blood stem cell mobilization: A systematic review and meta-analysis. Journal of Clinical Apheresis. 2017 Dec 1;32(6):517–42. doi:10.1002/jca.21550 PubMed PMID: 28485020.

129. Kayser S, Schlenk RF, Steiner M, Klüter H, Wuchter P. Predicting Successful Hematopoietic Stem Cell Collection in Healthy Allogeneic Donors. Transfus Med Hemother. 2023;50(5):396–402. doi:10.1159/000531236

130. Worel N, Ljungman P, Verheggen ICM, Hoogenboom JD, Knelange NS, Eikema DJ, et al. Fresh or frozen grafts for allogeneic stem cell transplantation: conceptual considerations and a survey on the practice during the COVID-19 pandemic from the EBMT Infectious Diseases Working Party (IDWP) and Cellular Therapy & Immunobiology Working Party (CTIWP). Bone Marrow Transplantation. 2023 Dec 1;58(12):1348–56. doi:10.1038/s41409-023-02099-w PubMed PMID: 37673982.

131. Tran MH, Cai Y, Stroncek D. Liquid Storage of Peripheral Blood Stem Cell Products: Effects of Time and Temperature on Product Quality. Transplantation and Cellular Therapy. 2024 Jan 1;30(1):120.e1-120.e10. doi:10.1016/j.jtct.2023.09.020 PubMed PMID: 37797720.

132. Sohn HS, Heo JS, Kim HS, Choi Y, Kim HO. Duration of in vitro storage affects the key stem cell features of human bone marrow-derived mesenchymal stromal cells for clinical transplantation. Cytotherapy. 2013 Apr;15(4):460–6. doi:10.1016/j.jcyt.2012.10.015 PubMed PMID: 23318345.

133. Yu NH, Chun SY, Ha YS, Kim HT, Kim DH, Kim J, et al. Optimal Stem Cell Transporting Conditions to Maintain Cell Viability and Characteristics. Tissue Engineering and Regenerative Medicine. 2018 Oct 1;15(5):639–47. doi:10.1007/s13770-018-0133-y

134. Jansen J, Nolan PL, Reeves MI, Akard L, Thompson JM, Dugan MJ, et al. Transportation of peripheral blood progenitor cell products: Effects of time, temperature and cell concentration. Cytotherapy. 2009;11(1):79–85. doi:10.1080/14653240802666050 PubMed PMID: 19153854.

135. Toda Y, Jo T, Arai Y, Harada K, Yanada M, Fukuda T, et al. Impact of CD34⁺ cell dose on outcomes of related allogeneic PBSCT in adult AML patients. Br J Haematol. 2026 Jul;209(1):210–21. doi:10.1111/bjh.70506

136. Araújo AB, Salton GD, Angeli MH, Furlan JM, Schmalfuss T, Röhsig LM. Effects of cell concentration, time of fresh storage, and cryopreservation on peripheral blood stem cells: PBSC fresh storage and cryopreservation. Transfusion and Apheresis Science. 2022 Feb 1;61(1). doi:10.1016/j.transci.2021.103298 PubMed PMID: 34696981.

137. Mitsuyasu RT, Champlin RE, Gale RP, Ho WG, Lenarsky C, Winston D, et al. Treatment of donor bone marrow with monoclonal anti-T-cell antibody and complement for the prevention of graft-versus-host disease. A prospective, randomized, double-blind trial. Ann Intern Med. 1986 Jul;105(1):20–6. doi:10.7326/0003-4819-105-1-20 PubMed PMID: 3521427.

138. Aversa F, Tabilio A, Velardi A, Cunningham I, Terenzi A, Falzetti F, et al. Treatment of high-risk acute leukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype. N Engl J Med. 1998 Oct 22;339(17):1186–93. doi:10.1056/NEJM199810223391702 PubMed PMID: 9780338.

139. Merli P, Algeri M, Galaverna F, Bertaina V, Lucarelli B, Boccieri E, et al. TCRαβ/CD19 cell-depleted HLA-haploidentical transplantation to treat pediatric acute leukemia: updated final analysis. Blood. 2024 Jan 18;143(3):279–89. doi:10.1182/blood.2023021336 PubMed PMID: 37738655.

140. Bleakley M, Sehgal A, Seropian S, Biernacki MA, Krakow EF, Dahlberg A, et al. Naive T-Cell Depletion to Prevent Chronic Graft-Versus-Host Disease. J Clin Oncol. 2022 Apr 10;40(11):1174–85. doi:10.1200/JCO.21.01755 PubMed PMID: 35007144; PubMed Central PMCID: PMC8987226.

141. Astigarraga CC, Mpms K, Iovino L, Milano F. Haploidentical transplantation: An optimal platform for graft manipulation and cellular therapies. Blood Rev. 2025 Jul;72:101286. doi:10.1016/j.blre.2025.101286 PubMed PMID: 40133165.

142. Brown M, Wittwer C. Flow Cytometry: Principles and Clinical Applications in Hematology. Clinical Chemistry. 2000 Aug 1;46(8):1221–9. doi:10.1093/clinchem/46.8.1221

143. Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. Journal of hematotherapy. 1996 Jun;5(3):213–26. doi:10.1089/scd.1.1996.5.213 PubMed PMID: 8817388.

144. Anthony RS, McKelvie ND, Cunningham AJ, Craig JIO, Rogers SY, Parker AC. Flow cytometry using annexin V can detect early apoptosis in peripheral blood stem cell harvests from patients with leukaemia and lymphoma. Bone

Marrow Transplantation. 1998;21(5):441–6. doi:10.1038/sj.bmt.1701134 PubMed PMID: 9535035.

145. Gaines H, Andersson L, Biberfeld G. A new method for measuring lymphoproliferation at the single-cell level in whole blood cultures by flow cytometry. Journal of Immunological Methods. 1996 Sep;195(1–2):63–72. doi:10.1016/0022-1759(96)00085-3

146. Marits P, Wikström AC, Popadic D, Winqvist O, Thunberg S. Evaluation of T and B lymphocyte function in clinical practice using a flow cytometry based proliferation assay. Clinical Immunology. 2014;153:332–42. doi:10.1016/j.clim.2014.05.010

147. Nilsson J, Granrot I, Mattsson J, Omazic B, Uhlin M, Thunberg S. Functionality testing of stem cell grafts to predict infectious complications after allogeneic hematopoietic stem cell transplantation. Vox Sanguinis. 2017 Jul;112(5):459–68. doi:10.1111/vox.12521

148. Leney-Greene MA, Boddapati AK, Su HC, Cantor JR, Lenardo MJ. Human Plasma-like Medium Improves T Lymphocyte Activation. iScience. 2020 Jan;23(1):100759. doi:10.1016/j.isci.2019.100759

149. Dunbar SA. Applications of Luminex® xMAP™ technology for rapid, high-throughput multiplexed nucleic acid detection. Clinica Chimica Acta. 2006 Jan;363(1–2):71–82. doi:10.1016/j.cccn.2005.06.023

150. Fulton RJ, McDade RL, Smith PL, Kienker LJ, Kettman JR. Advanced multiplexed analysis with the FlowMetrixTM system. Clinical Chemistry. 1997 Sep 1;43(9):1749–56. doi:10.1093/clinchem/43.9.1749

151. Vignali DA. Multiplexed particle-based flow cytometric assays. J Immunol Methods. 2000 Sep 21;243(1–2):243–55. doi:10.1016/s0022-1759(00)00238-6 PubMed PMID: 10986418.

152. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. Journal of the American Statistical Association. 1999 Jun;94(446):496–509. doi:10.1080/01621459.1999.10474144

153. Czerw T, Labopin M, Schmid C, Cornelissen JJ, Chevallier P, Blaise D, et al. High CD3+ and CD34+ peripheral blood stem cell grafts content is associated with increased risk of graft-versus-host disease without beneficial effect on disease control after reduced-intensity conditioning allogeneic transplantation from matched unrelated donors for acute myeloid leukemia - an analysis from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. Oncotarget. 2016 May 10;7(19):27255–66. doi:10.18632/oncotarget.8463 PubMed PMID: 27036034; PubMed Central PMCID: PMC5053647.

154. Rezvani K, Mielke S, Ahmadzadeh M, Kilical Y, Savani BN, Zeilah J, et al. High donor FOXP3-positive regulatory T-cell (Treg) content is associated with a low risk of GVHD following HLA-matched allogeneic SCT. Blood. 2006;108(4):1291–7. doi:10.1182/blood-2006-02-003996 PubMed PMID: 16627754.

155. Stanzani M, Martins SLR, Saliba RM, John LSSt, Bryan S, Couriel D, et al. CD25 expression on donor CD4+ or CD8+ T cells is associated with an increased risk for graft-versus-host disease after HLA-identical stem cell transplantation in humans. Blood. 2004 Feb 1;103(3):1140–6. doi:10.1182/blood-2003-06-2085

156. Brauninger S, Bialleck H, Thorausch K, Seifried E, Bonig H. Mobilized allogeneic peripheral stem/progenitor cell apheresis with Spectra Optia v.5·0, a novel, automatic interface-controlled apheresis system: results from the first feasibility trial. Vox Sanguinis. 2011 Oct;101(3):237–46. doi:10.1111/j.1423-0410.2011.01484.x

157. Zhang W, Smythe J, Frith E, Belfield H, Clarke S, Watt SM, et al. An innovative method to generate a Good Manufacturing Practice–ready regulatory T-cell product from non-mobilized leukapheresis donors. Cytotherapy. 2015 Sep;17(9):1268–79. doi:10.1016/j.jcyt.2015.05.015

158. Mohty M, Bagattini S, Chabannon C, Faucher C, Bardou VJ, Bilger K, et al. CD8+ T cell dose affects development of acute graft-vs-host disease following reduced-intensity conditioning allogeneic peripheral blood stem cell transplantation. Exp Hematol. 2004 Nov;32(11):1097–102. doi:10.1016/j.exphem.2004.07.020 PubMed PMID: 15539088.

159. Hassan H, Stockschläder M, Schleimer B, Krüger W, Zander A. Comparison of the content and subpopulations of CD3 and CD34 positive cells in bone marrow harvests and G-CSF-mobilized peripheral blood leukapheresis products from healthy adult donors. Transplant Immunology. 1996 Dec;4(4):319–23. doi:10.1016/S0966-3274(96)80054-2

160. Remberger M, Afram G, Sundin M, Uhlin M, LeBlanc K, Björklund A, et al. High incidence of severe chronic GvHD after HSCT with sibling donors. A single center analysis. Bone Marrow Transplant. 2016 Nov;51(11):1518–21. doi:10.1038/bmt.2016.159

161. Zheng H, Matte-Martone C, Jain D, McNiff J, Shlomchik WD. Central Memory CD8+ T Cells Induce Graft-versus-Host Disease and Mediate Graft-versus-Leukemia. The Journal of Immunology. 2009 May 15;182(10):5938–48. doi:10.4049/jimmunol.0802212

162. Muffly L, Sheehan K, Armstrong R, Jensen K, Tate K, Rezvani AR, et al. Infusion of donor-derived CD8+ memory T cells for relapse following allogeneic

hematopoietic cell transplantation. Blood Advances. 2018 Mar 27;2(6):681–90. doi:10.1182/bloodadvances.2017012104

163. Zhu P, Yang L, Wu Y, Shi J, Lai X, Liu L, et al. Graft CD8 T-cell-based risk system predicts survival in antithymocyte globulin-based myeloablative haploidentical peripheral blood stem cell transplantation. Clin & Trans Imm. 2024 Jan;13(1):e1484. doi:10.1002/cti2.1484

164. Reshef R, Huffman AP, Gao A, Luskin MR, Frey NV, Gill SI, et al. High Graft CD8 Cell Dose Predicts Improved Survival and Enables Better Donor Selection in Allogeneic Stem-Cell Transplantation With Reduced-Intensity Conditioning. JCO. 2015 Jul 20;33(21):2392–8. doi:10.1200/JCO.2014.60.1203

165. Saad A, Lamb L, Wang T, Hemmer MT, Spellman S, Couriel D, et al. Impact of T Cell Dose on Outcome of T Cell-Replete HLA-Matched Allogeneic Peripheral Blood Stem Cell Transplantation. Biology of Blood and Marrow Transplantation. 2019 Sep;25(9):1875–83. doi:10.1016/j.bbmt.2019.05.007

166. Chung H, Kook HW, Cho H, Jang JE, Cheong JW. Impact of CD34+ cell dose on outcomes of haploidentical peripheral blood stem cell transplantation in acute leukemia. Blood Res. 2025 Dec;60(1):42. doi:10.1007/s44313-025-00091-5

167. Mohty M, Bilger K, Jourdan E, Kuentz M, Michallet M, Bourhis JH, et al. Higher doses of CD34+ peripheral blood stem cells are associated with increased mortality from chronic graft-versus-host disease after allogeneic HLA-identical sibling transplantation. Leukemia. 2003 May 1;17(5):869–75. doi:10.1038/sj.leu.2402909

168. Avigan ZM, Dias AL, Dodge LE, Arnason JE, Joyce RM, Liegel J, et al. High CD34 -positive cell dose in matched unrelated donor allogeneic hematopoietic stem cell transplant is not associated with graft-versus-host disease or mortality. Transfusion. 2024 Jun;64(6):1068–75. doi:10.1111/trf.17864

169. Oyama T, Fujiwara S, Tominaga R, Yokoyama D, Noguchi A, Furuki S, et al. Effects of $CD34^+$ cell dose on engraftment and long-term outcomes after allogeneic bone marrow transplantation. Clinical Transplantation. 2024 Apr;38(4):e15313. doi:10.1111/ctr.15313

170. Singh AK, Savani BN, Albert PS, Barrett AJ. Efficacy of CD34+ Stem Cell Dose in Patients Undergoing Allogeneic Peripheral Blood Stem Cell Transplantation after Total Body Irradiation. Biology of Blood and Marrow Transplantation. 2007 Mar;13(3):339–44. doi:10.1016/j.bbmt.2006.10.029

171. Schultz KR, Paquet J, Bader S, HayGlass KT. Requirement for B cells in T cell priming to minor histocompatibility antigens and development of graft-

versus-host disease. Bone Marrow Transplant. 1995 Aug;16(2):289–95. PubMed PMID: 7581150.

172. Sarantopoulos S, Stevenson KE, Kim HT, Cutler CS, Bhuiya NS, Schowalter M, et al. Altered B-cell homeostasis and excess BAFF in human chronic graft-versus-host disease. Blood. 2009 Apr 16;113(16):3865–74. doi:10.1182/blood-2008-09-177840

173. Ratanatharathorn V, Logan B, Wang D, Horowitz M, Uberti JP, Ringden O, et al. Prior rituximab correlates with less acute graft-versus-host disease and better survival in B-cell lymphoma patients who received allogeneic peripheral blood stem cell transplantation. Br J Haematol. 2009 Jun;145(6):816–24. doi:10.1111/j.1365-2141.2009.07674.x

174. Shankar S, Stolp J, Juvet SC, Beckett J, Macklin PS, Issa F, et al. Ex vivo-expanded human CD19+TIM-1+ regulatory B cells suppress immune responses in vivo and are dependent upon the TIM-1/STAT3 axis. Nat Commun. 2022 Jun 3;13(1):3121. doi:10.1038/s41467-022-30613-z

175. Bao Y, Liu J, Li Z, Sun Y, Chen J, Ma Y, et al. Ex vivo-generated human CD1c+ regulatory B cells by a chemically defined system suppress immune responses and alleviate graft-versus-host disease. Molecular Therapy. 2024 Dec;32(12):4372–82. doi:10.1016/j.ymthe.2024.10.026

176. Luo XH, Chang YJ, Xu LP, Liu DH, Liu KY, Huang XJ. The impact of graft composition on clinical outcomes in unmanipulated HLA-mismatched/haploidentical hematopoietic SCT. Bone Marrow Transplant. 2009 Jan;43(1):29–36. doi:10.1038/bmt.2008.267

177. Xu LP, Luo XH, Chang YJ, Liu DH, Liu KY, Chen YH, et al. High CD4/CD8 ratio in allografts predicts adverse outcomes in unmanipulated HLA-mismatched/haploidentical hematopoietic stem cell transplantation for chronic myeloid leukemia. Ann Hematol. 2009 Oct;88(10):1015–24. doi:10.1007/s00277-009-0728-4

178. Chang YJ, Xu LP, Wang Y, Zhang XH, Chen H, Chen YH, et al. Controlled, Randomized, Open-Label Trial of Risk-Stratified Corticosteroid Prevention of Acute Graft-Versus-Host Disease After Haploidentical Transplantation. JCO. 2016 Jun 1;34(16):1855–63. doi:10.1200/JCO.2015.63.8817

179. Huttunen P, Taskinen M, Siitonen S, Saarinen-Pihkala UM. Impact of very early $CD4^+$ /$CD8^+$ T cell counts on the occurrence of acute graft-versus-host disease and NK cell counts on outcome after pediatric allogeneic hematopoietic stem cell transplantation. Pediatric Blood & Cancer. 2015 Mar;62(3):522–8. doi:10.1002/pbc.25347

180. Gaballa A, Stikvoort A, Önfelt B, Mattsson J, Sundin M, Watz E, et al. T-cell frequencies of CD8+ γδ and CD27+ γδ cells in the stem cell graft predict the

outcome after allogeneic hematopoietic cell transplantation. Bone Marrow Transplant. 2019 Oct;54(10):1562–74. doi:10.1038/s41409-019-0462-z

181. Stikvoort A, Gaballa A, Solders M, Nederlof I, Önfelt B, Sundberg B, et al. Risk Factors for Severe Acute Graft-versus-Host Disease in Donor Graft Composition. Biology of Blood and Marrow Transplantation. 2018 Mar;24(3):467–77. doi:10.1016/j.bbmt.2017.11.026

182. Fisher SA, Lamikanra A, Dorée C, Gration B, Tsang P, Danby RD, et al. Increased regulatory T cell graft content is associated with improved outcome in haematopoietic stem cell transplantation: a systematic review. Br J Haematol. 2017 Feb;176(3):448–63. doi:10.1111/bjh.14433

183. Törlén J, Ringdén O, Le Rademacher J, Batiwalla M, Chen J, Erkers T, et al. Low CD34 Dose Is Associated with Poor Survival after Reduced-Intensity Conditioning Allogeneic Transplantation for Acute Myeloid Leukemia and Myelodysplastic Syndrome. Biology of Blood and Marrow Transplantation. 2014 Sep;20(9):1418–25. doi:10.1016/j.bbmt.2014.05.021

184. Antonenas V, Garvin F, Webb M, Sartor M, Bradstock KF, Gottlieb D. Fresh PBSC harvests, but not BM, show temperature-related loss of CD34 viability during storage and transport. Cytotherapy. 2006;8(2):158–65. doi:10.1080/14653240600620994 PubMed PMID: 16698689.

185. Miyake Y, Asano K, Kaise H, Uemura M, Nakayama M, Tanaka M. Critical role of macrophages in the marginal zone in the suppression of immune responses to apoptotic cell–associated antigens. J Clin Invest. 2007 Aug 1;117(8):2268–78. doi:10.1172/JCI31990

186. Fisher V, Khuu H, David-OCampo V, Byrne K, Pavletic S, Bishop M, et al. Analysis of the recovery of cryopreserved and thawed CD 34+ and CD 3+ cells collected for hematopoietic transplantation. Transfusion. 2014 Apr;54(4):1088–92. doi:10.1111/trf.12428

187. Genestier L, Fournel S, Flacher M, Assossou O, Revillard JP, Bonnefoy-Berard N. Induction of Fas (Apo-1, CD95)-Mediated Apoptosis of Activated Lymphocytes by Polyclonal Antithymocyte Globulins. Blood. 1998 Apr 1;91(7):2360–8. doi:10.1182/blood.V91.7.2360

188. Watz E, Remberger M, Ringden O, Ljungman P, Sundin M, Mattsson J, et al. Quality of the hematopoietic stem cell graft affects the clinical outcome of allogeneic stem cell transplantation. Transfusion. 2015;55(10):2339–50. doi:10.1111/trf.13143 PubMed PMID: 25968813.

189. Toussirot E, Bonnefoy F, Vauchy C, Perruche S, Saas P. Mini-Review: The Administration of Apoptotic Cells for Treating Rheumatoid Arthritis: Current Knowledge and Clinical Perspectives. Front Immunol. 2021 Feb 25;12:630170. doi:10.3389/fimmu.2021.630170

190. Amison RT, Cheung TS, Giacomini C, Riffo-Vasquez Y, Galleu A, Savoldelli R, et al. Cryopreserved apoptotic mesenchymal stromal cells retain functional efficacy in suppressing an allergic inflammation in a murine model. Stem Cells Translational Medicine. 2024 Oct 10;13(10):979–84. doi:10.1093/stcltm/szae061

191. Mizrahi K, Yaniv I, Ash S, Stein J, Askenasy N. Apoptotic signaling through Fas and TNF receptors ameliorates GVHD in mobilized peripheral blood grafts. Bone Marrow Transplantation. 2014;49(5):640–8. doi:10.1038/bmt.2014.12 PubMed PMID: 24566711.

192. Gaballa A, Alagrafi F, Uhlin M, Stikvoort A. Revisiting the Role of γδ T Cells in Anti-CMV Immune Response after Transplantation. Viruses. 2021 May 29;13(6):1031. doi:10.3390/v13061031 PubMed PMID: 34072610; PubMed Central PMCID: PMC8228273.

193. Pitard V, Roumanes D, Lafarge X, Couzi L, Garrigue I, Lafon ME, et al. Long-term expansion of effector/memory Vdelta2-gammadelta T cells is a specific blood signature of CMV infection. Blood. 2008 Aug 15;112(4):1317–24. doi:10.1182/blood-2008-01-136713 PubMed PMID: 18539896; PubMed Central PMCID: PMC2515135.

194. Kheav VD, Busson M, Scieux C, De Latour RP, Maki G, Haas P, et al. Favorable impact of natural killer cell reconstitution on chronic graft-versus-host disease and cytomegalovirus reactivation after allogeneic hematopoietic stem cell transplantation. Haematologica. 2014 Dec 1;99(12):1860–7. doi:10.3324/haematol.2014.108407

195. Barron MA, Gao D, Springer KL, Patterson JA, Brunvand MW, McSweeney PA, et al. Relationship of Reconstituted Adaptive and Innate Cytomegalovirus (CMV)–Specific Immune Responses with CMV Viremia in Hematopoietic Stem Cell Transplant Recipients. CLIN INFECT DIS. 2009 Dec 15;49(12):1777–83. doi:10.1086/648423

196. Liu D, Wang X, Han Y, Wang J, Sun Y, Hou Y, et al. A donor PD-1+CD8+ TSCM-like regulatory subset mobilized by G-CSF alleviates recipient acute graft-versus-host-disease. Sig Transduct Target Ther. 2025 Apr 2;10(1):120. doi:10.1038/s41392-025-02183-1

197. Hu Y, Wang Y, Min K, Zhou H, Gao X. The influence of immune checkpoint blockade on the outcomes of allogeneic hematopoietic stem cell transplantation. Front Immunol. 2024 Nov 20;15:1491330. doi:10.3389/fimmu.2024.1491330

198. Herbaux C, Gauthier J, Brice P, Drumez E, Ysebaert L, Doyen H, et al. Efficacy and tolerability of nivolumab after allogeneic transplantation for relapsed Hodgkin lymphoma. Blood. 2017 May 4;129(18):2471–8. doi:10.1182/blood-2016-11-749556

199. Godfrey J, Liu H, Yu J, Tallarico M, Curran E, Artz A, et al. Pembrolizumab for the treatment of disease relapse after allogeneic hematopoietic stem cell transplantation. Blood Advances. 2023 Mar 28;7(6):963–70. doi:10.1182/bloodadvances.2022008403

200. Pham HP, Dormesy S, Wolfe K, Budhai A, Sachais BS, Shi PA. Potentially modifiable predictors of cell collection efficiencies and product characteristics of allogeneic hematopoietic progenitor cell collections. Transfusion. 2021 May;61(5):1518–24. doi:10.1111/trf.16370

201. Filosto S, Vardhanabhuti S, Canales MA, Poiré X, Lekakis LJ, De Vos S, et al. Product Attributes of CAR T-cell Therapy Differentially Associate with Efficacy and Toxicity in Second-line Large B-cell Lymphoma (ZUMA-7). Blood Cancer Discovery. 2024 Jan 8;5(1):21–33. doi:10.1158/2643-3230.BCD-23-0112

202. Müskens KF, Collot-d'Escury WNR, Dandis R, Haitjema S, Kuball J, De Witte MA, et al. Definitions matter: Multicenter investigation of incidence and outcome of poor graft function after hematopoietic cell transplantation. HemaSphere. 2024 Dec;8(12):e70059. doi:10.1002/hem3.70059