CONCISE REVIEW

STEM CELLS
TRANSLATIONAL MEDICINE

Optimizing allogeneic grafts in hematopoietic stem cell transplantation

Zheng-Li Xu | Xiao-Jun Huang ⓘ

Peking University People's Hospital, Peking University Institute of Hematology, National Clinical Research Center for Hematologic Disease, Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, Beijing, People's Republic of China

Correspondence
Xiao-Jun Huang, MD, Peking University People's Hospital, Peking University Institute of Hematology, National Clinical Research Center for Hematologic Disease, Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, No. 11 Xizhimen South Street, Xicheng District, Beijing 100044, People's Republic of China.
Email: huangxiaojun@bjmu.edu.cn

Abstract

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is widely used in the treatment of hematological diseases. It is well known that allogeneic grafts play a key role in predicting transplantation prognosis. Hematopoietic stem cells (HSCs) are a functional part of grafts and are capable of reconstructing hematopoiesis and immunity, but purified HSCs have not been identified or isolated to date. In clinical practice, allogeneic grafts have been optimized to improve transplantation outcomes. The optimized grafts are considered to engraft successfully, reconstruct immunity rapidly, and exert a graft-vs-leukemia (GVL) effect without causing severe graft-vs-host disease (GvHD). In the last several decades, considerable efforts have been made in searching for optimized grafts based on different graft manipulation approaches and different graft sources. Currently, there is no uniform standard for optimized grafts in allogeneic transplantation. In the future, sorting out the cellular elements responsible for the effects of allo-HSCT might be a research direction for further optimization of grafts. In this review, we propose the concept of optimized grafts and summarize the recent advances made in the process of optimizing grafts.

KEYWORDS
CD34+, hematologic malignancies, hematopoiesis, hematopoietic stem cells, hematopoietic stem cell transplantation

Significance statement
Allogeneic hematopoietic stem cell transplantation is widely used in the treatment of hematologic diseases. In clinical practice, allogeneic grafts have been optimized in order to improve transplantation outcomes. The optimized grafts are considered to engraft successfully, reconstruct immunity rapidly, and exert graft-vs-leukemia effect without causing severe graft-vs-host disease. Recently, considerable efforts have been made in searching optimized grafts based on different graft manipulation approaches and different graft sources. The present study explains the concept of optimized grafts and summarizes the recent advances made in the process of optimizing grafts.

1 | INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an established curative approach for an increasing number of patients with life-threatening hematological diseases. Allogeneic grafts are obtained from donors and transplanted into recipients to reconstitute both the hematopoiesis system and the immune system for curing diseases. In current allogeneic transplantation models, successful rates of engraftment have been close to 100% in both malignant and non-malignant diseases,[1,2] but graft-vs-host disease (GvHD), severe infection due to poor immune system reconstitution, and disease relapse remain major causes of transplantation failures.[3] In this sense, an ideal graft would successfully reconstruct hematopoietic and immunity, as well as exert a graft-vs-leukemia (GVL) effect without causing severe GvHD.

In clinical practice, clinicians always equate the concept of grafts with hematopoietic stem cells (HSCs) in allo-HSCT. HSCs are functional units of grafts, and healthy HSCs possess long-term engraftment and multilineage repopulation abilities among preconditioned recipients.[4] In addition to HSCs, grafts contain numerous downstream progenitors and mature blood cells that do not have long-term repopulating activity but are essential for immune reconstitution. In recent years, great progress has been made in identifying the respective roles of different graft compositions. To improve transplantation outcomes, researchers have been optimizing grafts mainly based on different graft manipulation approaches and different graft sources. In the current review, we will introduce what we have done and where we will go in searching for optimized grafts.

2 | TO OPTIMIZE GRAFT COMPOSITION BY GRAFT ENGINEERING

The composition of allogeneic grafts is of vital importance for clinical outcomes.[5] The key components of allogeneic grafts include hematopoietic stem/progenitor cells, T cells, B cells, NK cells, dendritic cells (DCs), and so on.[6] The optimal composition of grafts has not been determined, but there has been great progress in identifying the effects of various cellular components among grafts during recent decades.

2.1 | HSCs are the basic component of grafts

At minimum, a graft must include HSCs, which are capable of regenerating hematopoiesis in a recipient who has received myeloablative or non-myeloablative conditioning. As proven by human transplantation and xenograft repopulation studies, the bulk of HSCs are CD34 positive.[7] CD34 is expressed on 0.5% to 5% of human bone marrow cells, and this population comprises most long-term multipotent HSCs and far more numerous short-term progenitor cells.[8] Thus, CD34 is routinely used to identify and isolate human hematopoietic stem/progenitor cells for clinical use in HSCT.[9]

In the 1990s, CD34-positive selection technology was developed to enhance the elimination of other immune cell components in grafts. In 44 acute myeloid leukemia (AML) patients receiving human leukocyte antigen (HLA)-identical sibling grafts using CD34-positive selection as the sole form of immune suppression, neutrophil engraftment rates of 100% and grade II-IV aGvHD rates of 23% were both inspiring.[10] Nevertheless, it turned out to be disappointing that delayed immune reconstitution after transplantation was the major drawback of this approach. In the study by Aversa, a total of 26% of cases suffered from infection-related mortality due to delayed immune reconstitution in haploidentical transplants using CD34 purification.[11] From the perspective of immune reconstitution, CD34-positive selection might be a suboptimal choice.

For the CD34+ dose, the typical dose of CD34+ cells used for allo-HSCT is 2×10^6 cells/kg of recipient body weight or greater.[12] In the T-cell-depleted (TCD) model, the use of the "CD34+ mega dose" concept was introduced to overcome the risk of engraftment failure encountered with TCD. The initial experience showed that the infusion of approximately 10×10^6 CD34+ cells/kg resulted in sustained engraftment in over 90% of patients.[13] In a non-TCD model, the median value of nearly 3×10^6 CD34+ cells/kg resulted in 98% myeloid engraftment, even in heavily transfused aplastic anemia.[14] These results also suggest that T cells in grafts might play a role in promoting engraftment.

Above all, grafts containing only CD34-positive cells are not an optimized choice because depleted cell components might be responsible for rapid immune reconstruction. In addition, the optimal doses of CD34+ cells still vary among different transplant models.

2.2 | T cells in grafts: Good or Evil

On the one hand, T cells in grafts facilitate HSC engraftment, promote immune reconstitution, and achieve an enhanced GVL effect. On the other hand, donor T cells are the primary participant in the development of GvHD. The roles of T cells vary depending on the number of HSCs, the degree of HLA match between recipient and donor, and the intensity of the conditioning regimen.[6] In graft engineering, the major objective is to preserve selected subsets that can mediate GVL with minimal GvHD activity. The identification of the function of various subsets is useful in the optimization of grafts.

2.2.1 | Pan-TCD in the original experience

To reduce the incidence of GvHD, pan-T-cell removal was initially attempted.[15] Although the incidence of GvHD was obviously reduced, increased risks of graft failure and disease relapse were also observed with pan-TCD.[16,17] In 114 leukaemic recipients of HLA-identical sibling marrow depleted of pan-T cells, graft failure occurred in appropriately 17% of patients.[16] In another cohort of patients with chronic myelogenous leukemia in the chronic phase, the 3-y probability of relapse for 318 recipients of non-TCD bone marrow was 9%,

compared with a probability of 48% for 87 recipients of TCD marrow from HLA-identical siblings ($P < .0001$).[17] These results indicated that pan-TCD led to the loss of the GVL effect and an increased risk of graft failure. Thus, graft engineering by pan-TCD was not recommended.

2.2.2 | T-cell subset manipulation

T-cell subset depletion, such as CD8+ T-cell depletion, $\alpha\beta$+ TCR T-cell depletion, or CD45RA+ T-cell depletion, with negative selection has been progressively attempted with the aim of improving clinical results. The premise of this approach is to recognize the function of different T cell subsets.

CD8+ T-cell depletion

CD8+ T cells are regarded as effector cytotoxic cells that mediate GvHD tissue injury. An earlier randomized study included patients who received a CD8+-depleted donor lymphocyte infusion (DLI) and showed an obvious reduction in acute GvHD while GVL activity was preserved.[18] However, a later study applied CD8+ cell depletion in HLA-matched peripheral blood stem cell (PBSC) transplantation and failed to mitigate the risk of GvHD, with an incidence of acute GvHD grades II-IV of 61%.[19] One explanation was that CD8+ cells infused in PBSC grafts were much higher than those infused in purged DLI. More importantly, it is speculated that GvHD is not exclusively mediated by CD8+ T cells and that the remaining CD4+ T cells in the PBSC product are also important in the pathogenesis of GvHD. Thus, the markers CD4 or CD8 are not sufficient to distinguish between GvHD and GVL.

In addition, murine models have indicated that CD8+ memory T (T_M) cells can kill malignant cells without causing GvHD.[20] Phenotypic CD8 T_M cells were isolated using CD45RA depletion followed by CD8 + enrichment. Clinically, donor-derived phenotypic CD8+ T_M cells were infused in 15 relapsed patients after allo-HSCT. A total of 10 (67%) patients maintained or achieved a response, and only one developed GvHD.[21] This also suggests that different CD8 T-cell subsets might play different roles in the pathogenicity of GvHD.

$\alpha\beta$ + TCR T-cell depletion

T cells have either an $\alpha\beta$ T-cell receptor (TCR) or an $\gamma\delta$ TCR. $\alpha\beta$+ TCR T cells are implicated in mediating GvHD, and $\gamma\delta$+ TCR T cells might contribute to possible anti-leukemia and anti-infectious activities. In addition, B cells are implicated in EBV-driven post-transplantation lymphoproliferative disorders and may play a role in GvHD pathogenesis as well. Based on these theories, TCR $\alpha\beta$/CD19 depletion has been further developed. This selection strategy has led to acute GvHD rates of 13% to 22%, graft failure rates of 17% to 27%, and NRM of 3% to 9% in haploidentical or unrelated cohorts.[22,23] Subsequently, a prospective trial was conducted to evaluate the outcomes of children with acute leukemia who received haploidentical grafts with TCR $\alpha\beta$/CD19 depletion. Finally, leukemia-free, GVHD-free survival of patients given this type of haploidentical allograft is comparable to that of matched related donor (MRD) allografts.[24] For immune

reconstitution, rapid recovery of NK and $\gamma\delta$ T cells was shown with this type of transplant strategy, which was desirable given their important roles in protection against infections and the GVL effect.[24,25] The recovery of $\alpha\beta$ T cells and B cells was observed to occur more gradually. These studies suggest that TCR $\alpha\beta$/CD19 depletion might be a viable option for graft engineering.

CD45RA+ T-cell depletion

Naive T cells (Tn, CD45RA+/CD62L+) are mature, un-sensitized T cells and are the most alloreactive among the T cell subsets. Tn cells were indicated to cause more severe GvHD in animal models.[26] Thus, a novel graft-engineering strategy was attempted in which Tn cells were selectively depleted by a monoclonal antibody targeting CD45RA. A single-arm experience with a limited number of patients (n = 35) showed that the incidence of grade II-IV aGvHD was up to 66% in a MRD cohort, which was not reduced as expected, but the incidence of chronic GvHD was only 9%.[27] However, another phase I dose escalation study found that the maximum administered cell dose of 1×10^7 CD3+ cells/kg DLI depleted of CD45RA+ Tn cells did not result in clinically significant acute GvHD in patients following HLA-identical HSCT.[28] More recently, selective CD45RA T-cell depletion has been proven to reduce viremia and enhance early T-cell recovery compared to CD3-depleted T cells in haploidentical cohorts. The incidence of severe aGvHD in CD45RA-depleted recipients was similar to that in CD3-depleted recipients.[29] To date, clinical evidence in this regard is ongoing.

Selective adding back of regulatory T cells (Tregs)

In pre-clinical models, co-infusion with conventional T lymphocytes (Tcons) and Tregs reduced GvHD without compromising Tcon activity against tumor cells.[30] In a haploidentical donor cohort, 43 adults with high-risk AL received Treg-Tcon adoptive immunotherapy, and only 15% developed grade II-IV acute GvHD. The cumulative incidence of relapse was 5%, which was obviously better than that of historical controls.[31] These results demonstrate the potential of the Treg subset to suppress GvHD without a loss of the benefits of GVL activity.

T-cell engineering

A novel approach relies on the use of T-cell engineering. Donor T cells can be equipped with a "safety switch," which can help exert a GVL effect with the possibility of triggering induced cell apoptosis if severe GvHD occurs. HSV-TK cells,[32] iC9-T cells,[33] and BPX-501 cells[34] have been tested with positive preliminary results, but this strategy remains a cumbersome approach, requiring a time-consuming and costly manufacturing process. Nevertheless, this approach has provided a new method of graft optimization.

2.3 | NK cells: an integral component of the innate immune system

NK cells are the most rapidly reconstructed immune cells after HSCT and possess anti-infection and anti-leukemia effects. Russo et al recently documented early elimination of all mature NK cells,

including the alloreactive subset, in the PT-Cy model because donor-derived NK cells are sensitive to Cy-mediated killing.[35] In CD34-positive selection and PT-Cy models, the delayed recovery of mature NK cells might impair their GVL effect and protection against infections. Thus, high doses of ex vivo expanded NK cell infusion were studied in a PT-Cy-based haploidentical model. The infusion of expanded NK cells was found to be associated with improved NK cell number and function, lower viral infections, and a lower relapse rate post transplantation.[36] In another trial, 24 refractory AML patients received a transplant from a haploidentical relative and received adoptively transferred NK cells at day +1 after the transfer of purified CD34+ HSCs. A 2-y overall survival (OS) rate of 37% was observed, suggesting that adoptively transferred NK cells possibly contribute to long-term remission in refractory patients.[37]

NK cells were divided into immature NK cells (CD56highCD16dim) and mature cytotoxic NK cells (CD56dimCD16high) according to flow cytometry. A longitudinal analysis after allo-HSCT showed that the incidence of aGvHD was associated with a delayed expansion of the CD56high subset.[38] In addition, a high CD56dim/CD56high NK cell ratio early after transplantation was associated with better disease-free survival (DFS, 77% vs 28%; $P = .001$) due to lower relapse incidence (15% vs 37%; $P = .04$) in the αβ+ TCR/CD19+-depleted graft model.[39] These results suggest that the number and subsets of NK cells affect transplantation prognosis.

2.4 | DCs possess immunoregulatory functions

DCs are professional antigen-presenting cells (APCs) involved in the induction and regulation of immune responses.[40] According to their potential ability to induce Tn cell differentiation into Th1 and Th2 effector cells, two distinct lineages of DCs have been identified, namely, myeloid DCs (mDCs) and plasmacytoid DCs (pDCs).

Regarding DC reconstitution, both mDC and pDC counts recovered to pre-HSCT levels within 2 mo. In patients with cGvHD, decreased total DC counts and increased pDCs were found after day 100 post transplantation.[41] In patients with hematological

malignancies undergoing MRD HSCT, a high pDC content in the graft was observed to be significantly associated with a higher risk of relapse and lower OS and EFS.[42] This suggests a potential for pre-transplantation manipulation of this cellular subset in the graft.

To summarize this part of graft engineering, we have progressed from an era of "a megadose of CD34-positive cells with very few donor T lymphocytes" to a new era of a "better-designed graft" by selectively depleting or adding graft cellular subsets. With the deepening understanding of different cellular subsets in grafts, it is becoming clearer which graft subset is needed and which is not. Thus, graft engineering or manipulation will be optimized to reconstruct immunity and preserve a GVL effect while minimizing the incidence of GvHD.

3 | WHAT IS THE BEST GRAFT SOURCE?

3.1 | Different compositions of different graft sources

There are three sources of allogeneic grafts used for allo-HSCT: bone marrow (BM), PBSCs, and umbilical cord blood (UCB) (Table 1). Traditionally, grafts are collected from BM in allo-HSCT. In BM, approximately 1 in every 100 000 cells is a long-term blood-forming stem cell.[8] Currently, stem cells are also harvested from cytokine-mobilized PB and from UCB. The collection of PBSCs is usually performed by apheresis after mobilizing HSCs from BM niches under the effect of hematopoietic growth factors, including GM-CSF and G-CSF, or CXCR4 inhibitors, which have been shown to increase the numbers of circulating hematopoietic stem and progenitor cells by 30- to 1000-fold.[43] In the PB, approximately 5% to 20% of the cells collected would be regarded as true HSCs.[8] UCB is another abundant source of HSCs and progenitors, and the number of different types of hematopoietic progenitors is approximately 10 times higher than that observed in adult blood.

An important difference among graft sources is the amount of mature T cells present. PBSCs usually contain many more mature T cells than BM, and BM contains more T cells than UCB. In general, each of three HSC sources has its own advantages and disadvantages due to different graft compositions.[44] In BM sources, a higher level of CD34+ cells and a lower risk of GvHD have been established, but donors suffer from more invasive HSC collection. PBSCs are proven to be related to faster hematopoietic engraftment and immune reconstitution and an enhanced GVL effect but a higher risk of GvHD. UCB has the advantages of being a non-invasive, rapidly available source and possesses an increased level of HLA disparity tolerance and lower risks of GvHD and disease relapse. However, UCB has a relatively lower number of HSCs and slower immune reconstitution.

3.2 | Clinical outcomes from different graft sources

Although there are many differences between these three HSC sources, clinical survival after transplantation seems to be comparable.

TABLE 1 Comparison of hematopoietic stem cell sources

	BM	PB	UCB
Grafts			
Mature T cells	Lower	Higher	Lower
Red cells	Higher	Lower	Lower
Donors	More invasive collection	No need for general anesthesia	Non-invasive
Recipients			
Engraftment	Medium	Faster	Slower
Immune reconstitution	Faster	Faster	Slower
GvHD	Lower	Higher	Lower
Viremia	Higher	Higher	Lower

A meta-analysis involving 1521 patients with hematological malignancies indicated a statistically significant reduction in the incidence of chronic GvHD for patients transplanted with BM but no difference in OS or DFS outcomes when compared with PB from matched related, unrelated and haploidentical donors.[45] A phase III, multicenter, randomized trial of the transplantation of PB vs BM from unrelated donors also demonstrated similar survival rates, and PB may reduce the risk of graft failure, while BM may reduce the risk of GvHD.[46] High-level evidence is not available for UCB in comparison to BM or PBSCs. A single-center experience found that there was no apparent difference in OS, DFS, or the relapse rate for patients who received UCB, BM, or PB as graft sources.[47]

At present, there is no definite suggestion as to the source of the graft in allogeneic transplantation. Notably, different combinations of graft sources have also been applied in clinical use, such as the "Beijing protocol," which included G-CSF-mobilized bone marrow and peripheral blood grafts to combine their advantages and further promote engraftment and reduce the incidence of GvHD in both malignant and non-malignant diseases.[1,2]

4 | FUTURE DIRECTIONS ON OPTIMIZING GRAFTS

As the saying goes, "when water is clean, there are no fish." CD34-positive selection might be less than satisfactory because immune components such as γδ+ T cells, Tregs, and NK cells are lost during the procedure, and these cells play a role in promoting engraftment and improving the post-transplantation immune recovery of anti-infection and anti-leukemia agents. Although there is currently no exact answer for the optimization of HSCs, the optimization of grafts by engineering grafts with different compositions might be a future research direction.

In the future, the main objective should be to clarify the role of each immune cell among grafts and determine how to regulate their role in optimizing grafts. Perhaps the use of artificial intelligence technology for large data may help us clarify the proportion and relationship of various graft components. In detail, the quantity and function of various immune cells and the ways they act on the basis of receptors or signaling pathways in influencing clinical outcomes should be further explored. In this way, physicians can further identify and retain immune cells that promote immune reconstitution and enhance anti-tumor effects and deplete or engineer cells that cause negative effects after allo-HSCT. With this approach of an immune cell cocktail, the grafts will be optimized to the greatest extent.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

X.-J.H.: designed the review, wrote the manuscript; Z.-L.X.: wrote the manuscript; all authors gave final approval of the manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Xiao-Jun Huang https://orcid.org/0000-0002-2145-6643

REFERENCES

1. Wang Y, Liu QF, Xu LP, et al. Haploidentical versus matched-sibling transplant in adults with Philadelphia-negative high-risk acute lymphoblastic leukemia: a biologically phase III randomized study. *Clin Cancer Res*. 2016;22(14):3467-3476. https://doi.org/10.1158/1078-0432.CCR-15-2335.
2. Xu LP, Jin S, Wang SQ, et al. Upfront haploidentical transplant for acquired severe aplastic anemia: registry-based comparison with matched related transplant. *J Hematol Oncol*. 2017;10(1):25. https://doi.org/10.1186/s13045-017-0398-y.
3. Chang YJ, Xu LP, Wang Y, et al. Controlled, randomized, open-label trial of risk-stratified corticosteroid prevention of acute graft-versus-host disease after Haploidentical transplantation. *J Clin Oncol*. 2016;34(16):1855-1863. https://doi.org/10.1200/JCO.2015.63.8817.
4. Jurecic R. Hematopoietic stem cell heterogeneity. *Adv Exp Med Biol*. 2019;1169:195-211. https://doi.org/10.1007/978-3-030-24108-7_10.
5. Impola U, Larjo A, Salmenniemi U, et al. Graft immune cell composition associates with clinical outcome of allogeneic hematopoietic stem cell transplantation in patients with AML. *Front Immunol*. 2016;7:523. https://doi.org/10.3389/fimmu.2016.00523.
6. Biernacki MA, Sheth VS, Bleakley M. T cell optimization for graft-versus-leukemia responses. *JCI Insight*. 2020;5(9):134939. https://doi.org/10.1172/jci.insight.134939.
7. Notta F, Doulatov S, Laurenti E, et al. Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. *Science*. 2011;333(6039):218-221. https://doi.org/10.1126/science.1201219.
8. Mosaad YM. Hematopoietic stem cells: an overview. *Transfus Apher Sci*. 2014;51(3):68-82. https://doi.org/10.1016/j.transci.2014.10.016.
9. AbuSamra DB, Aleisa FA, Al-Amoodi AS, et al. Not just a marker: CD34 on human hematopoietic stem/progenitor cells dominates vascular selectin binding along with CD44. *Blood Adv*. 2017;1(27):2799-2816. https://doi.org/10.1182/bloodadvances.2017004317.
10. Pasquini MC, Devine S, Mendizabal A, et al. Comparative outcomes of donor graft CD34+ selection and immune suppressive therapy as graft-versus-host disease prophylaxis for patients with acute myeloid leukemia in complete remission undergoing HLA-matched sibling allogeneic hematopoietic cell transplantation. *J Clin Oncol*. 2012;30(26):3194-3201. https://doi.org/10.1200/JCO.2012.41.7071.
11. Aversa F, Terenzi A, Tabilio A, et al. Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse. *J Clin Oncol*. 2005;23(15):3447-3454. https://doi.org/10.1200/JCO.2005.09.117.
12. Mosaad YM. Immunology of hematopoietic stem cell transplant. *Immunol Invest*. 2014;43(8):858-887. https://doi.org/10.3109/08820139.2014.942460.

13. Aversa F, Tabilio A, Terenzi A, et al. Successful engraftment of T-cell-depleted haploidentical "three-loci" incompatible transplants in leukemia patients by addition of recombinant human granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells to bone marrow inoculum. *Blood*. 1994;84(11):3948-3955.

14. Cao LQ, Xu LP, Zhang XH, et al. Relationship of cell compositions in allografts with outcomes after haploidentical transplantation for acquired severe aplastic anemia: effects of CD34(+) and CD14(+) cell doses. *Chin Med J (Engl)*. 2018;131(18):2185-2192. https://doi.org/10.4103/0366-6999.240810.

15. Reisner Y, Kapoor N, Kirkpatrick D, et al. Transplantation for acute leukaemia with HLA-A and B nonidentical parental marrow cells fractionated with soybean agglutinin and sheep red blood cells. *Lancet*. 1981;2(8242):327-331. https://doi.org/10.1016/s0140-6736(81)90647-4.

16. Kernan NA, Bordignon C, Heller G, et al. Graft failure after T-cell-depleted human leukocyte antigen identical marrow transplants for leukemia: I. Analysis of risk factors and results of secondary transplants. *Blood*. 1989;74(6):2227-2236.

17. Goldman JM, Gale RP, Horowitz MM, et al. Bone marrow transplantation for chronic myelogenous leukemia in chronic phase. Increased risk for relapse associated with T-cell depletion. *Ann Intern Med*. 1988;108(6):806-814. https://doi.org/10.7326/0003-4819-108-6-806.

18. Soiffer RJ, Alyea EP, Hochberg E, et al. Randomized trial of CD8+ T-cell depletion in the prevention of graft-versus-host disease associated with donor lymphocyte infusion. *Biol Blood Marrow Transplant*. 2002;8(11):625-632. https://doi.org/10.1053/bbmt.2002.v8.abbmt080625.

19. Ho VT, Kim HT, Li S, et al. Partial CD8+ T-cell depletion of allogeneic peripheral blood stem cell transplantation is insufficient to prevent graft-versus-host disease. *Bone Marrow Transplant*. 2004;34(11):987-994. https://doi.org/10.1038/sj.bmt.1704690.

20. Dutt S, Baker J, Kohrt HE, et al. CD8+CD44(hi) but not CD4+CD44(hi) memory T cells mediate potent graft antilymphoma activity without GVHD. *Blood*. 2011;117(11):3230-3239. https://doi.org/10.1182/blood-2010-10-312751.

21. Muffly L, Sheehan K, Armstrong R, et al. Infusion of donor-derived CD8(+) memory T cells for relapse following allogeneic hematopoietic cell transplantation. *Blood Adv*. 2018;2(6):681-690. https://doi.org/10.1182/bloodadvances.2017012104.

22. Bertaina A, Merli P, Rutella S, et al. HLA-haploidentical stem cell transplantation after removal of alphabeta+ T and B cells in children with nonmalignant disorders. *Blood*. 2014;124(5):822-826. https://doi.org/10.1182/blood-2014-03-563817.

23. Balashov D, Shcherbina A, Maschan M, et al. Single-center experience of unrelated and Haploidentical stem cell transplantation with TCRalphabeta and CD19 depletion in children with primary immunodeficiency syndromes. *Biol Blood Marrow Transplant*. 2015;21(11):1955-1962. https://doi.org/10.1016/j.bbmt.2015.07.008.

24. Locatelli F, Merli P, Pagliara D, et al. Outcome of children with acute leukemia given HLA-haploidentical HSCT after alphabeta T-cell and B-cell depletion. *Blood*. 2017;130(5):677-685. https://doi.org/10.1182/blood-2017-04-779769.

25. Airoldi I, Bertaina A, Prigione I, et al. Gammadelta T-cell reconstitution after HLA-haploidentical hematopoietic transplantation depleted of TCR-alphabeta+/CD19+ lymphocytes. *Blood*. 2015;125(15):2349-2358. https://doi.org/10.1182/blood-2014-09-599423.

26. Dutt S, Tseng D, Ermann J, et al. Naive and memory T cells induce different types of graft-versus-host disease. *J Immunol*. 2007;179(10):6547-6554. https://doi.org/10.4049/jimmunol.179.10.6547.

27. Bleakley M, Heimfeld S, Loeb KR, et al. Outcomes of acute leukemia patients transplanted with naive T cell-depleted stem cell grafts. *J Clin Invest*. 2015;125(7):2677-2689. https://doi.org/10.1172/JCI81229.

28. Maung KK, Chen BJ, Barak I, et al. Phase I dose escalation study of naive T-cell depleted donor lymphocyte infusion following allogeneic stem cell transplantation. *Bone Marrow Transplant*. 2021;56(1):137-143. https://doi.org/10.1038/s41409-020-0991-5.

29. Triplett BM, Muller B, Kang G, et al. Selective T-cell depletion targeting CD45RA reduces viremia and enhances early T-cell recovery compared with CD3-targeted T-cell depletion. *Transpl Infect Dis*. 2018;20(1):12823. https://doi.org/10.1111/tid.12823.

30. Edinger M, Hoffmann P, Ermann J, et al. CD4+CD25+ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. *Nat Med*. 2003;9(9):1144-1150. https://doi.org/10.1038/nm915.

31. Martelli MF, Di Ianni M, Ruggeri L, et al. HLA-haploidentical transplantation with regulatory and conventional T-cell adoptive immunotherapy prevents acute leukemia relapse. *Blood*. 2014;124(4):638-644. https://doi.org/10.1182/blood-2014-03-564401.

32. Ciceri F, Bonini C, Stanghellini MT, et al. Infusion of suicide-gene-engineered donor lymphocytes after family haploidentical haemopoietic stem-cell transplantation for leukaemia (the TK007 trial): a non-randomised phase I-II study. *Lancet Oncol*. 2009;10(5):489-500. https://doi.org/10.1016/S1470-2045(09)70074-9.

33. Zhou X, Dotti G, Krance RA, et al. Inducible caspase-9 suicide gene controls adverse effects from alloreplete T cells after haploidentical stem cell transplantation. *Blood*. 2015;125(26):4103-4113. https://doi.org/10.1182/blood-2015-02-628354.

34. Naik S, Baumeister SHC, Aquino V, et al. Administration of BPX-501 cells following Aβ- T and B-cell-depleted HLA-haploidentical HSCT in children with fanconi anemia. *Blood*. 2018;132(Suppl. 1):4654.

35. Russo A, Oliveira G, Berglund S, et al. NK cell recovery after haploidentical HSCT with posttransplant cyclophosphamide: dynamics and clinical implications. *Blood*. 2018;131(2):247-262. https://doi.org/10.1182/blood-2017-05-780668.

36. Ciurea SO, Schafer JR, Bassett R, et al. Phase 1 clinical trial using mbIL21 ex vivo-expanded donor-derived NK cells after haploidentical transplantation. *Blood*. 2017;130(16):1857-1868. https://doi.org/10.1182/blood-2017-05-785659.

37. Killig M, Friedrichs B, Meisig J, et al. Tracking in vivo dynamics of NK cells transferred in patients undergoing stem cell transplantation. *Eur J Immunol*. 2014;44(9):2822-2834. https://doi.org/10.1002/eji.201444586.

38. Ullrich E, Salzmann-Manrique E, Bakhtiar S, et al. Relation between acute GVHD and NK cell subset reconstitution following allogeneic stem cell transplantation. *Front Immunol*. 2016;7:595. https://doi.org/10.3389/fimmu.2016.00595.

39. Diaz MA, Zubicaray J, Molina B, et al. Haploidentical stem cell transplantation in children with hematological malignancies using alphabeta(+) T-cell receptor and CD19(+) cell depleted grafts: high CD56(dim)/CD56(bright) NK cell ratio early following transplantation is associated with lower relapse incidence and better outcome. *Frontiers in Immunology*. 2019;10:2504. https://doi.org/10.3389/fimmu.2019.02504.

40. Wikstrom ME, Fleming P, Kuns RD, et al. Acute GVHD results in a severe DC defect that prevents T-cell priming and leads to fulminant cytomegalovirus disease in mice. *Blood*. 2015;126(12):1503-1514. https://doi.org/10.1182/blood-2015-01-622837.

41. Wiegering V, Peter L, Frietsch M, et al. Differences of immune reconstitution of dendritic cells in pediatric GvHD patients after allogenic stem cell transplantation. *J Pediatr Hematol Oncol*. 2019;41(2):e101-e107. https://doi.org/10.1097/MPH.0000000000001342.

42. Rajasekar R, Lakshmi KM, George B, et al. Dendritic cell count in the graft predicts relapse in patients with hematologic malignancies undergoing an HLA-matched related allogeneic peripheral blood stem cell transplant. *Biol Blood Marrow Transplant*. 2010;16(6):854-860. https://doi.org/10.1016/j.bbmt.2010.01.013.

43. Micallef IN, Stiff PJ, Nademanee AP, et al. Plerixafor plus granulocyte colony-stimulating factor for patients with non-Hodgkin lymphoma and

multiple myeloma: long-term follow-up report. *Biol Blood Marrow Transplant*. 2018;24(6):1187-1195. https://doi.org/10.1016/j.bbmt.2018.01.039.

44. Juric MK, Ghimire S, Ogonek J, et al. Milestones of hematopoietic stem cell transplantation - from first human studies to current developments. *Front Immunol*. 2016;7:470. https://doi.org/10.3389/fimmu.2016.00470.

45. Holtick U, Albrecht M, Chemnitz JM, et al. Comparison of bone marrow versus peripheral blood allogeneic hematopoietic stem cell transplantation for hematological malignancies in adults - a systematic review and meta-analysis. *Crit Rev Oncol Hematol*. 2015;94(2):179-188. https://doi.org/10.1016/j.critrevonc.2014.12.007.

46. Anasetti C, Logan BR, Lee SJ, et al. Peripheral-blood stem cells versus bone marrow from unrelated donors. *N Engl J Med*. 2012;367(16):1487-1496. https://doi.org/10.1056/NEJMoa1203517.

47. Chen YH, Xu L, Liu D, et al. Comparative outcomes between cord blood transplantation and bone marrow or peripheral blood stem cell transplantation from unrelated donors in patients with hematologic malignancies: a single-institute analysis. *Chin Med J*. 2013;126:2499-2503.