

Review Article

Immune reconstitution after T-cell replete HLA haploidentical hematopoietic stem cell transplantation using high-dose post-transplant cyclophosphamide

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As HLA haploidentical related donors are quickly available, HLA haploidentical hematopoietic stem cell transplantation (haploHSCT) using high-dose post-transplant cyclophosphamide (PTCy) is now widely used. Recent basic and clinical studies revealed the details of immune reconstitution after T-cell replete haploHSCT using PTCy. T cells and NK cells in the graft proliferate abundantly at day 3 post-haploHSCT, and the PTCy eliminates these proliferating cells. After ablation of proliferating mature cells, donor-derived NK cell reconstitution occurs after the second week; however, recovering NK cells remain functionally impaired for at least several months after haploHSCT. PTCy depletes proliferating cells, resulting in the preferential accumulation of Treg and CD4⁺ T cells, especially the memory stem T cell (T_{SCM}) phenotype. T_{SCM} capable of both self-renewal and differentiation into effector T cells may play an important role in the first month of immune reconstitution. Subsequently, *de novo* T cells progressively recover but their levels remain well below those of donor CD4⁺ T cells at the first year after haploHSCT. The phenotype of recovering T cells after HSCT is predominantly effector memory, whereas B cells are predominantly phenotypically naive throughout the first year after haploHSCT. B cell recovery depends on *de novo* generation and they are not detected until week 4 after haploHSCT. At week 5, recovering B cells mostly exhibit an unconventional transitional cell phenotype and the cell subset undergoes maturation. Recent advances in immune reconstitution have improved our understanding of the relationship between haploHSCT with PTCy and the clinical outcome.

Keywords: Immune reconstitution, haploidentical hematopoietic stem cell transplantation, post-transplant cyclophosphamide

INTRODUCTION

Donor availability remains one of the major challenges to the success of allogeneic hematopoietic cell transplantation (HSCT). A human leukocyte antigen (HLA)-matched sibling donor (MSD) or HLA-matched unrelated donor (MUD) can be identified for only around 50% of patients requiring HSCT. HLA haploidentical related donors are quickly available and can be identified for nearly all patients. However, historically, HSCT from a related donor mismatched for one HLA haplotype (HLA haploidentical transplantation, haploHSCT) was associated with high rates of graft failure and graft vs. host disease (GVHD). The Baltimore group developed post-transplant cyclophosphamide (PTCy) to overcome HLA barriers and omit the need for *ex vivo* T cell depletion following HSCT. Several studies demonstrated that haploHSCT with PTCy is associated with low incidences of graft failure, severe acute and chronic GVHD and non-relapse

mortality (NRM). Therefore, haploHSCT with PTCy represents a promising solution to enable all patients indicated for transplant who lack an HLA-matched donor to undergo allogeneic HCT.


As PTCy depletes proliferating cells and immune cell recovery depends on *de novo* generation rather than proliferation of mature cells in the graft, the graft cell content may have a lesser impact on the outcome after haploHSCT with PTCy.¹⁻³ To better understand the relationship between haploHSCT with PTCy and clinical outcome, we reviewed recent studies on immune reconstitution after T-cell replete haploHSCT using PTCy, although there are other approaches to haploHSCT using antithymocyte globulin (ATG) and intense immunosuppression (GIAC protocol) developed by the Peking group or T cell-depleted (TCD) haploHSCT using CD34-positive selection or CD3/CD19 cell depletion.

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Monocyte reconstitution

Monocytes are heterogeneous and can be subdivided into three subpopulations, classic (CD14⁺⁺CD16⁻), intermediate (CD14⁺CD16⁺) and non-classic (CD14⁺CD16⁺⁺) monocytes. Turcotte *et al.* reported that a higher absolute monocyte count and classic monocyte subsets at day 28 are associated with a reduced risk of relapse and treatment-related mortality (TRM), in addition to an improved 2-year overall survival (OS).⁴ Monocyte counts normalize by 1 month after conventional HSCT, whereas monocyte reconstitution has not been fully evaluated in the haploHSCT with PTCy setting. However, in patient groups receiving haploHSCT using the GIAC protocol, monocyte expansion was rapid, reaching normal values within 30 days of transplant;⁵ similar results were observed in patients receiving T cell-depleted haploHSCT using CD34-positive selection or CD3/CD19 cell depletion.⁶

Neutrophil reconstitution

The median times to neutrophil recovery are 16-19 days and 21-23 days in peripheral blood (PB) and bone marrow (BM) HLA-identical sibling transplantations, respectively (Table 1). The Center for International Blood and Marrow Transplant Research conducted a large scale comparison of outcomes for T cell replete haploHSCT with PTCy using either BM or PB grafts. The median time to neutrophil recovery was 1 day slower after transplantation of BM than after PB (17 vs. 16 days), but there were no significant differences in the rate of neutrophil recovery at day 28.⁷ The similar recovery after haploHSCT with PTCy contrasts the finding that neutrophil recovery occurs 4 to 6 days earlier with PB in the matched related and unrelated donors.⁸⁻¹⁰ Complete or near complete donor chimerism occurs quickly after haploHSCT^{10,11} and there were no differences in graft failure rates after transplantation of BM versus PB.⁷

Natural killer (NK) cell reconstitution

NK cells can be divided into two main subsets based on CD56 and CD16 surface expression. CD56^{bright}/CD16^{neg}-low (CD56^{br}) NK cells representing NKG2A⁺ KIR⁻ are immature and have regulatory functions.¹² Conversely, CD56^{dim}/CD16^{pos} (CD56^{dim}) NK cells representing

NKG2A⁻ KIR⁺ are mature and have cytotoxic functions. NK cells are the first lymphocytes to recover after transplantation and are considered powerful effector cells in HSCT in terms of their anti-leukemic and anti-infectious effects. The impact of NK cells in HSCT has been demonstrated mainly in HLA-mismatched haploHSCT with *in vivo* (ATG) or *ex vivo* T cell depletion.^{13,14} To a lesser degree, in HLA-matched T cell replete stem cell recipients, high early NK cell reconstitution is associated with better clinical outcomes.^{15,16} Although the frequencies and absolute counts of circulating NK cells reach normal levels a few weeks post-transplant, it takes much longer to mature and attain efficient effector functions.^{12,17-19} In recipients of HLA-matched HSCT, although reconstituting NK cells remain immature for more than 6 months post-transplantation, better phenotypic and functional reconstitution is related to better clinical outcomes.^{17,20}

Robert *et al.* reported that although donor derived NK cell reconstitution occurs the second week after haploHSCT, recovering NK cells are a functionally exhausted subset of unconventional CD56^{dim}/CD16^{neg} NK cells whose gene expression profile is intermediate between conventional CD56^{br} and conventional CD56^{dim} NK cells.¹⁹ NK cells on days 30 and 60 are often lower in haploHSCT with PTCy than in HLA-matched related HSCT in which NK cell reconstitution occurs the first week.²¹ These unconventional CD56^{dim} NK cells have impaired cytotoxicity and are characterized by significantly increased expression of NKG2A compared with their counterparts in healthy donors.¹⁹ Unconventional CD56^{dim} NK cells express high levels of activating receptors and lytic granules, and an *in vitro* study revealed that the cytotoxicity of these NK cells can be reversed by blocking the inhibitory receptor NKG2A.

Russo *et al.* extensively investigated NK cell reconstitution in haploHSCT recipients with PTCy.²² At day 3 after transplantation, NK cells proliferated to an even greater extent than T cells and eliminated proliferating NK cells. After ablation of proliferating mature NK cells, donor derived NK cell reconstitution occurred 15 days after haploHSCT and immature NK cells became highly prevalent. This suggests that NK cell recovery depends on *de novo* generation rather than proliferation of mature NK cells in the graft. Importantly, single KIR⁺ NK cells, considered to

Table 1. The median times to immune cell reconstitution

	HaploHSCT with PTCy	Conventional HSCT
Monocytes	by 30 days	by 30 days
Neutrophils	at 16-17 days	PBSCT at 16-19 days BM at 21-23 days
NK cells	at the second week (functionally exhausted)	at the first week
Conventional T cells	CD8: by 30 days CD4: over 1 year	CD8: PBSCT by 80 days BMT by 180 days CD4: PBSCT/BMT over 1 year
Regulatory T cells	by 15 days	by 30 days
B cells	at 49-77 days	over 180 days

include potentially alloreactive NK cells, were also eliminated by PTCy and had impaired anti-leukemic potential at day 30 after HSCT. Consequently, in an extended series of 99 cases of haploHSCT with PTCy, Russo *et al.* found that the KIR ligand mismatch model of NK cell alloreactivity did not correlate with any of the major HSCT end points in haploHSCT with PTCy.²² The absolute counts and relative proportion of mature NK cells at day 30 after HSCT may be a more reliable predictor of effective NK cell-mediated immunosurveillance against relapse after haploHSCT with PTCy.²²

These reports suggest that PTCy dampens the impact of KIR ligand mismatches on the HSCT outcome by eliminating the majority of mature alloreactive NK cells. However, in several studies of haploHSCT with PTCy, KIR mismatches (e.g. KIR receptor ligand mismatches, the KIR B/x haplotype with KIR2DS2 and inhibitory KIR gene mismatch) were associated with lower rates of relapse and better survival,²³⁻²⁶ as observed in HLA-matched donor HSCT.^{27,28} More recently, Ido *et al.* reported that KIR2DS1 positivity significantly reduced the risk of both relapse and mortality in the complete response (CR) group, but not in the non-CR group, after PTCy-haploHSCT.²⁹ Although patients with a high residual tumor burden require earlier exertion of graft vs. leukemia (GVL) effects to control leukemia/tumor progression, PTCy eliminates proliferating alloreactive NK cells and NK cell recovery may require more than 60 days.^{21,22} These findings regarding immune reconstitution may explain why GVL effects mediated by NK cells were observed when the tumor burden was low.

The role played by NK cells in GVHD is controversial and the exact NK cell-mediated mechanism for the prevention of GVHD onset remains unclear. Early studies suggested the involvement of NK cells in GVHD induction or exacerbation.³⁰ In both mice and humans, NK cells infiltrated the target tissues during GVHD; studies using murine models based on antibody depletion or genetic change in NK cells demonstrated a reduction in GVHD.³¹⁻³³ However, these approaches were unable to exclude the contribution of several immune cell subsets other than NK cells, including activated T cells,³⁴⁻³⁶ prompting further studies on the adoptive transfer of NK cells. Most studies involving adoptive transfer of NK cells failed to induce GVHD. Furthermore, Murphy *et al.* reported that in mice receiving splenocytes, activated NK cells prevented the development of GVHD.³⁷ This unexpected result was confirmed by several other reports.^{14,38-46} NK cells can suppress GVHD by killing activated T cells^{40,47} and antigen-presenting cells (APCs), which are necessary for T cell activation.^{14,46,48} In addition to their cytolytic potential, NK cells can alter immune responses through cytokine production. Increased NK cell IFN- γ production after HSCT in humans is associated with an increased incidence of acute GVHD.⁴⁹ Conversely, one clinical study reported that a high NK cell frequency in the first weeks after HSCT may prevent T cell proliferation through IL-10 production.⁵⁰

The quality of NK cells greatly affects the incidence of GVHD. Increased absolute counts and frequencies of

NKG2A+ NK cells reduce acute GVHD by inhibiting T cell proliferation and activation.⁵¹ Furthermore, increased frequencies of NKG2C+ NK cells are associated with a lower incidence of GVHD in allo-HSCT.⁵² Recent studies revealed that CMV infections/reactivations are beneficial for NK cell recovery after haploHSCT. In particular, CMV can accelerate NK cell maturation and induce the expansion of terminally-differentiated and alloreactive CD56dim NK cells that have potent GVL effects.⁵³

Innate lymphoid cells (ILC) are classified into two subpopulations, cytotoxic-ILC and helper ILC. The helper-ILC population is further subdivided into ILC1, ILC2 and ILC3, which functionally mirror the CD4+ Th1, Th2 and Th17 cell subsets, respectively.⁵⁴ ILC3 is a heterogeneous cell population that includes fetal lymphoid tissue inducer (LTi) cells, and two different ILC3 subsets have been identified in humans based on the expression of the natural cytotoxic receptor (NCR) NKp44.⁵⁴ NCR+ ILC3 are an important innate source of IL-22, a potent cytokine that acts directly on epithelial cells to induce proliferation, survival and repair.⁵⁵ In a mouse GVHD model, the ILC frequency and IL-22 amounts were reduced by GVHD and IL-22 deficient recipients had more acute GVHD tissue damage.⁵⁶

In haploHSCT with PTCy, ILC reconstitution has not yet been fully evaluated. Munneke *et al.* reported that ILCs disappear in the weeks after conventional allogeneic HSCT, and that reconstitution of ILC1, ILC2 and NCR- ILC3 was slower than that of neutrophils and monocytes.⁵⁵ After 3 months post-transplant, the levels of circulating ILC2 were still lower than those in healthy subjects. In contrast, NCR+ ILC3 reconstitution was apparent as early as 12 weeks after allogeneic HSCT, whereas such cells are not present in the circulation of healthy people.⁵⁵ The rapid appearance of NCR+ ILC3 was observed in the peripheral blood of patients who did not develop acute or chronic GVHD. In addition, increased proportions of skin-homing ILC1 and NCR- ILC3, and gut homing ILC2 in patients without acute GVHD of the skin and gut were observed.⁵⁵ ILC reconstitution is not affected by cyclosporine or corticosteroids,⁵⁵ whereas granulocyte-colony-stimulating factor (G-CSF) may affect ILC3 and NK cell differentiation *in vitro*.⁵⁷ Furthermore, ILC development may be impaired in patients with acute myeloid leukemia (AML).⁵⁸ Thus, after HSCT, ILC development may be affected by the presence of high residual leukemia burden or leukemia relapse.

Conventional T cell reconstitution

Several studies evaluated early T cell reconstitution following haploHSCT with PTCy.^{59,60} Roberto *et al.* reported that at day 3 post-haploHSCT, most CD3+ cells, particularly CD8+ T cells, expressed markers of proliferation (Ki-67) and activation.⁵⁹ Both naïve T cells and memory T cells divide in response to allogeneic stimulation, and naïve T cells rapidly acquire a memory/effector-phenotype; Ki-67-positive T cells exhibited exclusively memory phenotypes, and naïve T cells were Ki-67-negative among both CD4+ and CD8+ T cells at day 3 post-haploHSCT. Regulatory T cells (Tregs)

with a naive phenotype expressed lower markers of proliferation than conventional CD4⁺ T cells. PTCy depleted proliferating cells, resulting in preferential accumulation of CD4⁺ T cell and Treg following PTCy. Among both CD4⁺ and CD8⁺ T cells, recipient T cells accounted for less than 10% of the total at day 5, disappearing by day 15 post-HSCT.⁶⁰ At day 7, recipient T cells were preferentially memory, whereas donor naïve T cells disappeared from circulation and donor cells predominantly had the memory stem T cell (T_{SCM}) phenotype. *In vitro* incubation of naïve T cells with allo-APCs led to CD95 upregulation and the T_{SCM} phenotype.⁵⁹ Cieri *et al.* also reported that T_{SCM} exhibited low levels of apoptosis and were highly enriched at day 8 post-haploHSCT.⁶⁰ The serum concentration of IL-7 at day 1 was correlated with the number of circulating CD8⁺ and CD4⁺ T_{SCM} lymphocytes.⁶⁰ CD31, a marker preferentially expressed by early differentiated CD4⁺ recent thymic emigrant T cells, clustered predominantly within the T_{SCM} compartment. By day 30 post-HSCT, CD31 expression on CD4⁺ T_{SCM} was significantly reduced compared with day 8, whereas CD31⁺ naïve T cells were again detectable, consistent with *de novo* generation. Collectively, T_{SCM} observed following PTCy may originate from CD31⁺ CD4⁺ naïve T cells infused within the graft. These cells were gradually replaced by more differentiated central memory cells and effector memory cells over the following weeks. T_{SCM} represents the earliest developmental stage of memory T cells, and can differentiate into large numbers of effector T cells while maintaining their pool size through homeostatic self-renewal. T_{SCM} may play an important role in the first month of immune reconstitution. Although one study of 66 patients receiving haploHSCT with PTCy reported that CD3⁺ cell counts ≥ 120

cells/ μ L at day 30 were associated with a slightly longer OS and less relapse,⁶¹ the physiological roles and involvement of T_{SCM} in the transplantation outcome are largely unknown.

The majority of T cells that recover within the first 30-90 days after haploHSCT with PTCy are derived from the donor naïve compartment.⁶² Roberto *et al.* reported that T cells were undetectable in most patients up to 6 weeks post-haploHSCT.⁵⁹ From week 6, when mycophenolate mofetil was discontinued, CD3⁺ cells (particularly CD8⁺ T cells) increased (Fig. 1). CD8⁺ T cells recovered earlier than CD4⁺ T cells after haploHSCT, leading to an inverted ratio of CD4⁺ /CD8⁺ cells, as observed after HLA-matched HSCT. In myeloablative conditioning (MAC) haploHSCT with PTCy, CD8⁺ T cells neared normal levels by 60 days and achieved them by 180 days post-transplant.⁶³ This CD4⁺ and especially CD8⁺ T cell recovery was comparable with that after T cell-replete BM allografting using standard GVHD prophylactic regimens.⁶⁴ The generation of new naïve T cells by resumed thymic output did not begin until day 90 post-haploHSCT, and CD4⁺ thymic emigrant T cell or naïve T cells were absent during this time period. At day 90, recovering T cells predominantly exhibited a transitional memory, effector memory or terminal effector cell phenotype. Subsequently, T cells progressively recovered up to 1 year after haploHSCT, but remained well below normal donor CD4⁺ T cell levels at 1 year after haploHSCT.^{59,62} The low levels of thymic emigrant T cells suggested that the majority of T cell reconstitution in the first year after haploHSCT is due to the proliferation of cells present in the graft rather than from *de novo* generation.

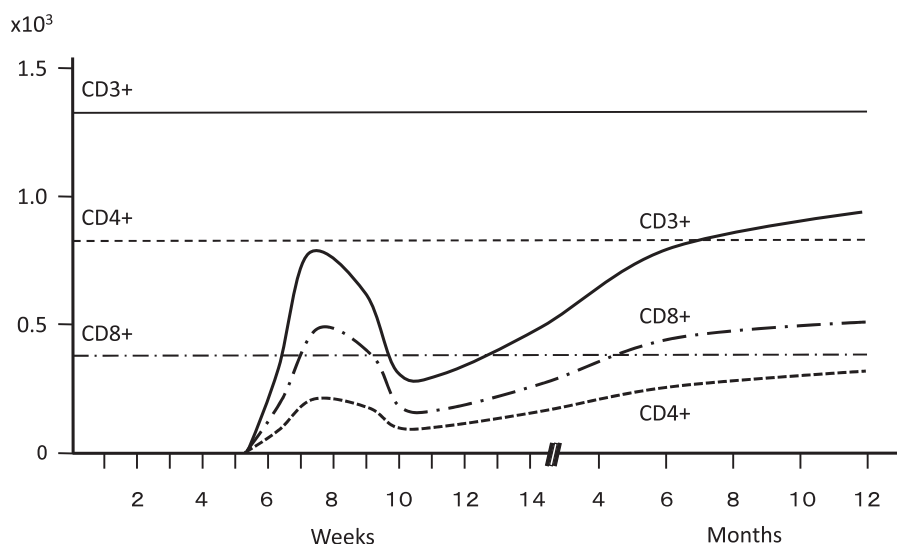


Fig. 1. Kinetics of T cell reconstitution after haploHSCT

At day 7, CD4⁺ T cells are predominantly of the memory stem T cell (T_{SCM}) phenotype. By day 30 post-HSCT, CD4⁺ T_{SCM} is significantly reduced, whereas CD31⁺ naïve T cells are again detectable, consistent with *de novo* generation. CD8⁺ T cells reach near normal levels by 60 days and achieve them by 180 days post-transplant, but CD4⁺ T cells remain below normal levels at 1 year after haploHSCT. This CD4⁺ and especially CD8⁺ T cell recovery was comparable with that after T cell-replete BM allografting using standard GVHD prophylactic regimens.

Regulatory T cell (Treg) reconstitution

Kanakry *et al.* revealed that CD4⁺ Foxp3⁺ T cells from patients and from allogeneic mixed lymphocyte reactions expressed relatively high levels of aldehyde dehydrogenase (ALDH), and concluded that ALDH expression drives Treg resistance to Cy. They also found that there was relative preservation of memory CD4⁺ Foxp3⁺ T cells after PTCy. Moreover, CD4⁺ CD45RA⁻ Foxp3^{hi} effector Tregs recovered rapidly.⁶⁵ Naïve Tregs defined as CD45RO⁻ CCR7⁺ CD45RA⁺ were increased in the early days following transplantation,⁵⁹ and Tregs significantly expanded at day 15 after transplantation compared with both leukapheresis samples and healthy controls.⁶⁶ Cieri *et al.* reported that values of circulating Treg <5% at day 15 after transplantation were predictive of subsequent GVHD. Another study also found that effector Tregs achieved normal donor levels at day 30.⁶³ Nakamae *et al.* compared immune reconstitution after haploHSCT with PTCy with that after conventional HCT and found that the Treg to conventional CD4⁺ T-cell ratio was significantly higher until day 90 in haploHSCT with PTCy.²¹

Using a murine haploHSCT with PTCy model, Matsuoka *et al.* evaluated early lymphocyte reconstitution.⁶⁷ Of note, Ki-67⁺ proliferating cells, including conventional T cells and Tregs, were depleted by Cy intervention; however, surviving T cells after Cy intervention had significantly higher BCL-2 expression levels than control recipients. Based on the increased anti-apoptotic elements, T cells in PTCy-treated recipients underwent aggressive homeostatic proliferation and the CD4 T cell subset, particularly Tregs, eclipsed that of the control recipient by day 14. CD8⁺ T cell proliferation after PTCy was less aggressive than the CD4 T cell proliferation, resulting in a higher ratio of Tregs to CD8 T cells in PTCy recipients than in controls.⁶⁸ At day 21 after HSCT, the number of Tregs in the spleen was significantly higher in PTCy-treated recipients than in the non-PTCy controls.⁶⁸

$\gamma\delta$ T-cell reconstitution

As they do not require further peripheral maturation or extensive clonal expansion to initiate their effector-functions, $\gamma\delta$ T cells are rapid responders to pathogens and tumors. Absolute counts of $\gamma\delta$ T cells do not influence the incidence or severity of GVHD^{69,70} and meta-analysis demonstrated that high $\gamma\delta$ T cell values are associated with less disease relapse and fewer viral infections.⁶⁹ However, there is no impact of $\gamma\delta$ T cells on infection or relapse after conventional transplant and their effect is context dependent, with a greater impact in the T cell-depleted transplant setting.⁶⁹ $\gamma\delta$ T cell reconstitution is evaluated mainly in recipients receiving $\alpha\beta$ and CD19-depleted grafts.⁷¹⁻⁷³ The majority of recovering $\gamma\delta$ T cells in the first weeks have a CD27⁺ CD45RA⁻ central memory phenotype and the same $\gamma\delta$ T cell clones found in the donor are present in the recipient after the transplant.^{70,74} This suggests that $\gamma\delta$ T cell recovery depends on peripheral expansion of graft-derived mature $\gamma\delta$ T cells. Subsequently, central memory $\gamma\delta$ T cells are replaced by naïve CD27⁺ CD45RA⁺ $\gamma\delta$ T cells originating from donor infused HSCs

within 14-60 days post-transplantation.^{70,73,75}

The dominant subset of circulating $\gamma\delta$ T cells in the peripheral blood of healthy subjects expresses the V δ 2 TCR paired with the V γ 9 TCR, whereas the minority express the V δ 1 TCR. After haploHSCT using the GIAC protocol, the recovery of V δ 2⁺ T cells was continuously delayed for at least 180 days after HSCT mainly, which was significantly correlated with the development of EBV reactivation.^{76,77} On the other hand, several studies in recipients receiving $\alpha\beta$ and CD19 depleted grafts reported that the recovery of the CDR3 of the TCR δ chain was almost complete 2 months after haploHSCT⁷¹⁻⁷³ and that reactivation of CMV (but not other viruses) was associated with the expansion of V δ 1⁺ T cells.⁷³ In haploHSCT with PTCy, $\gamma\delta$ T cell reconstitution remains to be fully characterized.

B-cell reconstitution

After immature B cells leave the bone marrow and enter the peripheral blood and lymphoid organs, they go through a transitional stage before becoming fully mature naïve B cells. The phenotype of recovering T cells after HSCT is predominantly effector memory, in contrast, B cells are predominantly phenotypically naïve throughout the first post-transplant year.⁷⁸ One study found that B cells were not detected until day 28 after haploHSCT⁷⁹ and recovering B cells were mostly Ki-67-negative at week 8, suggesting that B cell recovery depends on *de novo* generation rather than proliferation of cells present in the graft. B cells achieved normal donor frequency between days 49 and 77 post-haploHSCT.^{63,79} The phenotype of recovering B cells was largely immature/transitional (CD38 bright CD10⁺) until around day 60, when transitional B cells decreased. Subsequently, transitional cells were replaced by mature B cells. The majority of B cells are naïve up until 180 days after haploHSCT. The proportion of memory B cells is initially low, but can reach levels similar to that of marrow donors.⁷⁹ Repertoires of post-transplant B cells are highly diverse.⁷⁸

Roberto *et al.* investigated the steps of B cell maturation after haploHSCT and observed three distinct transitional subsets during reconstitution based on CD5 and CD21 surface expression: T1 (CD5⁺ CD21⁻), T2 (CD5⁺ CD21⁺) and CD5-CD21⁺.⁷⁹ In addition, they reported an additional CD5-CD21⁻ stage, named T0, which precedes the aforementioned T1 and T2 transitional stages. At week 5, recovering B cells mostly exhibited a T0 or, in smaller proportion, T1 phenotype. Subsequently, T0 cells progressively decreased, whereas T2 and CD5-CD21⁺ cells increased. The differentiation status of transitional B cells differed substantially among BM donors and patients up to 8-14 weeks post-haploHSCT. Transitional B cell subsets underwent a maturation process, as they progressively upregulated the naïve markers IgD, IgM and CD217. The surface expression of IgM at week 15 was significantly higher than that of transitional B cells from donors.⁷⁹

B cells affect chronic GVHD.^{80,81} Sarantopoulos *et al.* demonstrated that delayed recovery of B-cell homeostasis

and the persistence of high B cell activation factor /B-cell ratios are associated with an activated CD27+ B-cell pool in human chronic GVHD. Recent studies suggested that donor-derived follicular helper T cells (Tfh) play an important role in chronic GVHD pathogenesis by promoting the differentiation from naïve B cells into germinal center B cells, which may produce anti-host antibodies. Using a murine haploHSCT with PTCy model, Matsuoka *et al.* evaluated the reconstitution of B cells, Tfh cells and Tregs.⁸² Tfh cells were present at a low frequency in PTCy -treated recipients, whereas Tregs and B cells were present at higher frequencies than in the non- PTCy controls. This suggests that PTCy can induce the expansion of Tregs and increase naïve B cell recovery without causing the early emergence of Tfh cells in lymph nodes, resulting in diverse T and B cell reconstitution.

A subset of regulatory B cells (Bregs) in mice negatively regulate T cell immune responses through the secretion of regulatory cytokines, such as IL-10, as well as through direct cell-cell contact. Khoder *et al.* demonstrated that B cells with immunoregulatory properties are enriched within both the CD19+ IgM+ CD27+ memory and CD19+ CD24hi CD38hi transitional B-cell subsets in healthy human donors and IL-10-producing Bregs are less abundant in patients with chronic GVHD.⁸³ Further studies are needed to assess Breg reconstitution after PTC.

Dendritic cell (DC) reconstitution

Della Porta *et al.* analyzed the kinetics of reconstitution for two circulating dendritic cell (DC) subsets. The first was myeloid DCs, which express the myeloid antigens; CD33, CD13 and CD11c, and drive Th-1 differentiation. The second was lymphoid or plasmacytoid DCs, which lack myeloid markers and induce Th-2 differentiation. They found that a normal myeloid DC count was reached on day 365 after HSCT, whereas plasmacytoid DCs remained at a lower frequency than that in the controls.⁸⁴ Chang *et al.* reported the slower recovery of DCs, including myeloid DC1s (MDC1s), myeloid DC2s (MDC2s) and plasmacytoid DCs, at 15 and 30 days post-transplant in T cell replete haploHSCT using the GIAC protocol than in HLA-matched recipients.⁸⁵ MDC1s and plasmacytoid DCs reached normal levels at one year in both HLA-matched and haploHSCT recipients. Due to the impact of ATG on DCs, the early reconstitution of MDC1s, MDC2s and plasmacytoid DCs was significantly delayed after haploHSCT using the GIAC protocol.⁸⁵ In another study investigating haploHSCT using the GIAC protocol, the recipients had markedly reduced proportions of DCs, myeloid DCs and plasmacytoid DCs for at least 180 days post-haploHSCT compared with healthy subjects.⁷⁶ In haploHSCT with PTCy, DC reconstitution has not yet been fully evaluated.

CONCLUSION

Although recent basic and clinical studies revealed the details of immune reconstitution after T-cell replete haploHSCT

using PTCy, how haploHSCT using PTCy can reduce the risk of GVHD and/or relapse remains unclear. Advances in immune reconstitution will improve our understanding of the relationship between haploHSCT with PTCy and the clinical outcome, and further improve outcomes after HSCT.

CONFLICTS OF INTEREST

The author declared no conflicts of interest.

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