Journal of clinical and experimental hematopathology
Vol. 61 No.1, 1-9, 2021

Review Article

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

Yoshinobu Maeda

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.

Received: August 19, 2020. Revised: October 8, 2020. Accepted: October 16, 2020. J-STAGE Advance Published: February 6, 2021
DOI:10.3960/jslrt.20040
Department of Hematology and Oncology, Okayama University Hospital, Okayama, Japan
Corresponding author: Yoshinobu Maeda, M.D., Ph.D., Department of Hematology and Oncology, Okayama University Hospital, 2-5-1, Shikata-cho, Kita-ku, Okayama city, Okayama 700-8558, Japan. E-mail: yosmaeda@md.okayama-u.ac.jp

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. CD56bright/CD16neglow (CD56br) NK cells representing NKG2A+ KIR- are immature and have regulatory functions.[12] Conversely, CD56dim/CD16pos (CD56dim) 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 CD56dim/CD16neg NK cells whose gene expression profile is intermediate between conventional CD56br and conventional CD56dim 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 CD56dim NK cells have impaired cytotoxicity and are characterized by significantly increased expression of NKG2A compared with their counterparts in healthy donors.[19] Unconventional CD56dim 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/μL 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 naive 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.

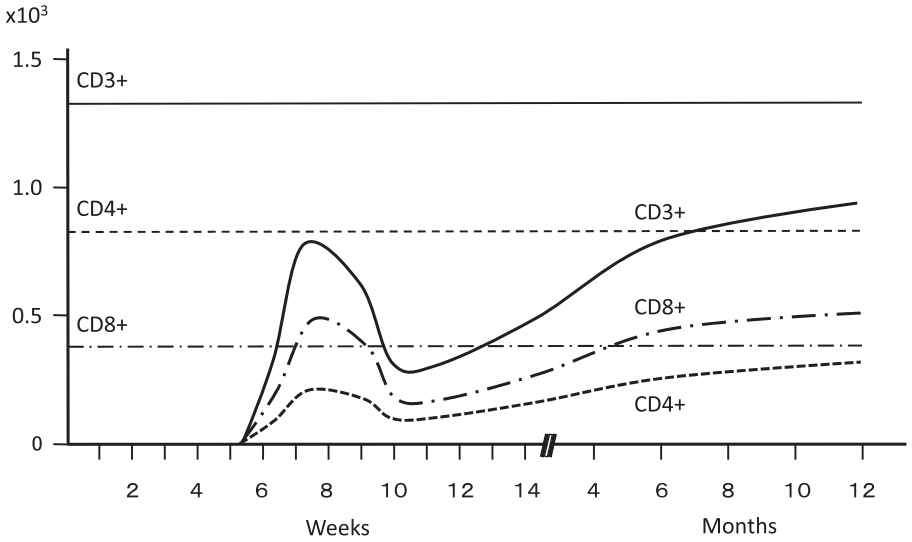

Fig. 1. Kinetics of T cell reconstitution after haploHSCT
At day 7, CD4+ T cells are predominantly of the memory stem T cell (TSCM) phenotype. By day 30 post-HSCT, CD4+ TSCM is significantly reduced, whereas CD31+ naïve T cells are again detectable, consistent with *de novo* generation. CD8 + T cells 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 the of 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]

γδ T -cell reconstitution

As they do not require further peripheral maturation or extensive clonal expansion to initiate their effector-functions, γδ T cells are rapid responders to pathogens and tumors. Absolute counts of γδ T cells do not influence the incidence or severity of GVHD[69,70] and meta-analysis demonstrated that high γδ T cell values are associated with less disease relapse and fewer viral infections.[69] However, these is no impact of γδ 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] γδ T cell reconstitution is evaluated mainly in recipients receiving αβ and CD19-depleted grafts.[71-73] The majority of recovering γδ T cells in the first weeks have a CD27+ CD45RA- central memory phenotype and the same γδ T cell clones found in the donor are present in the recipient after the transplant.[70,74] This suggests that γδ T cell recovery depends on peripheral expansion of graft-derived mature γδ T cells. Subsequently, central memory γδ T cells are replaced by naïve CD27+ CD45RA+ γδ T cells originating from donor infused HSCs within 14-60 days post-transplantation.[70,73,75]

The dominant subset of circulating γδ T cells in the peripheral blood of healthy subjects expresses the Vδ2 TCR paired with the Vγ9 TCR, whereas the minority express the Vδ1 TCR. After haploHSCT using the GIAC protocol, the recovery of Vδ2+ 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 αβ and CD19 depleted grafts reported that the recovery of the CDR3 of the TCR δ chain was almost complete 2 months after haploHSCT[71-73] and that reactivation of CMV (but not other viruses) was associated with the expansion of Vδ1+ T cells.[73] In haploHSCT with PTCy, γδ 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 naive throughout the first post-transplant year.[78] One study found that B cells were not detected until day 28 after haploHSCT[79] 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 naive 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.

REFERENCES

1 Garnier A, Guillaume T, Peterlin P, *et al*. Absence of influence of peripheral blood CD34+ and CD3+ graft cell counts on outcomes after reduced-intensity conditioning transplantation using post-transplant cyclophosphamide. Ann Hematol. 2020; 99 : 1341-1350.

2 Teofili L, Chiusolo P, Valentini CG, *et al*. Bone marrow haploidentical transplant with post-transplantation cyclophosphamide: does graft cell content have an impact on main clinical outcomes? Cytotherapy. 2020; 22 : 158-165.

3 Moiseev IS, Babenko EV, Epifanovskaya OS, *et al*. High prevalence of CD3, NK, and NKT cells in the graft predicts adverse outcome after matched-related and unrelated transplantations with post transplantation cyclophosphamide. Bone Marrow Transplant. 2020; 55 : 544-552.

4 Turcotte LM, Cao Q, Cooley SA, *et al*. Monocyte subpopulation recovery as predictors of hematopoietic cell transplantation outcomes. Biol Blood Marrow Transplant. 2019; 25 : 883-890.

5 Pei X, Zhao X, Wang Y, *et al*. Comparison of reference values for immune recovery between event-free patients receiving haploidentical allografts and those receiving human leukocyte antigen-matched sibling donor allografts. Front Med. 2018; 12 : 153-163.

6 Salzmann-Manrique E, Bremm M, Huenecke S, *et al*. Joint modeling of immune reconstitution post haploidentical stem cell transplantation in pediatric patients with acute leukemia comparing $CD34^+$-selected to CD3/CD19-depleted grafts in a retrospective multicenter Study. Front Immunol. 2018; 9 : 1841.

7 Bashey A, Zhang MJ, McCurdy SR, *et al*. Mobilized peripheral blood stem cells versus unstimulated bone marrow as a graft source for T-cell-replete haploidentical donor transplantation using post-transplant cyclophosphamide. J Clin Oncol. 2017; 35 : 3002-3009.

8 Blaise D, Kuentz M, Fortanier C, *et al*. Randomized trial of bone marrow versus lenograstim-primed blood cell allogeneic transplantation in patients with early-stage leukemia: a report from the Société Française de Greffe de Moelle. J Clin Oncol. 2000; 18 : 537-546.

9 Bensinger WI, Martin PJ, Storer B, *et al*. Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. N Engl J Med. 2001; 344 : 175-181.

10 Couban S, Simpson DR, Barnett MJ, *et al*. A randomized multicenter comparison of bone marrow and peripheral blood in recipients of matched sibling allogeneic transplants for myeloid malignancies. Blood. 2002; 100 : 1525-1531.

11 Retière C, Willem C, Guillaume T, *et al*. Impact on early outcomes and immune reconstitution of high-dose post-transplant cyclophosphamide vs anti-thymocyte globulin after reduced intensity conditioning peripheral blood stem cell allogeneic transplantation. Oncotarget. 2018; 9 : 11451-11464.

12 Zaghi E, Calvi M, Di Vito C, Mavilio D. Innate immune responses in the outcome of haploidentical hematopoietic stem cell transplantation to cure hematologic malignancies. Front Immunol. 2019; 10 : 2794.

13 Mancusi A, Ruggeri L, Urbani E, *et al*. Haploidentical hematopoietic transplantation from KIR ligand–mismatched donors with activating KIRs reduces nonrelapse mortality. Blood. 2015; 125 : 3173-3182.

14 Ruggeri L, Capanni M, Urbani E, *et al*. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002; 295 : 2097-2100.

15 Hattori N, Saito B, Sasaki Y, *et al*. Status of natural killer cell recovery in day 21 bone marrow after allogeneic hematopoietic stem cell transplantation predicts clinical outcome. Biol Blood Marrow Transplant. 2018; 24 : 1841-1847.

16 Minculescu L, Marquart HV, Friis LS, *et al*. Early natural killer cell reconstitution predicts overall survival in T cell-replete allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2016; 22 : 2187-2193.

17 Pical-Izard C, Crocchiolo R, Granjeaud S, *et al*. Reconstitution of natural killer cells in HLA-matched HSCT after reduced-intensity conditioning: impact on clinical outcome. Biol Blood Marrow Transplant. 2015; 21 : 429-439.

18 Nguyen S, Dhedin N, Vernant JP, *et al*. NK-cell reconstitution after haploidentical hematopoietic stem-cell transplantations: immaturity of NK cells and inhibitory effect of NKG2A override GvL effect. Blood. 2005; 105 : 4135-4142.

19 Roberto A, Di Vito C, Zaghi E, *et al*. The early expansion of anergic NKG2A ^{pos} /CD56 ^{dim} /CD16 ^{neg} natural killer represents a therapeutic target in haploidentical hematopoietic stem cell transplantation. Haematologica. 2018; 103 : 1390-1402.

20 Minculescu L, Fischer-Nielsen A, Haastrup E, *et al*. Improved relapse-free survival in patients with high natural killer cell doses in grafts and during early immune reconstitution after allogeneic stem cell transplantation. Front Immunol. 2020; 11 : 1068.

21 Nakamae H, Fujii K, Nanno S, *et al*. A prospective observational study of immune reconstitution following transplantation with post-transplant reduced-dose cyclophosphamide from HLA -haploidentical donors. Transpl Int. 2019; 32 : 1322-1332.

22 Russo A, Oliveira G, Berglund S, *et al*. NK cell recovery after haploidentical HSCT with posttransplant cyclophosphamide: dynamics and clinical implications. Blood. 2018; 131 : 247-262.

23 Solomon SR, Aubrey MT, Zhang X, *et al*. Selecting the best donor for haploidentical transplant: Impact of HLA, killer cell immunoglobulin-like receptor genotyping, and other clinical variables. Biol Blood Marrow Transplant. 2018; 24 : 789-798.

24 Willem C, Makanga DR, Guillaume T, *et al*. Impact of KIR/HLA incompatibilities on NK cell reconstitution and clinical outcome after T cell-replete haploidentical hematopoietic stem cell transplantation with posttransplant cyclophosphamide. J Immunol. 2019; 202 : 2141-2152.

25 Symons HJ, Leffell MS, Rossiter ND, *et al*. Improved survival with inhibitory killer immunoglobulin receptor (KIR) gene mismatches and KIR haplotype B donors after nonmyeloablative, HLA-haploidentical bone marrow transplantation. Biol Blood Marrow Transplant. 2010; 16 : 533-542.

26 Bastos-Oreiro M, Anguita J, Martínez-Laperche C, *et al*. Inhibitory killer cell immunoglobulin-like receptor (iKIR) mismatches improve survival after T-cell-repleted haploidentical transplantation. Eur J Haematol. 2016; 96 : 483-491.

27 Venstrom JM, Pittari G, Gooley TA, *et al*. HLA-C-dependent prevention of leukemia relapse by donor activating KIR2DS1. N Engl J Med. 2012; 367 : 805-816.

28 Cooley S, Weisdorf DJ, Guethlein LA, *et al*. Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia. Blood. 2010; 116 : 2411-2419.

29 Ido K, Koh H, Hirose A, *et al*. Donor KIR2DS1-mediated decreased relapse and improved survival depending on remission status at HLA-haploidentical transplantation with post-transplantation cyclophosphamide. Biol Blood Marrow Transplant. 2020; 26 : 723-733.

30 Simonetta F, Alvarez M, Negrin RS. Natural killer cells in graft-versus-host-disease after allogeneic hematopoietic cell transplantation. Front Immunol. 2017; 8 : 465.

31 Charley MR, Mikhael A, Bennett M, Gilliam JN, Sontheimer RD. Prevention of lethal, minor-determinate graft-host disease in mice by the in vivo administration of anti-asialo GM1. J Immunol. 1983; 131 : 2101-2103.

32 Varkila K. Depletion of asialo-GM1+ cells from the F1 recipient mice prior to irradiation and transfusion of parental spleen cells prevents mortality to acute graft-versus-host disease and induction of anti-host specific cytotoxic T cells. Clin Exp Immunol. 1987; 69 : 652-659.

33 Ghayur T, Seemayer TA, Lapp WS. Prevention of murine graft-versus-host disease by inducing and eliminating ASGM1+ cells of donor origin. Transplantation. 1988; 45 : 586-590.

34 Zeng D, Lewis D, Dejbakhsh-Jones S, *et al*. Bone marrow NK1.1(-) and NK1.1(+) T cells reciprocally regulate acute graft versus host disease. J Exp Med. 1999; 189 : 1073-1081.

35 Carlson GA, Marshall ST, Truesdale AT. Adaptive immune defects and delayed rejection of allogeneic tumor cells in beige mice. Cell Immunol. 1984; 87 : 348-356.

36 Halle-Pannenko O, Bruley-Rosset M. Decreased graft-versus-host reaction and T cell cytolytic potential of beige mice. Transplantation. 1985; 39 : 85-87.

37 Murphy WJ, Bennett M, Kumar V, Longo DL. Donor-type activated natural killer cells promote marrow engraftment and B cell development during allogeneic bone marrow transplantation. J Immunol. 1992; 148 : 2953-2960.

38 Asai O, Longo DL, Tian ZG, *et al*. Suppression of graft-versus-host disease and amplification of graft-versus-tumor effects by activated natural killer cells after allogeneic bone marrow transplantation. J Clin Invest. 1998; 101 : 1835-1842.

39 Lundqvist A, McCoy JP, Samsel L, Childs R. Reduction of GVHD and enhanced antitumor effects after adoptive infusion of alloreactive Ly49-mismatched NK cells from MHC-matched donors. Blood. 2007; 109 : 3603-3606.

40 Olson JA, Leveson-Gower DB, Gill S, *et al*. NK cells mediate reduction of GVHD by inhibiting activated, alloreactive T cells while retaining GVT effects. Blood. 2010; 115 : 4293-4301.

41 Kim DH, Sohn SK, Lee NY, *et al*. Transplantation with higher dose of natural killer cells associated with better outcomes in terms of non-relapse mortality and infectious events after allogeneic peripheral blood stem cell transplantation from HLA-matched sibling donors. Eur J Haematol. 2005; 75 : 299-308.

42 Larghero J, Rocha V, Porcher R, *et al*. Association of bone marrow natural killer cell dose with neutrophil recovery and chronic graft-versus-host disease after HLA identical sibling bone marrow transplants. Br J Haematol. 2007; 138 : 101-109.

43 Savani BN, Mielke S, Adams S, *et al*. Rapid natural killer cell recovery determines outcome after T-cell-depleted HLA-identical stem cell transplantation in patients with myeloid leukemias but not with acute lymphoblastic leukemia. Leukemia. 2007; 21 : 2145-2152.

44 Ludajic K, Balavarca Y, Bickeböller H, *et al*. KIR genes and KIR ligands affect occurrence of acute GVHD after unrelated, 12/12 HLA matched, hematopoietic stem cell transplantation. Bone Marrow Transplant. 2009; 44 : 97-103.

45 Clausen J, Kircher B, Auberger J, *et al*. The role of missing killer cell immunoglobulin-like receptor ligands in T cell replete peripheral blood stem cell transplantation from HLA-identical siblings. Biol Blood Marrow Transplant. 2010; 16 : 273-280.

46 Sivori S, Carlomagno S, Falco M, *et al*. Natural killer cells expressing the KIR2DS1-activating receptor efficiently kill T-cell blasts and dendritic cells: implications in haploidentical HSCT. Blood. 2011; 117 : 4284-4292.

47 Rivas MN, Hazzan M, Weatherly K, *et al*. NK cell regulation of CD4 T cell-mediated graft-versus-host disease. J Immunol. 2010; 184 : 6790-6798.

48 Ghadially H, Ohana M, Elboim M, *et al*. NK cell receptor NKp46 regulates graft-versus-host disease. Cell Rep. 2014; 7 : 1809-1814.

49 Cooley S, McCullar V, Wangen R, *et al*. KIR reconstitution is altered by T cells in the graft and correlates with clinical outcomes after unrelated donor transplantation. Blood. 2005; 106 : 4370-4376.

50 Chan YLT, Zuo J, Inman C, *et al*. NK cells produce high levels of IL-10 early after allogeneic stem cell transplantation and suppress development of acute GVHD. Eur J Immunol. 2018; 48 : 316-329.

51 Hu LJ, Zhao XY, Yu XX, *et al*. Quantity and quality reconstitution of $NKG2A^+$ natural killer cells are associated with graft-versus-host disease after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2019; 25 : 1-11.

52 Kordelas L, Steckel NK, Horn PA, Beelen DW, Rebmann V. The activating NKG2C receptor is significantly reduced in NK cells after allogeneic stem cell transplantation in patients with severe graft-versus-host disease. Int J Mol Sci. 2016; 17 : 1797.

53 Jin F, Lin H, Gao S, *et al*. Characterization of IFNγ-producing natural killer cells induced by cytomegalovirus reactivation after haploidentical hematopoietic stem cell transplantation. Oncotarget. 2017; 8 : 51-63.

54 Vacca P, Montaldo E, Croxatto D, *et al*. NK cells and other innate lymphoid cells in hematopoietic stem cell transplantation. Front Immunol. 2016; 7 : 188.

55 Munneke JM, Björklund AT, Mjösberg JM, *et al*. Activated innate lymphoid cells are associated with a reduced susceptibility to graft-versus-host disease. Blood. 2014; 124 : 812-821.

56 Hanash AM, Dudakov JA, Hua G, *et al*. Interleukin-22 protects intestinal stem cells from immune-mediated tissue damage and regulates sensitivity to graft versus host disease. Immunity. 2012; 37 : 339-350.

57 Moretta F, Petronelli F, Lucarelli B, *et al*. The generation of human innate lymphoid cells is influenced by the source of hematopoietic stem cells and by the use of G-CSF. Eur J Immunol. 2016; 46 : 1271-1278.

58 Vitale C, Ambrosini P, Montaldo E, *et al*. IL-1β-releasing human acute myeloid leukemia blasts modulate natural killer cell differentiation from CD34+ precursors. Haematologica. 2015; 100 : e42-e45.

59 Roberto A, Castagna L, Zanon V, *et al*. Role of naive-derived T memory stem cells in T-cell reconstitution following allogeneic transplantation. Blood. 2015; 125 : 2855-2864.

60 Cieri N, Oliveira G, Greco R, *et al*. Generation of human memory stem T cells after haploidentical T-replete hematopoietic stem cell transplantation. Blood. 2015; 125 : 2865-2874.

61 Perez-Corral A, Dorado N, Pradillo V, *et al*. Immune reconstitution impact on overall survival after hematopoietic haploidentical stem cell transplantation. Blood. 2016; 128 : 5779.

62 McCurdy SR, Luznik L. Immune reconstitution after T-cell replete HLA-haploidentical transplantation. Semin Hematol. 2019; 56 : 221-226.

63 McCurdy SR, Vulic A, Symons HJ, *et al*. Comparable and robust immune reconstitution after HLA-haploidentical or HLA-matched allogeneic transplantation (BMT) utilizing post-transplantation cyclophosphamide. Biol Blood Marrow Transplant. 2015; 21 : S71.

64 Storek J, Dawson MA, Storer B, *et al*. Immune reconstitution after allogeneic marrow transplantation compared with blood stem cell transplantation. Blood. 2001; 97 : 3380-3389.

65 Kanakry CG, Ganguly S, Zahurak M, *et al*. Aldehyde dehydrogenase expression drives human regulatory T cell resistance to posttransplantation cyclophosphamide. Sci Transl Med. 2013; 5 : 211ra157.

66 Cieri N, Greco R, Crucitti L, *et al*. Post-transplantation cyclophosphamide and sirolimus after haploidentical hematopoietic stem cell transplantation using a treosulfan-based myeloablative conditioning and peripheral blood stem cells. Biol Blood Marrow Transplant. 2015; 21 : 1506-1514.

67 Iwamoto M, Matsuoka K, Meguri Y, *et al*. Comprehensive analyses of early lymphocyte reconstitution after haploidentical HSCT with posttransplant cyclophosphamide: coordinated Treg-dominant T-cell reconstitution and stem cell-derived mature B-cell with broad BCR-repertoir diversity. Blood. 2016; 128 : 4542.

68 Iwamoto M, Matsuoka K, Meguri Y, *et al*. Essential role of regulatory T cells on early B cell reconstitution after haploidentical BMT with posttransplant cyclophosphamide. Blood. 2017; 130 : 4447.

69 Arruda LCM, Gaballa A, Uhlin M. Impact of γδ T cells on clinical outcome of hematopoietic stem cell transplantation: system-

atic review and meta-analysis. Blood Adv. 2019; 3 : 3436-3448.
70 Park M, Im HJ, Lee YJ, *et al*. Reconstitution of T and NK cells after haploidentical hematopoietic cell transplantation using αβ T cell-depleted grafts and the clinical implication of γδ T cells. Clin Transplant. 2018; 32 : e13147.
71 Bertaina A, Merli P, Rutella S, *et al*. HLA-haploidentical stem cell transplantation after removal of αβ+ T and B cells in children with nonmalignant disorders. Blood. 2014; 124 : 822-826.
72 Locatelli F, Merli P, Pagliara D, *et al*. Outcome of children with acute leukemia given HLA-haploidentical HSCT after αβ T-cell and B-cell depletion. Blood. 2017; 130 : 677-685.
73 Airoldi I, Bertaina A, Prigione I, *et al*. γδ T-cell reconstitution after HLA-haploidentical hematopoietic transplantation depleted of TCR-αβ+/CD19+ lymphocytes. Blood. 2015; 125 : 2349-2358.
74 Hirokawa M, Horiuchi T, Kawabata Y, Kitabayashi A, Miura AB. Reconstitution of γδ T cell repertoire diversity after human allogeneic hematopoietic cell transplantation and the role of peripheral expansion of mature T cell population in the graft. Bone Marrow Transplant. 2000; 26 : 177-185.
75 Ravens S, Schultze-Florey C, Raha S, *et al*. Human γδ T cells are quickly reconstituted after stem-cell transplantation and show adaptive clonal expansion in response to viral infection. Nat Immunol. 2017; 18 : 393-401.
76 Wang X, Liu J, Gao H, *et al*. Dendritic cells are critical for the activation and expansion of $V\delta2^+$ T cells after allogeneic hematopoietic transplantation. Front Immunol. 2018; 9 : 2528.
77 Liu J, Bian Z, Wang X, *et al*. Inverse correlation of $V\delta2^+$ T-cell recovery with EBV reactivation after haematopoietic stem cell transplantation. Br J Haematol. 2018; 180 : 276-285.
78 Kanakry CG, Coffey DG, Towlerton AMH, *et al*. Origin and evolution of the T cell repertoire after posttransplantation cyclophosphamide. JCI Insight. 2016; 1 : e86252.
79 Roberto A, Castagna L, Gandolfi S, *et al*. B-cell reconstitution recapitulates B-cell lymphopoiesis following haploidentical BM transplantation and post-transplant CY. Bone Marrow Transplant. 2015; 50 : 317-319.
80 Sarantopoulos S, Stevenson KE, Kim HT, *et al*. High levels of B-cell activating factor in patients with active chronic graft-versus-host disease. Clin Cancer Res. 2007; 13 : 6107-6114.
81 Sarantopoulos S, Stevenson KE, Kim HT, *et al*. Altered B-cell homeostasis and excess BAFF in human chronic graft-versus-host disease. Blood. 2009; 113 : 3865-3874.
82 Iwamoto M, Ikegawa S, Kondo T, *et al*. Post-transplantation cyclophosphamide restores early B-cell lymphogenesis that suppresses subsequent chronic graft-versus-host disease. Bone Marrow Transplant. 2020. doi./10.1038/s41409-020-01100-0. [Online ahead of print]
83 Khoder A, Sarvaria A, Alsuliman A, *et al*. Regulatory B cells are enriched within the IgM memory and transitional subsets in healthy donors but are deficient in chronic GVHD. Blood. 2014; 124 : 2034-2045.
84 Porta MD, Rigolin GM, Alessandrino EP, *et al*. Dendritic cell recovery after allogeneic stem-cell transplantation in acute leukemia: correlations with clinical and transplant characteristics. Eur J Haematol. 2004; 72 : 18-25.
85 Chang YJ, Zhao XY, Huo MR, *et al*. Immune reconstitution following unmanipulated HLA-mismatched/haploidentical transplantation compared with HLA-identical sibling transplantation. J Clin Immunol. 2012; 32 : 268-280.