Chapter

Assessment of Immune Reconstitution Following Hematopoietic Stem Cell Transplantation

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Abstract

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potential curative treatment for both congenital and hematological malignancies. Immune reconstitution after allogeneic hematopoietic stem cell transplantation is implicated in successful transplant outcomes such as overall survival and relapse-free survival. The reconstitution of immune cell subsets after HSCT occurs in different phases at different time points encompassing pre-engraftment, engraftment, and post-engraftment. The recovery of innate cellular immunity with the appearance of monocytes, dendritic cells, and natural killer cells in peripheral blood correlates with initiation of cellular engraftment. The cellular adaptive immunity is characterized by both thymic-independent expansion of T cells infused with graft and thymus-dependent expansion of naïve T cells derived from donor stem cells. The humoral immunity consists of B-cell reconstitution, which consists primarily of transitional and naïve subsets with the recovery of memory B cells that occur much later. In this review, we highlight the factors affecting immune reconstitution, the reconstitution of innate and adaptive immunity, techniques to assess immune reconstitution, and ways to enhance it.

Keywords: immune reconstitution, hematopoietic stem cell transplantation, innate immunity, adaptive immunity

1. Introduction

Hematopoietic stem cell transplantation (HSCT) is a choice of treatment for thousands of leukemic patients. The main outcome expected from HSCT is the lifetime engraftment of the donor graft. The preferred donor is a HLA matched-related donor; however, this is available in about 25% of the patients. Other options such as matched unrelated, matched cord blood units, and haploidentical-related donor also do exist. The success of HSCT is marred by conditions such as graft-versus-host disease (GvHD), relapse, treatment-related toxicity, and infection, which lead to higher morbidity and mortality [1]. The effectiveness of HSCT is dependent on the immune reconstitution in the host, which is linked to the number of active T and NK cells present in the graft. Delayed immune reconstitution results in unfavorable transplant outcomes; hence, faster immune reconstitution of donor origin is required for long-term survival of patients.

Soon after HSCT using myeloablative conditioning, the patient experiences a period of pancytopenia. It takes several months or years for immune reconstitution and for patients to regain immunocompetence after transplant. The immune cells start re-appearing in the following order: neutrophils (0.5 months), monocytes (1 month), NK cells (1 month), T cells (2 months), and B cells (3 months); however, the normal levels are reached much later (**Figure 1**) [2].

There are various factors affecting immune reconstitution after transplant such as

1. thymic damage (age-related or pre-transplant conditioning regimens)
2. source of stem cells
3. HLA disparity between donor and host
4. post-transplant immune suppressant
5. occurrence of graft-versus-host disease.

Age or pre transplant chemotherapy or radiation leads to thymic damage. The severity of the damage caused to the thymus depends on the dose of the drugs used and also on the age of the patients, which in turn affect the immune recovery. In younger patients (<18 years), there is faster thymic regeneration after chemotherapy than older patients [3]. The age of the donor also affects the engraftment and reconstitution potential of hematopoietic stem cells as shown in mouse models [4]. Moreover, the thymic recovery is faster and is associated with faster T-cell reconstitution and recovery of normal T-cell repertoire in autologous (9 months) than allo-HSCT (12 months) [5]. This delayed thymic-dependent immune reconstitution is further reduced by the occurrence of aGvHD after allogeneic HSCT [6, 7].

The source of stem cells used as graft could be either bone marrow, peripheral blood, or cord blood. Source of stem cells used predicts the rate of immune reconstitution. It has been observed that platelet (20 × 109/L) reconstitution is faster in peripheral blood (11–18 days) than bone marrow (17–25 days) HSCT. Similarly, neutrophil (>0.5 × 109/L) reconstitution is also faster in peripheral blood (12–19 days) than bone marrow (15–23 days) HSCT. This is because of the presence

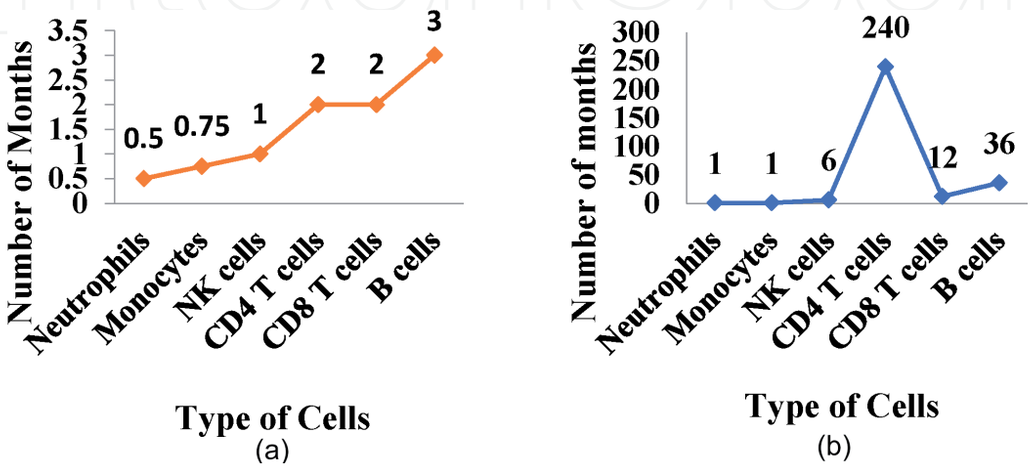

Figure 1.
The time taken for different immune subsets to (A) reappear in circulation and (B) reach normal levels after hematopoietic stem cell transplantation.

of long-term HSCs and more committed multipotent progenitors in the peripheral blood than bone marrow [8]. Further as compared to transplantation using in vivo or ex vivo T-cell depleted graft, faster immune reconstitution is seen in unmodified graft transplantation [9]. Using peripheral blood graft, faster reconstitution of CMV-specific cytotoxic T cells and CD4+ T cells is observed than stem cells from bone marrow source [10, 11]. The advantages of using umbilical cord blood units are its ready availability and its ability to cross the HLA barrier. The rates of engraftment and post-transplant outcomes are dependent on the number of total nucleated cells (TNCs) and CD34+ cell dose present in the graft source. Martin et al. [12] previously reported high TNC dose in association with positive transplant outcomes such as improved overall survival (OS), lower relapse rate (RR), and increased risk of chronic GvHD. Since there is a higher number of TNCs in the bone marrow and peripheral blood, there is faster engraftment (~14–21 days) after HSCT using this source of graft than umbilical cord blood source (~30 days) [13, 14]. Remberger et al. [15] reported faster engraftment but poor survival and higher relapse after HSCT using high CD34+ cell dose peripheral blood as graft source. Various researchers have reported immune cell reconstitution using different cell sources (**Table 1**).

Graft manipulations such as T-cell depletion (TCD) have resulted in lower chances of GvHD and graft rejection in unrelated and HLA mismatched transplants. However, T-cell depletion results in delayed immune reconstitution and increased morbidity and mortality due to infection [19–21]. An advantage of using T-cell depletion is that in case of malignancies it also leads to better GVL effect depending on the malignant disease being treated. For example, in CML, TCD is related to increased relapse rate [22], whereas in AML and AML cohorts, lower rate of relapse has been observed in TCD transplantation [23–25].

The degree of HLA mismatch is an important factor in immune reconstitution. It has been observed that the outcomes from matched unrelated transplantation are at par with that of matched related transplantation [1]. Chang et al. reported similar reconstitution of T-cell subsets, except for CD4+ cells and CD4+ naïve T cells, in haploidentical and HLA-matched transplantation [16]. Various researchers have reported reconstitution of immune cells following different transplant strategies. It has been observed that the immune reconstitution is best in matched sibling related followed by matched unrelated donor, haploidentical donor, T-cell replete, and T-cell depleted transplants.

Conditioning regimens deplete host immune system, eliminate the leukemic cells, and create space for engraftment of the donor cells. Although this eliminates the patient's leukemic cells, it also reduces the alloreactivity between host and donor cells after HSCT and further results in severe depletion of all immune cells. The use of drugs such as ATG or alemtuzumab depletes the host T cells further and results in

Cells/L type of transplant	NK cells 1 month	CD4+ T cells 90 days	CD8+ T cells 90 days	B cells 90 days	Reference
Matched sibling donor	—	220	645	33	[16]
Matched unrelated donor	253	198	447	43	[17]
Haploidentical donor	—	152	672	23	[16]
T-cell depleted	357	7	7	55	[18]
T-cell replete	183	127	181	64	[18]

Table 1.
Reconstitution of various immune subsets in different types of HSCT.

a delayed recovery of donor-derived T cells. Increase in the severity of the conditioning regimen results in prolonged immune deficiency after transplant [26].

Both thymus-dependent and thymus-independent T-cell reconstitutions are affected by the increase in HLA mismatch between the patient and the donor, probably because of higher risk of GvHD [27]. Clave et al. [28] reported higher reconstitution of both CD4+ and CD8+ T cells in transplants involving unrelated cord blood grafts (190 cells × 103/μL for CD4+ and 280 cells × 103/μL for CD8+) than CD34 selected peripheral blood haploidentical donor grafts (68 cells × 103/μL for CD4+ and 80 cells × 103/μL for CD8+). Mehta et al. [29] showed lower reconstitution of absolute CD4+ and CD8+ T cells at 3 months and higher B-cell counts (6 months) after unrelated cord blood HSCT than HLA matched HSCT (121.53 vs. 261.18 for CD4+, 36.03 vs. 190.56 for CD8+, and 210 vs. 31.2 for B cells). There was similar reconstitution of B cells but lower CD4+ and CD8+ T-cell reconstitution in single unit umbilical cord blood transplantation than HLA mismatched donor HSCT (11 vs. 9 for B cells, 15 vs. 21 for CD4+ cells, and 14 vs. 21 for CD8+ cells) [30].

Acute graft-versus-host disease occurs when donor lymphocytes react against normal host tissue to cause serious complications after allogeneic HSCT. Although there is faster recovery of the innate immune system after allo-HSCT, lymphocyte recovery is delayed due to aGvHD [3, 31]. The recovery of T cells depends on the thymic efficiency as well as the peripheral niche, which provides resources for T-cell survival. As GvHD targets the bone marrow, in patients with graft-versus-host disease, the peripheral resources are reduced because of which there is increased immunosuppression leading to delayed T-cell reconstitution in allogeneic HSCT as compared to autologous HSCT. The options to increase the efficiency of T-cell reconstitution must be selected in a manner so as to not aggravate the already present GvHD [32, 33]. Similarly, the drugs used to treat GvHD can also result in delayed immune reconstitution. Drugs such as cyclosporine A and methotrexate interfere with the T-cell receptor signaling and hence result in alteration of peripheral T-cell survival and B-cell differentiation [34, 35]. Tyrosine kinase inhibitors like imatinib mesylate used for controlling refractory cGvHD also lower T-cell survival by interfering with T-cell receptor (TCR) or IL7 signaling [36, 37]. Reconstitution of dendritic cells is decreased in GvHD [38]. Conversely, it has been suggested that depletion or inactivation of the host dendritic cells before allogeneic HSCT reduces the occurrence of GvHD [39–41].

2. Reconstitution of innate immunity

After HSCT, the first cells to engraft are the monocytes, followed by granulocytes, platelets, and NK cells [42]. Monocytes are primarily involved in phagocytosis and release of cytokines. They are classified into classical (CD14++CD16-), intermediate (CD14++CD16+), and nonclassical (CD14+CD16++) based on the expression of CD14 and CD16 [43, 44]. Monocytes remain below the normal levels for up to a year [45, 46].

The conditioning regimen used prior transplant results in a neutropenic phase till the neutrophils reconstitute, which takes approximately 11–12 days in T-cell depleted haploidentical HSCT [47, 48]. Although neutrophil counts rise to normal numbers within 2 weeks after transplant [49], they become functionally competent only after 2 months [50, 51]. The type of graft affects the reconstitution of neutrophils: 2 weeks in case of GCSF mobilized grafts, 3 weeks in case of bone marrow, and around 4 weeks in umbilical cord blood [1]. Use of peripheral blood has decreased the neutrophil recovery time from an average of 16 to 12 days [52].

NK cells recover in both number and function within the first few weeks after transplant [53], and functional reconstitution of NK cells is reached within 2 months [1]. The time taken for NK-cell reconstitution is dependent on the occurrence of GvHD [47, 54] and does not differ if the source of stem cells is peripheral blood or bone marrow [55]. However, the number of functional NK cells is higher when the transplant involves T-cell replete grafts than T-cell depleted grafts [56]. The most prominent functional NK cells after transplant are CD56brightCD16dim [57, 58]. Also, higher overall survival is seen in patients with high CD56bright NK cells at day 14 after unmanipulated haploidentical HSCT. The cytolytic function of NK cells is regulated by the interaction of inhibitory/activating killer immunoglobulin like receptors (KIRs) present on their surface and their specific HLA class I ligands. The reconstitution of the inhibitory and activating KIRs is dependent on factors such as conditioning regimen, T-cell deplete/replete graft, and immunosuppression used after transplant.

In a study evaluating NK-cell reconstitution after matched related/unrelated donor HSCT, it has been reported that the NK-cell counts are lower for longer period (2-3 months) after MUD (156/μL) than MRD (265/μL). The most frequent immature NK cells were CD56bright and NKG2A+CD57-CD56dim NK cells [59]. Russo et al. [60] reported that in haploidentical HSCT using after transplant cyclophosphamide, the immature NK cell starts appearing at 2 weeks; however, the mature NK cells expressing CD16 and CD56 and NKG2A appear at about a year.

Host dendritic cells that escape chemotherapy/radiation activate alloantigenic T cells in the donor and hence play an important role in GvHD. Since host dendritic cells present MHC antigens to donor CD8+ T cells after transplant, depleting these cells could result in lower risk of GvHD [61, 62]. Lower reconstitution of lymphoid dendritic cells has been associated with inferior overall survival [63].

Gamma delta T cells make up ~5% of the T-cell population, and their receptors are composed to gamma and delta chains. These T cells have been reported to enhance engraftment and graft-versus-leukemia effect without an increase in GvHD [64]. Gamma delta T cells reconstitute faster in patients in whom bone marrow (60 days) is used as the graft source than peripheral blood (200 days) [65].

3. Reconstitution of adaptive immunity

T-cell reconstitution is faster in transplantation with peripheral blood as graft source than bone marrow due to higher number of T cells present in the graft [55]. Ciurea et al. [18] reported better T-cell reconstitution in recipients of T-cell replete haploidentical HSCT than recipients of T-cell depleted haploidentical HSCT at 6 months after transplant. Use of ATG for T-cell depletion also affects the rate of immune reconstitution. This effect is more prominent in umbilical cord blood transplantation than bone marrow transplantation. T-cell reconstitution in allo-HSCT without the use of ATG is seen in about 7–12 months when using bone marrow and umbilical cord as stem cell source as compared to 6–24 months when using peripheral blood as stem cell source [66]. T cells recover primarily via peripheral expansion of memory T cells or endogenous T-cell development. Hence, functional thymus is required for effective reconstitution of T cells [67]. This is an issue in aging patients where there is thymus atrophy [68]. Due to this, although full immune recovery is possible in middle-aged patients, it is not possible in older patients and is a cause of morbidity and mortality [69]. Reconstitution of T cells is slow probably due to the prolonged depletion and reduced function of naïve T cells [70]. T cells that reconstitute are primarily from the donor origin in case of T-cell replete transplant or host T cells that have escaped the conditioning

regimen in case of T-cell depleted transplant. Naïve T cells/T-cell receptor excision circles (TRECs) are lower for approximately 10–30 years after transplant [71, 72]. Reconstitution of functional T cells as observed by their ability to secrete interferon gamma and interleukin-4 to normal levels returns in 30 days after haploidentical HSCT for patients in whom acute GvHD is not observed [73]. Recipients of T-cell depleted haploidentical HSCT show higher CD31+ naïve CD4+ T cells than their donors at approximately 4–6 years [74]. Homeostatic peripheral expansion is induced by various homeostatic cytokines such as IL7 and IL15, inflammatory cytokines, and viral exposure. Peripheral homeostatic expansion leads to an inverse CD4/CD8 ratio in patients for several months after transplant. CD4 counts are considered as the best predictive marker for the recovery of immune competence after HSCT, and its recovery has also been associated with lower risk of infections and improved transplant outcomes [1]. CD4+ T-cell counts are as low as <200 cells/μL in the first 3 months and reach levels of 450 cells/μL at about 5 years after transplant [55, 75]. CD8+ T-cell counts increase rapidly during the first 3 months after transplant possibly due to the expansion of herpesvirus-specific CD8 T cells [55, 76]. GvHD reduces the number of CD4+ T cells by inhibiting the thymic output, whereas CD8+ cells increase in number during GvHD or CMV reactivation [77, 78]. The reconstituting CD4+ T cells have a higher expression of CD11a, CD29, CD45RO, and HLA-DR and a lower expression of CD28, CD45RA, and CD62L than normal individuals [79, 80]. The early reconstituting CD8+ T cells are mostly memory or effector cells. Naïve or TREC+CD8+ T cells recover at a slower rate [77, 81]. The number of regulatory T cells (Tregs) is much higher after transplant than normal individuals and may contribute to remission [82, 83]. A Treg:CD4+ T cell ratio of less than 9% has been associated with higher risk of aGvHD [84]. Chang et al. [16] reported lower CD4+ T cells, dendritic cells, and higher CD28 expression on CD4+ and CD8+ T cells in patients receiving haploidentical HSCT than patients receiving HLA matched HSCT.

B-cell reconstitution is also delayed after HSCT: ~6 months for autologous and ~9 months after allogeneic transplantation and is mainly due to GvHD or its treatment. In the first 2 months after transplant, B-cell counts are low but rise higher than the normal levels in approximately 1–2 years [55, 85]. Since restoration of full humoral immune functioning requires both naïve and memory B cells, all patients who have undergone HSCT remain susceptible to infections for at least a year after transplant [1]. The reconstituted B cells express higher levels of CD1c, CD38, CD5, membrane IgM, and membrane IgD and lower levels of CD25 and CD26L than normal individuals [86].

A number of studies have reported comparisons between reconstitution of different immune cells depending on the graft source. Faster reconstitution of

Cell type and numbers	Bone marrow	Peripheral blood	Unrelated cord blood	Reference
Neutrophils (>0.5 × 109/L)	16 days	15 days	19 days	[87]
Natural killer cells (>0.1 × 109/L)	1.5 months	4 months	4 months	[16, 87]
T cells (>0.5 × 109/L) CD4	2–3 months	6 months	3 months	[28, 88]
Naïve T cells (>0.5 × 109/L)	9 months	24 months	12 months	[87, 89]
Cytotoxic T cells (>0.25 × 109/L	3 months	9 months	8 months	[65, 90]
T helper cells (>0.2 × 109/L)	4 months	10 months	1 months	[65, 90]

Table 2.
Reconstitution of different immune cells depending on the graft source.

different immune cells was observed when bone marrow was used as graft source as compared to peripheral blood or cord blood (**Table 2**).

4. Assessment of post-transplant immune recovery

There are different methods to assess the immune recovery after transplant, such as estimation of absolute lymphocyte count (ALC), levels of immune cell subsets (NK cells, B cells, and T cells), and antibody titers to assays for T- and B-cell repertoires [91].

ALC levels have been reported in association with overall survival and rate of relapse. ALC >500 cells/μL on day 15 is linked with better OS and lower relapse after autologous as well as allogeneic transplantation [92, 93]. An increase in the levels of CD16+ monocytes has been associated with aGvhD [94].

Early recovery of CD4+ T cells is associated with overall survival, nonrelapse mortality, and risk of infections [95, 96]. Admiral et al. [97] reported the time taken by circulating CD4+ T cells to reach 0.5× 109/L as a strong marker for probability of relapse. In myeloablative allogeneic HSCT, higher levels of CD3+, CD8+ T cells, regulatory T cells, and myeloid dendritic cells are correlated with relapse-free survival [98].

Recently, flow cytometric analysis has been used to differentiate between the T, B, and NK-cell subpopulations. Low levels of NK cells within the first few weeks after transplant have been associated with poor transplant outcomes like lower overall survival and higher risk of infection [99, 100]. Surface markers such as CD45RA, CD28, CD27, CD62L, and CCR7 can be used to differentiate naïve, effector, effector memory, and central memory CD4+ and CD8+ subsets [101, 102]. The surface markers expressed by naïve T cells are CD45RA+CCR7+; central memory T cells are CD45RA-CCR7+; effector memory T cells are CD45RA-CCR7–; and effector T cells are CD45RA+CCR7– [91]. CD4+ T cells also include regulatory T cells (CD25+FoxP3+) and Th17 cells [103, 104]. The expression of CD27, IgM, and IgD helps in distinguishing between naïve B cells (CD27-IgD+), memory B cells (CD27+IgD+), and isotype switched memory B cells (CD27+IgD-) [105]. Myeloid and plasmacytoid dendritic cells can be distinguished based on the expression of CD123 and CD11c: CD123low CD11c+(myeloid) and CD123bright CD11c- (plasmacytoid) [106].

TRECs have been suggested as a marker for reconstitution of naïve T cells (CD4+CD45RA+) derived from the thymus. TRECs, however, remain low up to 6 months after HSCT [107]. Due to thymic atrophy with age, older patients have T cells with low TCR repertoire, which leads to higher risk of infections leading to lower transplant outcomes [108, 109]. Thymopoiesis can also be evaluated by measuring the number of TRECs by real-time quantitative in purified CD4+ and CD8+ T cells [110]. Lewin et al. [111] reported faster recovery of TRECs in younger patients and patients who received conventional grafts as compared to T-cell depleted grafts. Lower levels of TRECs are associated with GvHD and opportunistic infections [77, 112].

Certain cytokines can also be used as predictive markers for transplant outcomes. One such marker is IL7, which can be used to evaluate successful T-cell recovery. Increased IL7 is associated with delayed reconstitution and increased mortality and aGvHD [113]. High levels of IL6, GCSF, and IL2α have also been indicated in association with risk of aGvHD [96, 114]. For assessing chronic GvHD, high levels of IL8 and low levels of IL17A have been suggested [103, 115]. Min et al. [104] have also correlated high levels of IL6 and IL10 with poor transplant-related outcomes.

Further, T- and B-cell receptor repertoire gene arrangements can be evaluated by molecular techniques such as next generation sequencing [116, 117]. Michalek et al. [118] have demonstrated β chain sequencing of the T-cell receptor in order to identify the T-cell clones that could mediate either graft-versus-host disease or graft-versus-leukemia effect. Brink et al. [9] reported higher diversity in CD4+ T cells than CD8+ T cells following allogeneic HSCT. Greater diversity was observed in cord blood grafts, followed by unmanipulated grafts and T-cell depleted grafts.

5. Strategies to improve immune reconstitution

Many strategies, such as administration of recombinant cytokines, adoptive cell therapy, and hormone-based therapies, have recently been used to improve immune reconstitution after transplantation.

IL7 cytokine has been shown to effectively enhance reconstitution of T and B lymphoid cells by enabling thymopoiesis [105, 119]. It has been demonstrated that IL7 increased the CD3+, CD4+, and CD8+ T-cell levels to more than four folds and also leads to increase in functional and diverse T cells [120]. Administering IL-7 predominantly increases the naïve CD8+ T cells. The timing of administering is, however, important, as administering early after transplant aggravates GvHD [116, 121], whereas administering it at a later stage after HSCT results in lower risk of GvHD. This is contributed by the activation of alloreactive T cells that express lower IL-7Rα levels [32, 38]. Other cytokines that enable immune reconstitution are insulin-like growth factor 1(IGF-1), IL22, IL15, and IL12 [122–124]. IL15 has been shown to significantly increase the reconstitution of CD8+ T cells and NK cells and improve the GvL effect in haploidentical murine models [125]. Sauter et al. [126] reported better lymphocyte reconstitution after IL-15 administration in T-cell depleted allogeneic HSCT; however, it has been shown to worsen GvHD.

Recently, it has been suggested that modulating the function of dendritic cells could reduce GvHD while maximizing GvL [127]. Studies on reconstitution of dendritic cells after HSCT have been contradictory. Maraskovsky et al. [128] have shown that treatment with Flt3-L can expand DC subsets; however, when administered after HSCT, it can worsen GvHD [38]. Gauthier et al. [38] have demonstrated that SDF-1α therapy can expand the DC1 subsets and lower the severity of GvHD. Because of their immunosuppressive properties, mesenchymal stem cells have recently been used for suppressing GvHD [129–131]. Mesenchymal stem cells release cytokines such as IL-7, which improve T-cell survival and promote reconstitution of dendritic cells by secreting SDF-1α [132].

NK-cell immunotherapy is one of the novel strategies underway to reduce GvHD and enhance graft-versus-leukemia effect in a KIR-HLA mismatched haploidentical HSCT [133–135].

6. Future directions

Recently, few studies have identified the association of reconstitution of certain immune subsets with predicting post-HSCT outcomes. However, these studies are often limited by small sample size, lack of detailed immune reconstitution, and secretome profile, which could be used as biomarkers to predict immune reconstitution. Prospective studies involving a large number of patients should be conducted to determine which immune factors and tests to detect the same could have prognostic value and understand the impact of such predictive risk factors on transplant outcomes. This is most beneficial, especially for recipients of

haploidentical HSCT, in which a routine strategy could be adopted to result in faster immune reconstitution and hence lower probability of poor transplant outcomes, such as TRM, relapse, and GvHD.

Conflict of interest

The authors declare no conflict of interest.

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