Strategies before, during, and after Hematopoietic Cell Transplantation to improve T-cell Immune-Reconstitution

Short title: IMPROVING IMMUNE-RECONSTITUTION AFTER HCT

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Abstract
T-cell immune reconstitution (IR) after allogeneic hematopoietic cell transplantation (allo-HCT) is highly variable between patients and may take several months to even years. Patients with delayed or unbalanced T-cell IR have a higher probability of developing transplantation-related-morbidity, -mortality, and relapse of disease. Hence, there is a need for strategies to better predict and improve IR to reduce these limitations of allo-HCT. In this review, we provide an update of current and in near-future clinically relevant strategies before, during, and after transplantation to achieve successful T-cell IR. Potent strategies are choosing the right HCT-source (e.g. donor-recipient matching, cell-dose, graft manipulation), individualized conditioning and serotherapy (e.g. Anti-Thymocyte-Globulin), nutritional status, exercise, home care, modulation of microbiota, enhancing homeostatic peripheral expansion, promoting thymopoiesis, and the use of adjuvant targeted cellular immunotherapies. Strategies to prevent Graft-versus-Host-Disease (GvHD) are important as well, for this complication and subsequent need for immunosuppression affects T-cell IR and function. These options aim for personalized precision-transplantation, where allo-HCT therapy is designed to boost a well-balanced T-cell IR and limit complications in individual patients, resulting in overall lower morbidity and higher survival chances.
Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) nowadays is a widely accepted potentially curative treatment strategy for patients with refractory hematological malignancies and a variety of benign disorders. Immune-reconstitution (IR) after allo-HCT, especially of the adaptive immune system, is highly variable between patients and can take several months to even years. Especially a timely and balanced T-cell IR is important for sustained Graft-versus-Leukemia (GvL) effects, protection against opportunistic infections, and survival chances after allo-HCT.1–3

Hence, there is a clear need to develop strategies to better predict and enhance T-cell recovery and function. The choice of the transplantation unit (e.g. cell-source, cell-dose, donor-recipient matching, graft manipulation), and conditioning regimens are important variables pre-HCT that influence T-cell IR and subsequent complications (Figure 1). Also peri-transplantation parameters, such as the nutritional status of the patient and the composition of the gut microbiome may influence outcome. Post-transplantation, T-cell reconstitution is dependent on two mechanisms: homeostatic peripheral expansion (HPE) and thymopoiesis. Therefore, T-cell IR would improve with strategies that stimulate HPE and thymic function. Graft-versus-Host-Disease (GvHD) also is a major factor affecting T-cell IR,4 in part due to the immunosuppression needed to treat this complication after allo-HCT. As the thymus is especially sensitive to damage, such as during GvHD, even minor grade GvHD might affect thymopoiesis.4–6 Therefore, strategies to prevent GvHD might improve T-cell IR as well.

In this review, we comprehensively discuss current and in near-future clinically relevant strategies to better predict and improve T-cell immune-reconstitution and -function after allo-HCT.

Pre-Transplantation Strategies

Choice of HCT-source

Transplantation with bone marrow (BM) or peripheral blood (PB) (stem) cells from a matched family donor is considered the standard for allo-HCT. Alternative choices are BM/PB from a volunteer unrelated matched or mismatched donor, a haplo-identical or mismatched family donor, and unrelated cord blood (CB). In addition, in attempt to lower the risk of GvHD, the physician may choose for different strategies: CD34+ positive selection, ex vivo T-cell depletion (TCD) using negative selection with CD3+ or CD3αβ+ beads, in vivo TCD using serotherapy (T-cell binding antibodies) such as ATG or alemtuzumab, or for post-transplant cyclophosphamide (in haplo-identical setting). The choice of the HCT-source and its manipulation are important factors in the probability of T-cell IR, and therefore different HCT-sources
are associated with different probabilities for various outcomes; e.g. probability on GvHD (acute and chronic), relapse, and immune-recovery. Use of BM/PB grafts from younger donors is shown to improve survival chances, which may be due to enhanced immune-recovery probability. Overall, all HCT-sources have advantages and disadvantages, and no clear superior cell-source exists. The donor choice highly depends on institutional practices: some centers are more confident with CB, while others prefer BM/PB from matched unrelated donors, or haplo-identical transplants.

**HLA-matching**

The availability of a donor is largely based on matching human leukocyte antigens (HLA) between patient and donor. For BM/PB, donor and recipient are typically matched on high resolution for HLA-A, HLA-B, HLA-C, HLA-DRB1, and some centers also match for HLA-DQB1 and HLA-DP. For CB transplantation (CBT) most centers use less stringent HLA-matching criteria; HLA-A, HLA-B (on low resolution), and HLA-DRB1 (on high resolution). This mismatching in CBT is permissive without increasing the probability on GvHD (in particular chronic). For BM/PB, mismatching at one or more HLA-loci (on high resolution) increases the incidence of transplant-related-mortality, resulting in lower survival chances. A mismatch of donor-specific antibodies (DSA) may influence engraftment as well. Interestingly, in CBT a certain degree of mismatching is suggested to be associated with increased GvL while GvHD-probability is not increased. Importantly, to influence the probability on finding a (better matched) donor, recruitment of minority BM/PB donors, donor retention, improved efficiency of searches, and for CB donor selection a larger global inventory of diverse units in public banks is needed.

**Cell-dose**

To permit any chance on T-cell recovery, myeloid engraftment needs to occur first. For this, the graft needs to contain a sufficient amount of (nucleated/CD34+) cells as a low cell-dose hampers engraftment. CB-units generally contain lower cell numbers than do BM/PB, which may be a problem for transplanting adults or larger children. Therefore, CB-units are selected on cell-dose, which is disease (malignant/non-malignant) and HLA-matching dependent. Furthermore, recently, strategies to identify the better cryopreserved CB-units are developed as well: such as selection based on the total Colony-Forming-Unit, or the amount of Aldehyde-dehydrogenase-Bright-cells. These quality checks suggest to better predict neutrophil-engraftment, and may subsequently enhance the T-cell IR potential after CBT. For BM/PB, cell-dose from HCT in adults is usually sufficient (although a minimum number is still required), since an adequate amount of cells can readily be harvested from the donor (bone marrow/-apheresis).
For CB-grafts that do not contain enough cells for transplantation, \textit{ex vivo} expansion-strategies are available: Delta1 Notch ligand,\textsuperscript{18} co-culture with mesenchymal-cells (\textit{e.g.} Mesoblast),\textsuperscript{19} aryl hydrocarbon receptor antagonist StemRegenin 1 (SR1, \textit{e.g.} HSC835),\textsuperscript{20} nicotinamide (\textit{e.g.} NiCord),\textsuperscript{21} and the copper chelator tetraethylenepentamine (\textit{e.g.} TEPA; StemEx).\textsuperscript{22} These expansion-methods were successfully used in various clinical phase I/II studies and showed to be safe and effective. Overall, over 95\% neutrophil-engraftment was noted after a median time of 10-15 days. Another approach to overcome low cell-dose is the transplantation of two CBT-units; double-CBT. In this, both CB-units contribute to engraftment and reconstitution, but only one provides durable neutrophil-engraftment.\textsuperscript{23,24} Neutrophil-engraftment after double-CBT (of usually inadequately dosed units for single unit CBT) is successful and shows 85-100\% engraftment at a median time of 24 days, which is comparable to single-CBT with sufficient cell-dose. Some reports suggested that double-CBT is associated with lower relapse probability,\textsuperscript{25} compared to single CBT. However, randomized controlled trials failed to prove this effect on relapse,\textsuperscript{26,27} and higher GvHD rates are shown after double-CBT.\textsuperscript{28} Co-infusion with CD34+ cells from a haplo-identical donor (haplo-cord) can also overcome the lower cell-dose for CBT. Compared to double-CBT, haplo-cord showed faster neutrophil recovery, lower risk of GvHD, but slower T-cell IR, and is suggested to be associated with lower relapse risk.\textsuperscript{29} Randomized controlled studies are, however, lacking.

\textit{Immune-reconstitution}

Differences in IR rates between cell sources and manipulations might also influence the choice of HCT-source. In adults, T-cell IR generally is considered most rapid after HCT with PB compared to BM, without \textit{ex vivo} TCD.\textsuperscript{30,31} Not surprisingly, T-cell IR is more rapid after PB/BM from a HLA-matched donor compared to haplo-identical BM/PB, conditioned with Anti-Thymocyte-Globulin (ATG).\textsuperscript{32} Nevertheless, the effect of the nowadays-popular option of post-haplo cyclophosphamide on T-cell IR remains to be investigated. After \textit{ex vivo} TCD with serotherapy, T-cell IR is similar in recipients of PB or BM.\textsuperscript{33} Obviously, T-cell reconstitution is severely hampered after HCT with CD34-selected PB/BM.\textsuperscript{34,35} T-cell IR is prolonged in adult recipients of \textit{ex vivo} TCD unrelated-BM, compared to pediatric recipients of unrelated-BMT and adults receiving related-BMT.\textsuperscript{36} In children, data on PB are scarce as BM and CB are most frequently used as graft sources. Without serotherapy, overall T-cell IR is comparable after BMT or CBT in children, although CD8+ T-cell reconstitution is faster after BMT, while regulatory T-cells (Tregs) and CD4+ T-cell recovery are faster after CBT.\textsuperscript{37} The use of ATG (aiming for \textit{in vivo} T-cell depletion) appears to be critical and cell source dependent; especially T-cell IR after CBT with ATG is severely hampered compared to BMT.\textsuperscript{38}
Improving donor-recipient matching

PIRCHES

Recently, an additional donor-recipient matching strategy was presented based on the probability of indirect HLA-molecule recognition represented by the number of predicted indirectly recognizable HLA-epitopes (PIRCHES). The number of PIRCHES presented on HLA class I and II (PIRCH-I and -II, respectively) is highly correlated with allo-reactivity. For BM/PB donors with low PIRCH-I and -II the outcomes were similar to 10/10 HLA-matching, while in CB a high PIRCH-I is related to higher GvL.

HLA-KIR-matching

NK-cells are one of the first cells that reconstitute after allo-HCT, making them important for immunity when T-cell counts are still low. NK-cell function is regulated by the balance of activating and inhibitory signals via killer immunoglobulin-like receptors (KIRs), which interact with specific HLA class I ligands. This may have implications for GvL; when inhibitory KIRs are mismatched for HLA type (mainly HLA-C), donor NK-cells recognize and kill recipient leukemia cells. However, KIR-mismatching can also lead to GvHD-like syndromes. Future prospective trials should assess the effect of HLA-KIR-matching on the balance between unwanted effects from mismatching (e.g. graft failure and GvHD) and its wanted effects (e.g. GvL).

Conditioning prior to HCT

Chemotherapeutics and total body irradiation

Conditioning prior to HCT includes chemotherapy, such as cyclophosphamide, fludurabine, and busulfan, and/or total body irradiation. In addition, serotherapy, such as ATG or alemtuzumab, is added to reduce the risk of graft rejection and GvHD. Both chemotherapeutics and irradiation may severely affect T-cell IR after HCT, mainly due to thymic damage. Notably, T-cell IR is delayed with myeloablative conditioning compared to nonmyeloablative conditioning, without serotherapy. Therefore, T-cell IR could be enhanced by optimizing the dosage of these chemotherapeutics via pharmacokinetic and -dynamic modelling, and thymic shielding during irradiation, aiming for minimal thymic damage. Furthermore, some chemotherapy with longer half-life (e.g. Fludarabine) may still be present during the infusion of the cells and can subsequently deplete T-cells in vivo. Also sex steroid hormone ablation may be applied to prevent thymic damage by chemotherapy/irradiation (described below).

Individualized serotherapy to improve T-cell IR

ATG was introduced to the conditioning regimen for in vivo TCD, to reduce GvHD risk, which can reach a cumulative incidence of over 40-70% even with a well-matched unrelated donor. A major drawback of
conditioning with serotherapy is a hampered early T- and B-cell recovery.\textsuperscript{48,49} This effect is partly through direct binding and killing of T- and B-cells, but also through “off-target” cytotoxic effects on thymus cells, affecting thymopoiesis.\textsuperscript{50} However, abandoning ATG from the conditioning is associated with significantly higher incidences of graft rejection and GvHD.\textsuperscript{51}

Recently, high ATG-exposure after allo-HCT was associated with detrimental effects on IR.\textsuperscript{38} The highly variable pharmacokinetics of ATG between patients causes under- or over-exposure in a significant number of patients.\textsuperscript{52} CB-cells were affected more by ATG compared to BM/PB; the tolerated post-HCT ATG-exposure, that does not affect IR, is lower for CBT (<20 active units [AU]*day/mL) compared to BMT/PBCT (<50 AU*day/mL).\textsuperscript{38} CBT without ATG, and CBT with very low ATG-exposure, were associated with excellent IR potential,\textsuperscript{49,51} stressing the importance for individualization. Interestingly, these studies also suggest that not ATG-exposure after, but exposure before transplantation prevented GvHD (acute and chronic) in children, but also in adults (unpublished data). Sufficiently high exposure before CBT also prevented rejection. This indicates that there still is an important role for ATG, at least for HCT in non-malignant diseases for graft rejection prevention, but earlier in the conditioning. In adult BM/PB recipients, this positive effect from conditioning with ATG on lower GvHD risk, but negative effects on T-cell IR, was also found.\textsuperscript{48,53} In HCT for hematopoietic malignancies, the decreased T-cell IR from post-transplantation ATG should especially be avoided, as this increases relapse risk and decreases survival.\textsuperscript{54,55} Taken together, these data suggest that personalized ATG-dosing impacts T-cell IR potential after HCT, resulting in better survival chances.

**Peri-Transplantation Factors**

**Nutrition and Microbiotics**

Despite careful assessment and food supplementation, nutritional deficiency is common in allo-HCT patients. Nutritional requirements increase (up to 130-150% of normal) due to catabolic stress, such as during IR and complications.\textsuperscript{56–58} Post-HCT calorie intake is directly related to time of neutrophil-engraftment,\textsuperscript{56} and calorie requirements increase to $\geq 35$ kcal/kg/day in addition to 1.5-2 g/kg/day of protein in the setting of GvHD.\textsuperscript{57}

To maintain nutritional status during and after allo-HCT treatment, patients often receive enteral nutrition or total parenteral nutrition (TPN).\textsuperscript{59} Recently, enteral nutrition in the form of an oral elemental diet showed to be superior to TPN in decreasing the duration and severity of mucositis and duration of hospitalization.\textsuperscript{60} Even during intestinal GvHD, outcome (\textit{e.g.} diarrhea and time to complete dietary recovery) after enteral nutrition was comparable to TPN.\textsuperscript{61} TPN, compared to enteral nutrition, resulted in
significantly higher morbidity and mortality. Therefore, recent studies suggest that enteral nutrition, seems to be the best approach to maintain nutritional status after allo-HCT, even in patients with mucositis or intestinal GvHD.

The composition of nutrients is important for IR after allo-HCT; a lack of zinc, selenium, iron, and antioxidant vitamins can lead to clinically significant immune deficiency and infections. On the other hand, immune-modifying nutrients like vitamin D could be used to dampen GvHD, as it increases Treg numbers. Notably, vitamin D supplementation is only beneficial in decreasing GvHD-risk for patients with the low active AA or Aa vitamin D receptor (VDR) phenotype. Vitamin D increases the risk for GvHD in patients with the active aa VDR phenotype. This poses opportunities to improve allo-HCT-outcome using vitamin D supplementation, at least in patients with low VDR-activity, which is currently evaluated in prospective trials.

Recent studies investigating the relation between intestinal microbiotics and GvHD in the human HCT-setting found that a lower diversity of microbiota relates to a higher risk of GvHD and mortality. Specifically, a shift towards higher presence of Enterococcus faecium and Enterococcus faecalis was found in GvHD patients, while genus Blautia bacteria was associated with a lower probability on GvHD. Therefore, choosing the right antibiotic regime may be of importance for its effects on microbiome diversity and GvHD-probability. As pre- and probiotics maintain and restore microbiotic diversity, the use of probiotics to reduce GvHD risk is currently under investigation. The first results indicate that probiotic treatment prior to and after allo-HCT is safe, and might even reduce GvHD severity associated with better T-cell IR.

Exercise and Home care

Daily exercise before- and after HCT may fasten T-cell IR, although not all studies found a significant effect. Nevertheless, there is evidence indicating beneficial effects of physical exercise in the distribution and function of immune cells in healthy subjects. Prospective trials are warranted to prove this.

Some advantageous effects of home care in the neutropenic phase of the treatment are reported. Home care correlated with lower aGvHD incidence, and survival was at least as high as historic controls. Especially since home care would improve quality of life, reduce costs, and shows to be safe, further research on outcome and the effect on IR would be of interest.
Post-Transplantation Interventions

For T-cell recovery, homeostatic peripheral expansion is the most important mechanism in absence of thymopoiesis, which takes at least 6-12 months. Improving HPE will, therefore, ultimately improve early T-cell IR. Some cytokine-based therapies for improving IR through HPE are currently under investigation (Figure 2). Nevertheless, early T-cell immunity through HPE is limited due to a limited T-cell receptor (TCR) diversity, as thymopoiesis is needed to provide new TCR clones in naïve T-cells. Treatment of patients before receiving allo-HCT (e.g. conditioning, steroids, chemotherapy) causes damage to the thymus. GvHD after HCT can also significantly damage the thymus, while, on the other hand, immunosuppressive treatment of GvHD (e.g. steroids, Cyclosporine, Tacrolimus) can hamper thymopoiesis as well. Therefore, thymic damage from GvHD needs to be balanced from that of immunosuppression. In this, therapeutic drug monitoring, such as for levels of Tacrolimus, Cyclosporine, and Mycophenolate can reduce the probability on thymic damage by GvHD, while limiting their effects on thymopoiesis by targeting to the appropriate exposure. Furthermore, of interest are several therapies linked to normal thymic function that are currently investigated to directly promote thymopoiesis after allo-HCT (Figure 2).

Improving T-cell IR through homeostatic peripheral expansion (HPE)

IL-2
Interleukin-2 (IL-2) was introduced in the HCT setting for it’s role in the differentiation of T-cells into effector cells and promotion of T-, B-, and NK-cell proliferation. Although interleukin 2 (IL-2) can have severe side effects at higher dosage (e.g. capillary-leak syndrome), low-dose IL-2 administration after HCT reduced relapse probability, probably due to an increased GvL response. It also restored homeostasis of CD4+ T-cells, and increased Treg counts, associated with clinical improvement of cGvHD, while no adverse events were noted. Therefore, low-dose IL-2 therapy may improve T-cell IR, contributing to GvL, without increasing GvHD-probability.

IL-15
IL-15 is another interesting cytokine for improving HPE, as it is important for the expansion of B- and T-cells and for the survival of NK-cells. In a murine HCT-model, IL-15 during the first month after HCT was associated with early T-cell and NK-cell IR. In humans, no systemic IL-15 therapy after allo-HCT has been evaluated so far. Only IL-15-stimulated CD3/CD19-depleted graft-cells, from part of the HCT-graft, or IL-15-activated cytokine-induced killer cells have been studied, albeit in few patients. Interestingly, this therapy increased NK, NKT, and T-cell numbers at 15 to 30 days after receipt, without
exacerbating GvHD, and showed GvL potential when given at the initiation of relapse. IL-15-stimulation of stem cells would, therefore, be interesting to evaluate in future studies.

**Improving T-cell IR through thymopoiesis**

**IL-7**
The administration of IL-7 was investigated in the HCT setting, as it naturally promotes the differentiation and proliferation of naïve T-cells. Treatment with recombinant IL-7 after HCT enhanced TCR-diversity, induced a doubling of (mainly memory) CD4 and CD8 T-cells, with no effects on Tregs, NK-, or B-cells. Interestingly, IL-7 also increased the amount of functional T-cells, including virus-specific T-cells, while no significant increase in GvHD or other serious toxicities were seen. These first results show promise for IL-7 in enhancing T-cell IR after HCT.

**KGF**
Keratinocyte growth factor (KGF) promotes proliferation and maturation of immature T-cells and plays a role in postnatal thymic regeneration. KGF, or palifermin, is used as standard-of-care peri-HCT therapy in some centers, and is FDA-approved to prevent mucositis. Treatment of HCT-patients with KGF, during 3 days before and 3 days after conditioning therapy, indeed decreased mucositis, but also prevented damage to the thymus. Although not all studies found any noteworthy impact of KGF on T-cell IR directly, this protection from thymic damage showed enhanced thymopoiesis, with early T-cell recovery probably caused by induction of IL-7, and lower GvHD incidence.

**Sex steroid ablation**
Sex steroids are known to inhibit thymic function. Thus, sex steroid ablation (SSA) may be applied to improve thymopoiesis. Administration of luteinizing hormone-releasing hormone (LHRH) agonists after HCT showed enhanced TCR excision circle production and T-cell repertoire regeneration, with enhanced total and naïve CD4+ T-cell regeneration, indicating thymopoiesis. However, a major limitation of this approach is the surge in sex steroid after LHRH-agonist treatment, so a more rational approach could be to use LHRH-antagonists. Another strategy for SSA; leuprolide acetate (Lupron), in combination with KGF, showed increased reconstitution of naïve CD4+ and CD8+ T-cells, with a more diverse T-cell repertoire, in mice. Currently, phase II clinical studies are underway evaluating the effect of Lupron in the human allo-HCT setting (ClinicalTrials.gov Identifier: fNCT01338987).

**Thymosin alpha 1**
Thymosin alpha 1 (Tα1) is recently investigated in the HCT-setting, as the thymus naturally promotes T-cell development by secreting this hormone. Tα1 (Thymalfasin/Zadaxin) is a FDA approved orphan drug
for treatment of chronic hepatitis B, with no adverse effects found in recipients from 13 months to 99 years old.\textsuperscript{94} In the human HCT-setting, T\textalpha{}1 administration shows encouraging first results on T-cell IR. Subcutaneous administrations of T\textalpha{}1, twice weekly for 4 weeks after allo-HCT, caused an increased CD4+ T-cell recovery and pathogen-specific T-cells to appear already at 1 month after HCT, which was earlier and in higher levels compared to controls.\textsuperscript{95} Additionally, T\textalpha{}1 lowered the cumulative incidence of non-relapse-mortality (mainly infection-related) and increased event-free-survival.\textsuperscript{94,95} Therefore, T\textalpha{}1 therapy might be a promising novel therapy to enhance T-cell IR after allo-HCT.

**Cellular immunotherapies to improve immunity**

The lack of effector immune cells after allo-HCT may be compensated with adjuvant cell-based immunotherapies. Due to high costs, regulatory burden, and complicated production, cellular immunotherapies are currently not widely being used in the HCT-setting. They mainly have potency to be used to treat life-threatening complications such as relapse, viral reactivations, GvHD, but could also be used prophylactically. Here, we discuss the currently most potent cellular immunotherapies to improve immunity after allo-HCT (Figure 3).

**DC-vaccination**

Dendritic cells (DCs) are highly specialized antigen-presenting-cells (APCs) that regulate the balance between action (relevant for GvL) and suppression of the immune system (relevant for GvHD).\textsuperscript{96} Therefore, DC-vaccination is of major interest as a tool to modulate immune responses after allo-HCT. For BMT/PBCT, DCs can either be obtained by culturing monocytes into monocyte-derived DCs, or by directly isolating plasmacytoid DCs or conventional (myeloid) DCs from the peripheral blood of the donor. However, the low number of circulating DCs complicates their clinical application. In case of CBT, CD34+ stem cells from part of the CB-unit can easily be differentiated \textit{ex vivo} into potent CB-DCs targeting viral- and fungal infections after HCT, and even relapse.\textsuperscript{97,98} In addition, tolerogenic DCs might be a future therapy to suppress GvHD, as shown in a murine HCT-model.\textsuperscript{99}

**\gamma\delta T-cells**

Increased numbers of donor \gamma\delta T-cells early after allo-HCT correlate with better overall survival of leukemia patients, without increased risk of GvHD.\textsuperscript{100} Therefore, \gamma\delta T-cells hold promise to improve outcome after allo-HCT. To date, trials using \gamma\delta T-cells focus only on activated autologous V\gamma9V\delta2 \gamma\delta T-cells, combined with IL-2, or activated and amplified \textit{ex vivo}.\textsuperscript{101} This stresses the need for future evaluation of the implementation of these potent cells in the allogeneic HCT-setting, as is currently investigated in a setting that combines \gamma\delta T-cells and NK-cells.\textsuperscript{102,103}
NK-cells
Adoptive NK-cell immunotherapy in the allo-HCT setting is under investigation as well. Results from one of the earliest studies suggest limited adverse events and no induction of GvHD.\textsuperscript{104} More recently, the combination of NK- and γδ T-cells was evaluated as a cell-based immunotherapy after allo-HCT. It is suggested that infusion of NK-cells and γδ T-lymphocytes around the time of HCT may protect the patient from early relapse, viruses (in particular cytomegalovirus; CMV), and graft rejection.\textsuperscript{105} Initial efforts are currently underway using TCR-αβ/CD19-depleted cell populations, which contain CD34+ progenitors, as well as mature NK-cells and γδ T-cells.\textsuperscript{102,103}

NKT-cells
For their immunomodulatory properties, immunotherapy using natural-killer-T (NKT)-cells could be useful in the allo-HCT setting as well. NKT-cells play a role in both tumor surveillance and GvHD,\textsuperscript{106} with early reconstitution suggested to be predictive of lower relapse and GvHD risk, and higher survival.\textsuperscript{107,108} Clinical trials implementing expanded NKT-cells in the allo-HCT setting are currently underway.

Engineered T-cells
One method to improve T-cell recovery is donor lymphocyte infusion (DLI). This non-specific T-cell therapy is, however, associated with increased risk on GvHD.\textsuperscript{109} Furthermore, because of the low frequency of T-cells reactive against many common viruses within the circulation, higher DLI doses may be required to confer clinical benefit against viruses, consequently increasing the risk of GvHD.

A more targeted method to enhance T-cell immunity after allo-HCT is with adoptively transferred antigen-specific T-cells. These T-cells can be expanded \emph{ex vivo} after stimulation with APCs presenting specific antigens, such as cytomegalovirus, epstein-bar-virus, and adenovirus,\textsuperscript{110} or against malignant cells to induce GvL.\textsuperscript{111} A drawback of this therapy is the long time needed for production of specific T-cells. Gamma capture, based on IFN-γ production, of functional antigen-specific T-cells from a donor is a faster alternative. But for this method the presence of specific T-cells is essential and demands a seropositive donor. This is a challenge when the donor lacks viral immunity, or with CB which generally contains virus-naïve immune cells. Nevertheless, virus-specific T-cells can also be cultured from naïve CB T-cells.\textsuperscript{112}

Another way is T-cell gene therapy to produce Chimeric Antigen Receptor (CAR) T-cells or engineer T-cells to express specific TCRs (TCR-T-cells). Based on the chosen CAR or TCR binding domain these modified T-cells can be used to target viruses and leukemic cells to target relapse (\emph{e.g.} CART19, WT1-TCR-T-cells).\textsuperscript{113–115} But they can potentially also be used as an alternative myeloablative therapy.\textsuperscript{116}
Drawbacks of genetically modified T-cell therapy, however, are possible cytotoxic effects on normal cells that express target antigens. Nevertheless, the specificity and potency of this therapy is shown by the finding that donor-derived anti-CD19-CAR T-cells induce complete remission in relapsed patients, without exacerbating GvHD. In addition, these cells have been shown to persist for over 3 years providing long-term protection against their target.

Tregs and MSCs

Tregs and Mesenchymal stromal/stem cells (MSCs) can inhibit immune responses, and have been investigated as adjuvant cell-therapies to treat or prevent GvHD. The non-specific MSC therapy did not prevent GvHD when co-transplanted alongside the HCT-graft, while even increased relapse rates and mortality were noted. However, reduced aGvHD risk was seen when given after HCT before GvHD-onset. Co-infusion of Tregs alongside the HCT-graft decreased the occurrence of acute- and chronic GvHD, while maintaining GvL. This also resulted in high frequencies of pathogen-specific CD4+ and CD8+ T-cells already at 2 months after HCT, decreased incidence of CMV disease, and showed strong antiviral protection after pandemic influenza vaccination. No negative effects due to the Treg infusion were reported. Furthermore, transplantation with CB-derived Tregs directly after CBT showed an increase in viral infections within 30 days infusion, but lowered the occurrence of GvHD without increased risk of relapse. This may make Treg-immunotherapy a promising strategy to decrease the risk for GvHD associated with better T-cell IR potential.

Concluding remarks and future perspective

We have summarized a broad variety of novel strategies before, during, and after HCT to improve T-cell IR, aiming to decrease the risk of complications and increase survival chances. Selecting the appropriate HCT source, and graft manipulations, are the first critical factors affecting T-cell IR after transplantation. Individualizing the conditioning regimen is crucial as well, especially for compounds that dramatically influence T-cell IR, such as ATG. Furthermore, enhancing homeostatic peripheral expansion, preventing thymic damage, and promoting thymopoiesis are important in enhancing T-cell IR. Better disease control may be achieved with adjuvant (specific) cellular immunotherapies to prevent GvHD and promote immunity against pathogens and cancer cells. Probably a combination of these pre-, peri-, and post-HCT strategies will most potently enable a timely and balanced T-cell recovery.

Immunomonitoring will be of major importance to identify patients at risk, which would provide opportunities for early immune interventions. For instance, patients with low T-cell counts early after HCT, and/or a low TCR-diversity, are at risk for HCT-related complications and could benefit from
adjuvant immunotherapies; such as low-dose IL-2, administration of Tregs, or leukemia/virus-specific DCs and/or -specific-T-cells. New functional measures of immune-recovery would, therefore, also add to guide clinicians in the assessment of IR beyond simple absolute lymphocyte counts. In addition, it is important to continue adequate supportive care with continuation of anti-microbial prophylaxis and PCR monitoring for viruses until adequate immune recovery occurs.

Standardization and harmonization of immunomonitoring would enable better comparison between multiple allo-HCT studies, in order to better understand the biology of IR, and find biomarkers. These biomarkers will help the physician to guide the developing immune system, and anticipate on complications in order to avert them. This will pave the path to precision-transplantation in the future; in which allo-HCT therapy will be personalized, using a combination of strategies to boost T-cell IR, subsequently resulting in lower morbidity and higher survival after allo-HCT.

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Contributors

CdK wrote and edited the report, SN and JJB reviewed the manuscript and provided critical comments. All authors reviewed and approved the final report.

Declaration of interests

All authors declare no competing interests.

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Figures

Figure 1: Pre-, peri-, and post-Hematopoietic Cell Transplantation factors affecting T-cell immune-reconstitution. Depicted in the bars are relative immune cell levels: red: < reference values; risk for complications, green: ≥ reference values; adequate immunity.

Figure 2: Overview on timing of non-cellular post-transplantation interventions to improve T-cell immune-reconstitution via homeostatic peripheral expansion (HPE) and thymopoiesis.

Figure 3: Overview on timing of cellular immunotherapies to improve immunity after allogeneic hematopoietic cell transplantation.
 allo-HCT

conditioning

immune-reconstitution

Pre-HCT:
- choice of HCT source
- graft manipulation
- cell-dose
- donor-recipient matching
- conditioning regimens
- serotherapy

Post-HCT:
- homeostatic peripheral expansion
- thymic function
- complications (infections, GvHD, relapse)
- GvHD treatment (immunosuppressive drugs)
- adjuvant immunotherapy

Peri-HCT:
- nutrition
- exercise
- environment: home care
- microbiotics
Improving HPE

allo-HCT

- low-dose IL-2 (daily for 8 weeks)
  - increased CD4+ T-cell and Treg counts

IL-15 stimulated graft cells
  - increased counts of NK, NKT, and T-cells 15-30 days after administration

Improving thymopoiesis

- IL-7 (2 weeks, starting at day 14)
  - enhanced TCR-diversity, improved T-cell recovery

- KGF (daily, 3 days pre- and 3 days post-HCT)
  - prevents thymic damage, enhanced thymopoiesis

- Tα1 (twice weekly, 4 weeks)
  - increased CD4+ T-cell recovery, early pathogen-specific T-cells
Figure 3

**Pre-HCT**
- **Tregs / MSCs** (prevent GvHD)
- **NK + γδ T-cells** (prevent early relapse, graft rejection, viral reactivation)
- **CAR T-cells** (myeloablation, T-cell-depletion)

**Post-HCT**
- **NK, iNKT, DLI** (enhance immunity, lower TRM)
- **DCs** (stimulate antigen-specific T-cell reconstitution)
- **virus-specific T-cells, CAR/TCR T-cells** (target viral reactivation, infections, relapse, lower TRM)
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