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NK Cells and γδT Cells for Relapse Protection After Allogeneic Hematopoietic Cell Transplantation (HCT)

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Abstract

Purpose of review—The outcome of allogeneic stem cell transplantation (allo-HCT) is still compromised by relapse and complications. NK cells and γδT cells, effectors which both function through MHC-unrestricted mechanisms, can target transformed and infected cells without inducing Graft-versus-Host Disease (GVHD). Allo-HCT platforms based on CD34+ selection or αβ-TCR depletion result in low grades of GVHD, early immune reconstitution (IR) of NK and γδT cells and minimal usage of GVHD prophylaxis. In this review we will discuss strategies to retain and expand the quantity, diversity and functionality of these reconstituting innate cell types.

Recent findings—Bisphosphonates, IL-15 cytokine administration, specific antibodies, checkpoint inhibitors and (CMV based) vaccination are currently being evaluated to enhance IR. All these approaches have shown to potentially enhance both NK and γδT cell immuno-repertoires.

Summary—Rapidly accumulating data linking innate biology to proposed clinical immune interventions, will give unique opportunities to unravel shared pathways which determine the Graft-versus-Tumor effects of NK and γδT cells.

Keywords

NK cells; γδT cells; allogeneic stem cell transplantation; immune reconstitution

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Compliance with Ethical Standards

Conflict of Interest

Moniek A. de Witte has no conflict of interest.

Human and Animal Rights and Informed Consent

This article refers to published studies with both human and animal subjects performed by the authors. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

1.1 Introduction

Allogeneic stem cell transplantation (allo-HCT) is the only accepted curative option for many patients with advanced hematological malignancies. The chief long-term goal of allo-HCT is to develop low-toxicity platforms that will not only reduce morbidity and mortality but also allow disease-specific interventions to be applied after transplantation. Graft manipulation using profound T cell depletion with antibodies like Campath reduces acute and chronic Graft-versus-Host Disease (GVHD), but with an increase in relapse and infectious complications, most likely attributable to delayed immune reconstitution (IR) [1, 2]. More recently, there has been a surge of interest in the usage of post-transplantation high dose cyclophosphamide (PTCy) as short-term GVHD-prophylaxis. Incidence of severe aGVHD and cGVHD is low [3], but it appears that as transplant-related mortality declines, relapse is unacceptably high. Clearly, further modifications are needed to reach the goal of long-term disease free survival[4]. To avoid long-term immune deficiency mediated by circulating Campath after transplantation or PTCy acting early after transplantation on all engrafted immune cells, graft manipulation by CD34+ selection[5] or αβ TCR depletion[6–8] has been explored. Such strategies have been associated with lower incidences of aGVHD and cGHVD, in some cases without the need for post-transplant immune suppression and without an increase in relapse [5].

Individual variations in IR remain a challenge. Even in unmanipulated grafts, αβ T cell-based immunity is severely impaired the first months post allo-HCT[9], with a substantial variability between individuals. In response, patient-tailored grafts and individualized dosing of conditioning regimens have been proposed to equalize immune reconstruction [10]. Innate immune subsets including NK cells and γδT cells present an additional opportunity to improve tumor control shortly after transplant. These cells reconstitute swiftly post allo-HCT [11, 9] and are not associated with GVHD[12]. NK cells recover faster in numbers after αβ TCR depletion as compared to CD34+ selection, presumably due the co-transferred NK cells within the graft [13, 14]. Moreover, γδT cells can be detected at physiological numbers in the first month post allo-HCT after αβ TCR depletion [7]. The major challenge for both subpopulations is to retain functionality and / or diversity even in the absence of early immune suppression. This challenge is very well illustrated in recipients of *ex vivo* expanded NK and γδT cells. Although some successes are reported, clinical efficacy is often disappointing, most likely due to loss of function of the transferred innate cells [15, 16]. Interventions aiming to improve early outcome after allo-HCT should result in profound enrichment and improved functionality of both NK and γδT cells [17]. Administration of bisphosphonates, cytokines and bispecific or trispecific immune engagers, checkpoint inhibitors, and “controlled infection” or vaccination (Figure 1) are all examples of such interventions and will therefore be the focus of this review.

1.2 Basic biology of NK and γδT cells

1.2.1 NK cells—NK cells and γδT cells are major factors in cancer immune surveillance [18, 19]. NK cells are large granular lymphocytes with the ability to lyse virally-infected cells and tumor targets without MHC-restriction or prior sensitization. Human NK cells comprise around 10–15% of the lymphocyte pool. NK cells can kill infected or transformed

cells both by direct cytotoxicity or by the production of cytokines [20]. NK cell reactivity is tightly regulated via activating and inhibiting receptors, the balance of which will dictate the fate of an NK cell [20]. In order to react with a target cell, NK cells require engagement of an activating receptor and functional competence through inhibitory receptor signaling. This process, referred to as NK cell education or licensing, prevents auto reactivity of NK cells not expressing inhibitory receptors recognizing self-MHC [21]. By contrast, chronic triggering of activating receptors can lead to loss of function of an NK cell [21]. Thus, NK cell function is adaptable or fluid.

Activating receptors detect ligands representing a cell in ‘distress’. These receptors include natural cytotoxicity receptors (NCRs), c-type lectins (including NKG2D which can engage with MICA/MICB or ULBPs and NKG2C/CD94 which can engage with HLA-E), DNAM-1 (which is specific for poliovirus receptor (PVR) and Nectin-2) [22], and the low affinity Fc receptor CD16 [23]. Inhibitory receptors include the killer immunoglobulin-like receptors (KIRs) and the C-type lectin NKG2A/CD94, which also recognizes HLA-E. KIRs are polymorphic molecules that are not only inhibitory, but can also be activating. The ligand for inhibitory KIRs are HLA class I molecules [24]. Four types of KIRs can provide inhibitory signals through engagement with defined HLA molecules. When inhibitory molecules engage with HLA molecules, the target cells will be considered as ‘self’. In allo-HCT (especially haplo-HCT), donor-recipient KIR mismatches (‘missing self’) are reported to contribute to the Graft-versus-Leukemia (GVL) effect [25].

NK cells are divided in $CD56^{dim}$ and $CD56^{bright}$ subsets (Table 1). In a steady state situation, 90% of the NK cells in peripheral blood (PB) are $CD56^{dim}CD16+$. $CD56^{dim}$ NK cells highly express granzyme/perforin and can mediate direct cytotoxicity. $CD56^{bright}$ cells lack perforin, but are strong producers of cytokines such as IFNγ upon stimulation (e.g. with IL-15, IL-12 and IL-18) [26].

1.2.2 γδT cells—γδT cells are CD3+ lymphocytes and comprise 1–5% of the PB lymphocyte pool. γδT cells can also be found in secondary lymphoid organs and peripheral tissue like skin, lung and intestine, where they can represent up to 50% of the T cell population [27]. γδT cells express a range of activating and inhibitory molecules, many of which are shared with NK cells [28, 29]. Like NK cells, activation of γδT cells depends on the net balance of activating and inhibitory signals. In cases of ‘distress’, γδT cells are activated rapidly and contribute to the ‘lymphoid stress-surveillance’ response. This immune response includes direct cytotoxicity and production of cytokines as well as more adaptive features like antigen presentation [29].

γδT cells are sometimes also referred to as ‘unconventional T cells’ (Table 1). Unlike conventional (αβ)T cells, the TCR of unconventional T cells does not bind with antigens presented on MHC molecules, but instead with alternative ligands on target cells[30]. The most extensively characterized γδTCR is the vγ9δ2 TCR [27], which senses elevated levels of isopentenyl pyrophosphate (IPP) of a dysregulated mevalonate pathway[31]. This concept will be expanded in section 1.3.1. Other ligands for γδTCRs include MICA/B, CD1 and endothelial protein C receptor (EPCR)[28].

γδT cells are commonly divided into vδ2+ and vδ2- γδT cells (Table 1). Most vδ2+ T cells express the vγ9δ2. They represent the up to 95% of the γδT cells in PB. The majority of γδT cells in lymphoid organs and peripheral tissue like skin, lung and intestine express either the vδ1 or vδ3 TCR chain, and not vδ2. They are often collectively referred to as vδ2- γδT cells. γδT cells can recognize a range of hematological and solid malignancies [18]. Recently, analysis of tumor-infiltrating lymphocyte revealed that γδT cells have the strongest association with a beneficial outcome in a large series of diverse cancers, suggesting an important role in tumor immune surveillance for these cells●●[32].

1.3 Potential strategies to simultaneously enhance NK and γδT cell immunity post allo-HCT

1.3.1 Bisphosphonates

Bisphosphonates (BPs) like pamidronate (PAM) or zoledronate (ZOL) are routinely administered to prevent osteoporosis and prevent bone loss in cancer patients. In multiple myeloma (MM) patients the development of an acute phase reaction after administration of BPs was associated with expansion of vγ9δ2 γδT cells [33]. Vγ9δ2 γδT cells target tumor cells by sensing elevated levels of isopentenyl pyrophosphate (IPP) from the dysregulated mevalonate pathway (MVA) [34]. The MVA pathway, an essential metabolic pathway for many tumor types, uses acetyl-CoA to produce sterols (including cholesterol) and isoprenoid metabolites[35]. BPs disrupt the MVA pathway, resulting not only in impaired protein prenylation in the osteoclast, but also in accumulation of IPPs in a wide range of cell types. Vγ9δ2 γδT cells recognize a conformational change of the ubiquitously expressed surface protein butyrophilin A1 (BTN3A1). This conformational change is caused by accumulation of IPPs and depends on an altered cellular localization of the small GTP-ase Rho-B[36, 37]. An altered localization of Rho-B can be found in many tumors, but only in a few non-transformed cells (e.g. APCs)●[36]. Administration of BPs has contributed to γδT cell-mediated immune responses in a number of cancer patients[38]. Accumulating knowledge that BPs can induce γδT cell-mediated tumor reactivity [39, 40], and in particular reduce the frequency of EBV-induced lymphoma in mouse models [39], as well as awareness that bone loss and osteoporosis is a common complication post allo-HCT [41], suggest that BPs are logical agents to stimulate γδT cell immunity post allo-HCT. Clinical trials have shown that administration of BPs after allo-HCT is safe and results in increased bone densities[42]. In addition, administration of ZOL shortly after αβ TCR / CD19-depleted haploidentical allo-HCT results in activation of vδ2+ γδT cells, associating with improved clinical outcomes ● [43]. Interestingly, administration of ZOL also resulted in an increase of vδ1+ γδT cells with increased cytotoxicity [43].

BPs may also impact alternative immune mediated anti-tumor responses. Pre-clinical studies have shown that ZOL can activate NK cells[44, 45]. An indirect mechanism is hypothesized in which ZOL stimulates DC-like cells and γδT cells, subsequently enhancing NK cell cytotoxicity [45]. Osteoclasts have also been shown to contribute to an immunosuppressive microenvironment in cancer patients. Part of the immunosuppressive nature of osteoclasts is mediated by expression of checkpoint inhibitors like PD-L1, expression of T-cell metabolism regulators like indoleamine 2, 3-dioxygenase (IDO) [46] and stimulation of

regulatory T cells [47], mechanisms likely to also negatively impact NK and γδT cells. Inhibition of the osteoclastogenesis is therefore proposed to create a more pro-inflammatory microenvironment in the bone marrow niche ●[46].

1.3.2 Cytokines (IL-2 and IL-15)

Cytokine therapy can promote NK cell antitumor reactivity [16]. NK cells respond to both IL-2 and IL-15, which signal via the common γ chain receptors. High dose IL-2 was the first cytokine to show, in metastatic melanoma patients, that it can be used to prime the immune system to destroy tumor cells[48]. It is currently the only lymphocyte cytokine approved by the FDA [49]. Clinical translation of this observation to a broadly applicable immune intervention is challenged by the considerable toxicity of high dose IL-2 and the induction of regulatory T cells (even with low dose IL-2). When IL-2 was used to promote γδT cells, clinical trials showed only modest efficacy and significant toxicity [38].

In contrast to IL-2, IL-15 does not induce the proliferation of regulatory T cells [50, 51]. IL-15 induces the association of IL-15Rα, IL-2Rβ and γc, which promotes NK cell development, expansion and homeostasis [52]. In contrast to other γc cytokines, IL-15 requires trans presentation of IL-15Rα to activate NK cells [53]. The cleaved IL-15/IL-15Rα heterodimer is bioactive, whereas the single-chain IL-15 is poorly secreted and unstable. Therefore, serum IL-15 is present in heterodimeric forms [54]. A recently identified mechanism on how IL-15 promotes immune responses is via activation of the mTOR pathway ●[55], which results in subsequent phosphorylation STAT-3 and STAT-5 [52]. In that light, the use of the mTOR inhibitor rapamycin is likely not the optimal immunosuppressive agent for restoration of NK cell function post allo-HCT.

IL-15 also plays an important role in γδT cell function. Loss of IL-15 or any component of its receptor complex in mice not only results in a severe reduction of NK cells, but also of γδT cells, NKT cells and a population of memory CD8 T cells [56]. IL-15 promotes the maturation of a naïve γδT cell repertoire both in cord blood[57] and in thymocytes [58] towards a more cytotoxic repertoire. It also enhances the function of vδ2+ γδT cells in the presence of bisphosphonates [59]. In addition, IL-15 deficiency has been reported to deplete tumor-infiltrating γδT cells, resulting in loss of tumor control ●●[60]. Not surprisingly and in line with *ex vivo* NK cell expansion [16], IL-15 has recently been described as the key cytokine to generate GMP grade γδT cells active against many hematological malignancies [61, 62]. Like NK cells, IL-15 also signals via the JAK/STAT pathway in γδT cells [59, 58], suggesting a common pathway for IL-15 signaling in both innate immune subsets.

In the first clinical phase I trial using recombinant IL-15 (rh-IL15) in cancer patients, 0.3ug of rh-IL15 daily was determined as the maximum tolerated dose (MTD) ●●[63]. NK cells and γδT cells showed the most marked expansion, followed by a contraction in subsequent weeks. Objective clinical responses were observed in a small fraction of patients. Current efforts therefore focus on reducing toxicity and increasing efficacy of rh-IL15 administration. In line with the increased biological activity of trans-presented heterodimeric IL-15, superagonistic formulas of synthetic IL-15 coupled to the IL-15Rα significantly promoted NK cell development and differentiation *in vivo* [64]. These include soluble IL-15Rα coupled to the human portion of IgG1 associated with recombinant rh-IL15[65] or

a mutant form of rhIL-15 (ALT-803) [66], as well as rhIL-15 directly linked to the high affinity IL-15Rα 'sushi+ domain' (CYP0150) [67]. Pre-clinical data showed superior NK activation of these superagonists as compared to conventional rhIL-15. Clinical trials are in progress to determine safety and efficacy. In the context of allo-HCT, (superagonistic) rh-IL15 appears to be a logical intervention to promote early IR of NK and γδT cells, especially after αβ TCR depletion, where the reconstituting immune repertoire primarily consists of NK and γδT cells and the duration of immunosuppression is anticipated to be short.

1.3.3 Bi-and trispecific antibodies

An effective adaptive immune response is characterized by a clonal expansion directed towards a few high- affinity antigens, a concept which is being implemented in all tumor vaccination programs. As NK cells do not perform antigen-specific clonal expansion, alternative approaches will need to be explored to induce NK-mediated immune responses with a magnitude and specificity comparable to adaptive immune responses. A unique feature of NK cells is the high expression of CD16 (FcγRIII), which allows NK cells to perform antibody-dependent cytotoxicity (ADCC). Even though CD16 is a low affinity binding receptor to natural Fc, NK cells are key contributors to the anti-tumor effect seen with the first FDA-approved monoclonal antibody rituximab [68].

To further optimize NK-cell mediated ADCC, bi-and trispecific killer engagers (BiKEs and TriKEs) have been designed which bind CD16 at higher affinity than a natural Fc binder to create a specific immune synapse between NK cells and tumor cells[69]. BiKEs bind to NK cells through a single chain variable fragment (scFv) spaced with an inert linker and combined in a single sequence to a scFv directed against one or more desired tumor antigens (e.g. CD19/CD22/CD33). Upon BiKE administration, NK cells are activated through CD16 signaling and gain specificity via the scFv fragment towards for instance CD33 [70]. Importantly, levels of CD16 expression are not a limiting factor as patient-derived NK cells with lower CD16 expression also respond upon administration of a BiKE [71]. However, even high affinity binding of CD16 with a BiKE does not deliver a proliferation signal to NK cells., We hypothesized that addition of an an IL-15 linker between the scFV against antigen and CD16 would result in promotion of expansion, activation and enhance the survival of NK cells and therefor developeda 161533 TriKE. Compared to the 1633 BiKE, the 161533 TriKE showed superior NK cell function both in patient-derived NK cells as well as in murine models ●[72]. Based on this data, TriKEs are targeted for clinical development.

Although the capacity of NK cells to mediate ADCC via the abundantly expressed FcγRIII CD16 is well-recognized [73], γδT cell-mediated responses upon antibody based immunotherapy are generally not reported. This is somewhat remarkable since it has been long-known that γδT cells express CD16 [74], albeit at much lower and heterogeneous levels as compared to NK cells. In addition, γδT cells have been shown to be able to induce ADCC upon incubation with both monomeric antibodies, as well as CD19 and CD33-based BiKEs and TriKEs [75, 76].

Based upon these promising pre-clinical data, clinical trials planned in the near future may elucidate how TriKEs can improve NK cell and perhaps γδT cell-mediated immune

responses in cancer patients. Pending results, FDA- approved antibodies such as rituximab or daratumumab may have potential to induce NK and γδT cell-mediated tumor-specific immune responses shortly post allo-HCT indirectly via CD16 (FcγRIII).

1.3.4 Keeping innate cells functionally active

To prevent uncontrolled immune activation, lymphocytes express a range of inhibitory checkpoint molecules. Blockade of 'programmed death-1' (PD-1) or 'cytotoxic T-lymphocyte–associated antigen' (CTLA-4) mediated signaling can restore αβ-T cell mediated tumor responses. After decades of translational research, administration of 'checkpoint inhibitors' is implemented in daily clinical practice for many types of malignancies[77]. As NK cells[78] and also γδT cells[79] express checkpoint proteins, administration of checkpoint inhibitors is expected to increase NK and γδT cell responses. For example, blocking CTLA-4 in melanoma patients showed an association between tumor control and reconstitution of γ9δ2T cell repertoires [80].

After allo-HCT, checkpoint inhibitors have thus far been administered to only a small series of allo-HCT patients, given the potential risk of inducing severe GVHD, [81, 82]. Larger clinical trials are necessary to establish the safety and efficacy of checkpoint blockade after allo-HCT. If the risk of detrimental side effects of immune checkpoint inhibitors is predominantly determined by αβ T cells, T cell depletion platforms may be a more suitable platform for implementation as compared to T cell replete allo-HCT.

1.3.5 CMV exposure exerts a profound effect on the innate immune repertoire

CMV is a herpes virus which establishes a lifelong latent infection following primary infection. CMV infection is common, with seroprevalence rates ranging from 45–100% [83]. Post allo-HCT, CMV reactivation is observed in a 30–60% of recipients, with GVHD and T cell depletion as risk factors [84].

During a primary CMV infection, NKG2C+ NK cells expand and become strong producers of IFNγ. Importantly, increased frequencies of $CD56^{dim}$CD57+NKG2C+ NK cells persist, with an ability to expand and become functionally active upon a CMV reactivation [85]. These CMV-induced 'adaptive NK cells' share DNA methylation patterns similar to cytotoxic effector T cells but different from conventional NK cells ●[86]. Consistent with other studies [87, 88], we have recently shown that allo-HCT recipients with reactivated CMV had lower leukemia relapse, associating with both higher frequencies and greater absolute numbers of these adaptive NK cells ●[89]. Collectively, these data reinforce the concept that CMV can result in NK cells with properties of immune memory. These properties include priming after secondary target exposure, which may potentially result in lasting malignant disease relapse protection [89].

Vδ1+ T cells are the primary γδT cell subset proliferating upon CMV infection/reactivation [90]. After allo-HCT, CMV induced vδ1+ T cells are capable of recognizing both CMV-infected cells and primary leukemic blasts ●[8, 91]. Next generation sequencing of the γδTCRs revealed that CMV reactivation post allo-HCT induced a clonal proliferation of distinct γδT cell clones, suggesting a γδTCR-mediated response of γδT cells upon CMV infection ●●[92]. Longitudinal analysis of γδT cells in series of allo-HCT recipients

demonstrated that γδT cell numbers are significantly higher in recipients with CMV viraemia as compared to recipients without CMV reactivation [93–95, 91]. Memory-like γδT cell responses are increasingly recognized, but many questions remain, including the driving ligands and other signal-driving γδT cell responses [96].

Harnessing the beneficial impact of CMV infection/reactivation on the innate immune repertoire remains challenging. Theoretically, development of a CMV-based vaccine seems the most straightforward approach. Currently there is no FDA-approved CMV vaccine, but over 25 phase 1–3 clinical trials are currently evaluating CMV vaccination [97]. Amongst candidate CMV vaccines are those expressing T cell epitope(s) and/or the CMV glycoprotein B (gB). The CMVPepVax (chimeric peptide composed of a the HLA A*0201 restricted pp65 CD8 T-cell epitope and a tetanus T-helper epitope) and the TransVax vaccine (a CMV DNA vaccine encoding the pp65 epitope and gB) have an attractive safety profile and lower CMV reactivation post allo-HCT in patients still on immunosuppressive medication[98, 99]. Interestingly, a higher relapse- free survival was observed in recipients of CMVPepVax, warranting confirmation in a phase 2 trial ●●[99]. Open questions concern the effectiveness of peptide-based vaccines aiming for a CD8 T cell-based immune response in situations where the αβ T cell repertoire is more strongly impaired and what alternatives are suitable for patients who do not have the desired HLA haplotype.

Though γδT cell responses have also been observed after vaccination with CMV vaccines developed for inducing αβ T cell repertoires[100], those inducing strong (adaptive) NK and γδT cells responses probably have alternative requirements. Two approaches for enhancing NK and γδT cells responses are conceivable. The first involves identifying CMV vaccines with a potent effect on NK and γδT cells. In particular, analysis of the NK and γδT cell repertoire will be of special interest following use of attenuated or replication-incompetent CMV vaccines currently under clinical evaluation [97]. A second approach to harness NK and γδT cell immune responses entails indirect stimulation of already existing (donor derived) adaptive NK and γδT cell repertoires. For example, vaccination with a seasonal influenza vaccine in healthy individuals has shown long-lasting NK cell responses, in which the CMV serostatus impacts the outcome [101]. This finding suggests that the recommended influenza vaccination for allo-HCT recipients[102] might result in adaptive innate responses that are potentially cross-reactive towards other infections or malignant cells. Additionally, within EBV infected individuals, NK and γδT cell repertoires show differential, both redundant and non-redundant, activation patterns [103], which might also apply to CMV infection. Therefore, a next - generation vaccine is required to fulfill the needs synergistic activation of both subsets.

1.4 Concluding remarks

For more than 6 decades, allo-HCT has been the principle mode of cellular immunotherapy. Traditionally, focus has been upon the anti-tumor effect αβ T cells, in which separating GVL from GVHD remains challenging [104]. Adoptive transfer of tumor specific αβ-T cells has been proposed as a way to overcome these challenges. Both *ex vivo* expanded tumor-infiltrating lymphocytes and genetically engineered αβ-CAR-T cells have shown the impressive capacity of tumor-specific αβ T cells to induce sustained responses [105, 106].

However, widespread clinical implementation is challenged by a limited number of the target antigens suitable for CAR-T cells and by logistic and financial hurdles associated with CAR-T cells manufacturing [107].

Allo-HCT is currently providing hundreds of patients with an array of allogeneic NK and γδT cells [108]. Such allogeneic immune cells can recognize malignant cells, but do not cause GVHD. NK and γδT cells share many innate features, yet they also have been shown to be pleiotropic and diverse [17, 20]. In particular, the interaction between NK and γδT cells in the context of immunological challenge has only recently undergone appraisal [103]. Reconstituting innate repertoires will provide unique possibilities to further elucidate these interactions. A factor to consider is that the composition and function of the tumor-infiltrating lymphocyte repertoire is different as compared to that of circulating lymphocytes [109]. Recently developed high-resolution methods for cell analysis like Cytometry by time of Flight (CyTOF) ●[110], next-generation sequencing of γδ TCRs[92] as well as single-cell RNA-seq [109] have all contributed to new insights in complex (innate) immunological networks.

Substantial progress has been made to develop allo-HCT platforms facilitating early NK and γδ T cell reconstitution. The challenge for immediate future is to build on these efforts by implementing early ‘off the shelf ’ therapies which specifically stimulate reconstituting innate subsets in the timeframe in which αβ T cell IR is still incomplete. This will fulfill an urgent need, as mortality rates of HCT-recipients are highest in the first months after transplantation, due largely to impaired control of infections and relapse.

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Jürgen Kuball is scientific co-founder and CSO of Gadeta BV (www.gadeta.nl) and is a shareholder. He is also an inventor on different patents given to Gadeta via the University Medical Centre Utrecht that deal with γδTCR, processing strategies, and their ligands. In addition, he has received grants from Novartis and Miltenyi Biotech.

Jeffrey S. Miller is a consultant and scientific advisory for Fate Therapeutics and Oxis Biotech. He also is on the scientific advisory board for Celgene. In addition, he has pending patents via the University of Minnesota for Fate Therapeutics and Oxis Biotech.

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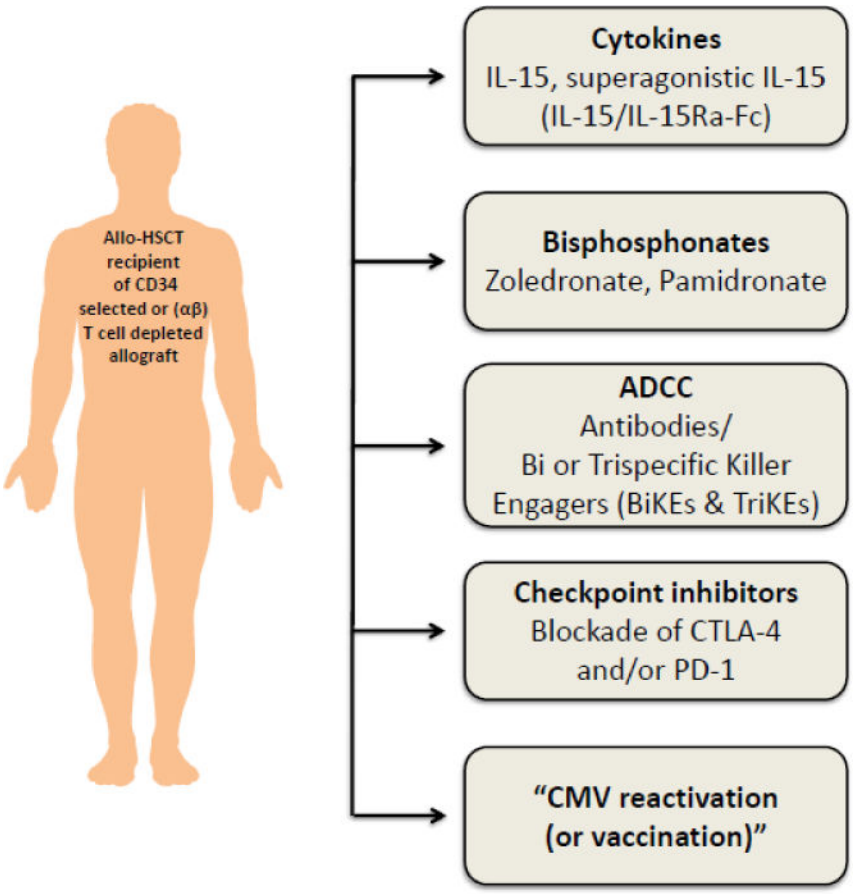

Figure 1.

Potential strategies to induce NK and γδ T cell based anti-tumor responses after allo-HCT. ADCC (antibody-dependent cytotoxicity); Allo-HCT (allogeneic hematopoietic cell transplantation); CMV (cytomegalo virus); CTLA-4 (cytotoxic T-lymphocyte–associated antigen); IL-15 (interleukin 15); PD-1 (programmed death-1).

Table 1

Potential strategies to induce NK and γδ T cell-based anti-tumor responses after allo-HCT

Name	Brief description	Ref.
CD56 bright NK cells	10% NK cells in PB with as most important function cytokine production	[26]
CD56dim NK cells	90% NK cells in PB with as most important function cytotoxicity	[26]
Adaptive NK cells	NKG2C+CD56+CD57 NK cells which are induced by CMV infection. Adaptive NK cells persist long time and can expand upon reactivation of CMV.	[83]
Unconventional T cells	CD3+ lymphocytes (including γδ T cells and NKT cells) with unconventional TCR recognizing peptides, lipids or other molecules representing an altered metabolic state of a target cell.	[30]
Vδ2+ T cells	γδ T cells with the vγ9δ2 TCR. Normally up to 95% of γδ T cells in PB. Vδ2+ T cells recognize derivatives of an altered mevalonate pathway.	[37, 36]
Vδ2- T cells	γδ T cells not expressing a vδ2 delta chain of the γδ TCR. The majority of vδ2- γδ T cells are vδ1+. Vδ2- γδ T cells usually comprise the minority of circulating γδ T cells in PB, but up to 50% of tissue residing γδ T cells. CMV infection can result in proliferation of vδ1+ γδ T cells.	[89, 88]