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Adoptive Cellular Therapy After Hematopoietic Stem Cell Transplantation

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Abstract

Hematopoietic stem cell transplantation (HSCT) remains one of the most effective forms of treatment for patients with high-risk hematologic malignancies. Treatment-related mortality (TRM) associated with this procedure has improved over time and can be further decreased with reduced-intensity conditioning regimens; however, decreasing intensity of the conditioning regimen exposes patients to higher risk of relapse. Disease relapse is now the most important cause of treatment failure, and adoptive cellular therapy (ACT) could potentially be used to decrease relapse post-transplant, and improve transplant outcomes. Donor lymphocyte infusion, as the first form of ACT post-transplant, has been used with limited efficacy and significant risk of developing graft-versus-host disease. Effective ACT using CD19 chimeric antigen receptor (CAR) T-cells for the treatment of patients with advanced B-cell malignancies, raises the question whether administration of such therapies post-transplant could reduce relapse and improve survival in this setting. Moreover, several early phase clinical studies have shown potential beneficial effects of administration of tumor associated antigen-specific T-cells and natural killer (NK) cells post-transplant for patients with myeloid malignancies to decrease relapse and possible improve survival. In this article, we present an in-depth review of ACT after transplantation, as administration of cellular therapy after transplant has the potential to significantly improve the efficacy of this procedure and revolutionize this field.

Introduction

Hematopoietic stem cell transplantation (HSCT) is an important therapeutic procedure with curative potential for both benign and malignant hematologic diseases. Major complications include infections and graft-versus-host disease (GVHD), with significant impact on treatment-related mortality (TRM). Despite progress in decreasing TRM using reduced-intensity conditioning (RIC) regimens, as well as better treatment of infectious complications, improved pre-transplant evaluation of recipients and the use of post-transplant cyclophosphamide (PTCy) for alternative donors transplants, disease relapse has remained relatively unimpacted from the beginning of transplantation, and is now the most common cause of treatment failure, especially for patients with high-risk or advanced disease at transplantation[1,2]. Many approaches have been evaluated for prevention and treatment of relapse, including salvage chemo-immunotherapy, consolidation or maintenance therapy with study drugs, monitoring measurable residual disease (MRD), and adoptive cellular therapy (ACT). Donor lymphocyte infusion (DLI) has been initially studied as treatment or prevention of relapse after HSCT. However, DLI as non-targeted T-cell therapy, is relatively inefficient and carries a significant risk for the development of GVHD. Recently, successful use of CD19 chimeric antigen therapy (CAR) T-cell therapy for patients with an advanced B-cell lymphoid malignancies has sparked interest to use this therapy after transplant, while several studies of natural killer (NK) cell therapy administration and tumor-associated antigen (TAA) specific T cell therapy (TAA-T) after transplant have suggested that it is possible to improve relapse post-transplant for patients with myeloid malignancies. Improving the graft-versus-tumor (GVT) effect without increasing risk of GVHD or major toxicities, has long been the ultimate goal of transplantation, and might come to fruition with the administration of cellular therapy post-transplantation. In this article, we aim to comprehensively review the current state of the new field of cellular therapy after transplantation, with a focus of TAA-T, T-cell receptor (TCR)-gene modified T-cell therapy, CAR T-cell therapy and NK cell therapy (Figure 1).

Donor lymphocyte infusion after stem cell transplantation

Unmodified DLI has been long investigated to treat or prevent disease relapse post-transplant, due to potential of enhancing GVT effect. In 1990, Kolb and colleagues reported that all 3 patients with relapse of chronic myeloid leukemia (CML) after transplantation achieved complete hematologic and cytogenetic remission after DLI with interferon (IFN) alfa. Two of these patients developed GVHD[3]. Subsequent studies demonstrated some efficacy of DLI in patients with relapsed disease after transplantation, including acute myeloid leukemia (AML)[4-6], myelodysplastic syndromes (MDS)[5,6], acute lymphoblastic leukemia (ALL)[5,7], non-Hodgkin lymphoma (NHL)[8], Hodgkin lymphoma (HL)[9] and multiple myeloma (MM)[10-12]. Response rate to therapeutic DLI ranged between 20% to 60% across studies[4,5,7-13]. Low tumor burden was the most relevant factor associated with improving the response to therapeutic DLI[4,6,14]. Therefore, the use of immuno-chemotherapy or hypomethylating agents,

appeared to be important to lower the tumor burden before DLI administration[13,15]. Treatment before DLI with hypomethylating agents or or venetoclax may improved efficacy for patients with relapsed myeloid malignancies[16]. Azacytidine has been used dose in different doses varying from 24mg/m^2 to 100mg/m^2 [17-19], which, in addition to reducing leukemic burden, may also have an immunomodulatory effect [17,19]. An escalationg dose strategy of DLI administration with CD3+ doses starting at 1×10^7 for HLA matched donor transplants and 1×10^6 in haploidentical stem cell transplantation (haplo-SCT), respectively[20,21].

DLI has also been administered as pre-emptive or as prophylaxis therapy. Pre-emptive DLI has been studied post-transplant in patients who developed mixed chimerism (MC) or have MRD positivity. Pre-emptive DLI administration in MC increased the percentage of donor chimerism [22,23], potentially impacting survival[24]. In patients with MRD detected after transplantation, DLI administration showed lower relapse rate and better survival when compared with non-DLI or interleukin (IL)-2 cohort[25,26]. Results from an European group for Blood and Marrow Transplantation (EBMT) study showed that DLI after 6 months from was associated with better survival [27] while in haplo-SCT with PTCy, prophylactic DLI administration had superior 2-year cumulative incidence of relapse ($P = 0.002$), disease-free survival (DFS) ($P = 0.002$) and overall survival (OS) ($P < 0.001$) when compared with therapeutic and pre-emptive DLI[28]. Schmid et al. compared outcomes of 89 patients treated with prophylaxis DLI with a matched pair cohort from the EBMT registry, demonstrating a survival benefit only in high-risk AML patients[29]. Reccomended dose of CD3+ in pre-emptive and prophylaxis purposes in haplo-SCT was 1×10^5 cells/kg[21]. The timing of first dose of pre-emptive or prophylaxis DLI varies across the studies starting early os day 30-60 in high-risk patients[30,31].

Although extensively investigated, there are major limitations associated with using DLI early post-transplant, including limited efficacy, heterogeneity in CD3+ dose, timing of administration, and, most importantly, the significant risk of developing GVHD. The incidence of grade II-IV acute GVHD (aGVHD) and chronic GVHD (cGVHD) occurs in approximately 30%-40% after DLI infusion[32,33]. Several studies aimed to optimize lymphocyte subsets to augment the GVT effect and reduce the risk of GVHD. However, the effectiveness of these approaches remain limited[34,35]. Hence, novel approaches to augment anti-tumor effect using cell therapy administration post-transplant are needed to overcome this unmet clinical need. TAA-T, TCR-gene modified T-cell therapy, CAR T-cell therapy and NK cell therapy could potentially fit this need.

Tumor-associated antigen specific T-cell therapy after stem cell transplantation

TAA specific T-cells are generated by expanding and stimulation of CD8+ cytotoxic T-cells (CTLs) with dendritic cells that present specific tumor antigens in the presence of specific cytokines. The monoclonality of final cell clones is tested by TCR analysis using the polymerase chain reaction method[36].

Single tumor-associated antigen T-cell therapy

The Wilms' Tumor Gene (WT1), a tumor-suppressor gene, encodes a zinc finger transcription factor, which actively engages in cell growth and differentiation[37]. The WT1 gene is overexpressed in leukemic cells at levels 10 times higher than in normal hematopoietic progenitor cells[38], and has been studied as potential target for leukemic cells. Gao and colleagues reported in a pre-clinical study that WT1-specific CTLs could eliminate leukemic cell line and inhibit transformed CD34+ progenitor cells isolated from patients with CML[39]. Chapuis et al. reported a phase I clinical study involving 11 post-allogeneic HSCT patients with AML, MDS, or ALL featuring high-risk characteristics, subsequently treated with donor-derived WT1-specific CTLs using an escalation dose with maximum target dose of 1×10^{10} WT1-specific CTL/m^2. Six of 11 patients had evidence of leukemia before infusion and WT1 expression in leukemia was confirmed in all 10 evaluable patients. A correlation between the reduction in leukemic blasts, the elevation WBC and the concurrent appearance of infused WT1-specific CTLs in the peripheral blood (PB) was observed. Complete remission (CR), relapse and progressive disease (PD) were achieved in 5, 3 and 3 patients, respectively. None of the patients developed de novo GVHD. Furthermore, WT1-specific CTLs generated in the presence of IL-21 were introduced in the last 4 patients. The IL-21-exposed group exhibited higher median CTLs peak level, sustained CTLs presence as detected by IFN-gamma producing cells, long-lasting memory CTLs markers, and increased Ki-67 expression within the CTLs clones, in contrast to the non-exposed group. The median WT1-specific CTLs frequencies detectable in PB mononuclear cells and bone marrow (BM) were 0.45% and 1.31%, respectively ($P<0.001$), suggesting a preference for BM[36].

The incidence of Epstein-Barr virus (EBV) reactivation after transplantation varies between 2% to 50%, depending on patient, disease and transplant-related factors[40,41]. EBV reactivation can be associated with post-transplant lymphoproliferative disorders (PTLD), a post-transplant complication with high mortality[40,41]. To treat or prevent these complications, EBV-specific T-cell therapy has been developed, either form donor-derived or third party sources[42]. The Baylor group reported the long-term efficacy and safety of EBV-specific T-cells for prevention and treatment of EBV reactivation and PTLD. Adoptive immunity against EBV persisted up to 9 years.[43-47]. After a median follow-up of 10.5 years of 114 patients who received EBV-specific T cells, all 101 patients with prophylaxis treatment did not develop PTLD and 11 of 13 PTLD treated patients remained in CR[47]. Doubrovina et al. reported an overall response rate (ORR) of 68% from total 19 PTLD patients treated with EBV-specific T-cell therapy[48]. EBV-associated tumor antigens have also been evaluated as target antigens for T-cell therapy. The latent membrane protein (LMP) of EBV is expressed in most EBV-associated lymphoproliferative diseases[49]. Donor-derived LMP-specific T-cell therapy (LMP-T) has been evaluated in 26 post-allogeneic HSCT patients with EBV+ HL or NHL and EBV-associated NK/T lymphoproliferative disease. Among the 26 patients, 19 received LMP-T as adjuvant treatment, while 7 patients received LMP-T during active disease. At 8-week after infusion, 4 out of the 19 patients in adjuvant therapy

group experienced disease relapse, while 2, 1 and 4, patients with active disease, achieved partial response (PR), stable disease (SD) and PD, respectively. The 2-year event-free survival (EFS) and OS rates were 46% and 68%, respectively. Patients with B-cell lymphoma or those receiving LMP-T as adjuvant therapy while in CR tended to have better survival outcomes. In the responder group, higher frequencies of circulating LMP-specific T cells in the PB and higher specificity for LMP2/EBV antigen in the products were observed. No immediate infusion reactions were reported; however, a case of grade 4 hepatic necrosis, probably related to LMP-T, was identified. New onset grade I skin aGVHD, reactivation of aGVHD and cGVHD occurred in 1, 2 and 3 cases, respectively[50].

Multiple tumor-associated antigens T-cell therapy

The failure of single leukemic antigen-specific T-cell therapy may be related to the downregulation of the specific antigen. Therefore, the potential of a T-cell therapy targeting multiple leukemic antigens has been evaluated. Lulla and colleagues reported on the safety and efficacy of multiple leukemia-specific antigen T-cell therapy (mLST), targeting WT1, PRAME, NY-ESO-1, and survivin, in 25 AML/MDS patients receiving transplantation. Among these patients, 17 were at high-risk of relapse (receiving as adjuvant therapy), while 8 patients experienced relapse post-transplant with active disease. At a median follow-up of 1.9 years, 11 out of 17 patients in the adjuvant therapy group remained in CR, while 6 patients experienced relapse. This was linked to declining of TCR-vβ clone tracking and one of the types of evidence in immune escape, including upregulation of PD-L1, tumor relapse in immune-privileged sites, decreased major histocompatibility complex (MHC) class II and loss of target antigen expression. In the active disease group, 1 CR and 1 PR were noted. There was no report of grade > II aGVHD and extensive cGVHD. No cytokine release syndrome (CRS), neurotoxicity or persistent myelosuppression was reported[51]. Kinoshita et al. conducted a phase I study of mLST targeting WT1, PRAME and survivin involving 23 patients with high-risk (N=12) or relapsed/refractory (r/r) (N=11) post-transplant AML (N=20) or ALL (N=3). Nine out of 11 patients with r/r disease achieved CR prior mLST. The median survival in high-risk and r/r patients was not reached and 255 days, respectively. Leukemia-specific T-cells were detected in PB by TCR sequencing up to 1 year post infusion. Patients who experienced disease relapse within 6 months post-transplant and did not undergo a second HSCT prior to mLST were identified as the poorest prognostic factor, with1-year OS of 25%. All mLST infusions in the study were outpatient based. No infusion reaction, CRS or neurotoxicity was reported. New onset of grade III and grade I/II aGVHD developed in 1 and 3 patients, respectively[52]. Focusing on preventing relapse in post-transplant high-risk ALL patients, Naik and colleagues reported on the safety and efficacy of mLST targeting WT1, PRAME, and survivin in 11 patients with post-transplant ALL. Six out of 8 evaluable patients maintained CR with MRD negativity for a median duration of 46.5 months after the infusion. Tumor-reactive T cells were detected shortly after infusion in CR patients, whereas these cells were absent in 2 relapsed patients. Moderate cGVHD developed in 1 patient with previous history of aGVHD. No significant mLST-related toxicity was reported[53].

Furthermore, mLST with viral-specific activity has been evaluated in a phase I/II study. Modified CD8+ T-cell therapy directed against multiple tumor-associated antigens (WT1, PRAME, NY-ESO-1, RHAMM, proteinase 3), viral-specificity (EBV, cytomegalovirus [CMV], adenovirus) and minor histocompatibility antigens has been infused as prophylaxis treatment for 24 post-transplant hematologic malignancies patients. Disease progression occurred in 5 patients without expansion of mLST. Additionally, 8 patients had reactivations of CMV and/or EBV. No infusion reaction or severe GVHD was reported[54].

T-cell receptor-gene edited T-cell therapy after stem cell transplantation

EBV-specific CD8+, donor-derived T-cells were genetically modified TCR to express a high-affinity WT1, as reported safety and efficacy by Chapuis and colleagues. The manufacturing process included isolating the TCR with high-affinity for WT1 antigen (TCR_{C4}) from normal donors with HLA-A*02:01. Subsequently, this TCR_{C4} was inserted into EBV specific donor CD8+ T-cells using a lentiviral vector. Twelve post-transplant AML patients at high risk of relapse were included in a study assessing the use of TCR-gene edited T-cell therapy for relapse prevention. At a median of 44 months post-infusion without lymphodepletion therapy, the relapse-free survival (RFS) rate was 100%, compared to the comparative group, which showed an RFS rate of 54% (P=0.002). TCR-edited T-cells persisted in PB for at least 1 year in 4 patients. Moreover, expression of phenotypic markers of long-lived memory cells were identified. Grade III aGVHD and cGVHD developed in 1 and 6 patients respectively. In 2 patients treatment was complicated by grade 3 CRS[55].

Chimeric antigen receptor T-cell therapy after stem cell transplantation

CAR T-cells express genetically engineered receptors, which bind to tumor-specific antigens (CD19, B-cell maturation antigen [BCMA], etc.). After tumor cells are recognized, an intracellular signal is activated leading to CAR T-cell proliferation, resulting in killing of the targeted cancer cells[56]. CAR T-cells can be generated either from recipients' mononuclear cells (autologous CAR T-cells) or from the healthy donors (allogeneic CAR T-cells). The use of allogeneic CAR T cells can be associated with development of GVHD; however, the cost and time to administration can decrease for large-scale production (off-the-shelf product). Autologous CAR T-cells may persist longer; however, there are higer production costs and longer time to treat the patients[57]. Most of the studies using CAR T-cell therapy after transplantation to date focus on treatment of disease relapse, especially for B-cell malignancies and plasma cell neoplasms. Studies using adjuvant CAR T-cell therapy with transplantation as prophylaxis or pre-emptive treatment after transplantation are still very limited.

CAR T-cell therapy for the treatment of relapsed disease after allogeneic stem cell transplantation

Several CAR T-cell therapies targeting AML antigens have undergone evaluation in preclinical and phase I/II studies. However, data in post-transplant setting are very limited. Jin and colleagues reported outcomes of C-type lectin-like molecule 1 (CLL-1) CAR T-cells in 10 patients with r/r AML treated with transplantation. CR was achieved in 4 out of 5 treated patients[58]. Other studies which used CAR T-cells targeting CD33, CD38, CD123 and NKGD2L, had a limited number of participants as well as response outcomes[59-62].

Cruz et al. reported the results of a phase I study which included 8 patients post allogeneic HSCT (4 with r/r chronic lymphocytic leukemia [CLL], 2 patients with r/r ALL and 2 ALL patients at high-risk for disease relapse). Patients received donor-derived CD19 CAR combined with multivirus-specific T-cells (VSTs) with CD28 costimulatory domain administered without lymphodepletion. VSTs were generated by stimulated donor PB mononuclear cells with viral antigens from antigen-presenting cells, viral vector and cytokines. Objective antitumor activity was shown in 2 of 6 patients with relapsed disease. CAR-VSTs were detected in the PB between 1-12 weeks. No GVHD occurred in this study[63]. Likewise, Lapteva and colleagues demonstrated that expansion of CD19 CAR-VSTs and B-cell aplasia was observed only in patients with EBV reactivation, whose TCRs were stimulated by viral antigen[64]. Another phase I study with higher number of patients was reported by Brudno and colleagues. Twenty patients (CLL 5, ALL 5, mantle cell lymphoma [MCL] 5, diffuse large B-cell lymphoma [DLBCL] 5) with relapsed disease after HLA-matched sibling (MSD) (N=13) or ≥9 HLA-matched unrelated donor (MUD) (N=7) allogeneic HSCT received donor-derived anti-CD19 CAR T-cells without lymphodepletion. Six patients achieved CR (ALL 4 with negative MRD, CLL 1, DLBCL 1) and 2 patients achieved PR (CLL 1, MCL 1). Patients who responded after CAR T-cells had higher levels of circulating CAR T-cells compared to non-responders (P=0.001). Grade 3-4 CRS developed in 12 patients. None of the patients had a new onset of aGVHD after CAR T-cells infusion. Mild cGVHD was reported in 2 cases[65,66]. In relapsed post haplo-SCT setting, Chen et al. reported outcomes of 6 B-ALL patients with donor-derived anti-CD19 CAR T-cells. Five patients achieved MRD negative CR (83.3%); however, 4 patients relapsed within 2-7 months. Grade 1-3 CRS and grade II-IV GVHD occurred in 5 and 3 patients, respectively[67].

Apart from donor-derived CAR T-cells, recipient-derived (autologous CAR T-cell therapy) after allogeneic HSCT has also been utilized. Park and colleagues studied infusion of autologous anti-CD19 CAR T-cells to treat 53 patients with r/r B-ALL. Nineteen cases (36%) received an allogeneic HSCT. The CR rate in the post-transplant group was 84%, which did not appear to differ significantly from the group without previous transplantation (CR rate 82%). Previous HSCT did not increase risk of developing CRS or neurotoxicity[68]. Importantly, there are 2 case reports of monoclonal CAR T-cell malignancies following piggyBac transposon system-manufactured CAR T-cell therapy from a phase I study involving 10 patients with B-cell lymphoma who relapsed or persisted after allogeneic HSCT. The exact pathogenesis has not yet been definitively identified[69]. Several other studies have shown safety and efficacy of CAR T-cell therapy post-allogeneic HSCT[70-75] (Table 1). Currently, an ongoing phase I clinical trial is enrolling participants for CD19 CAR T-

cell therapy, specifically targeting relapsed B-cell malignancies post-allogeneic HSCT (NCT02050347).

Patients with r/r ALL after transplant can receive FDA-approved CD19 CAR T-cell therapies, including tisagenlecleucel (tisa-cel) and brexucabtagene autoleucel (brexu-cel)[76-78]; however, the number of patients with NHL treated with CD19 CAR T-cell therapies after disease relapse following allogeneic HSCT is still very limited[79-82].

CAR T-cell therapy for the treatment of relapsed disease after autologous stem cell transplantation

Axicabtagene Ciloleucel (axi-cel) is approved by the US FDA for adult patients with refractory to first-line chemoimmunotherapy large B-cell lymphoma (LBCL) or that relapses within 12 months, and r/r follicular lymphoma (FL) after two or more lines of systemic treatment, according to ZUMA-1 and ZUMA-5 studies, respectively[83,84]. Twenty-one of the total 111 patients in the ZUMA-1 trial had disease relapse after autologous HSCT. ORR at 6-month of this group was 76% and 2-year ORR was 52%[83,85]. Recently, Hamadani et al. reported outcomes of 181 patients with DLBCL relapsing after autologous HSCT from the CIBMTR registry. The 1-year cumulative incidence of relapse and non-relapse mortality (NRM) were 39.5% and 4.8%, and 1-year PFS and OS were 55.7% and 73.4%, respectively. The incidence of grade ≥3 CRS and neurotoxicity were 9.9% and 20.9%, respectively. Additionally, a CIBMTR prognostic model, which included Karnofsky performance score < 80%, autologous HSCT prior to CAR-T cell therapy interval <1 year and chemoresistant disease at CAR-T cell therapy, separated patients into 3 risk groups, which correlated with survival. The 1-year PFS and OS in low-, intermediate-, and high/very high-risk groups were 75.8%, 54.3%, 34.9% (P<0.001) and 88.4%, 76.4%, 52.8% (P<0.001), respectively[86].

For r/r DLBCL patients treated with tisa-cel, a phase II study included 56 patients with prior autologous HSCT out of a total of 115 patients (49%). Long-term outcome analysis showed that the 3-month ORR in post-autologous HSCT group was 37.5%[87-89]. Lisocabtagene maraleucel (liso-cel) and brexu-cel have been reported to be safe and have efficacy post autologous HSCT for patients with LBCL and MCL, respectively[82,90].

Currently, there are 2 anti-BCMA CAR T-cell therapies FDA approved for patients with r/r MM after ≥4 prior lines of therapy, including an immunomodulatory agent, a proteasome inhibitor and an anti-CD38 monoclonal antibody[91,92]. Hansen et al. reported real-world data of idecabtagene vicleucel (ide-cel) in 159 patients with 75% of patients who did not meet eligible criteria for KarMMa trial. One hundred thirty-four (84%) patients had a prior autologous HSCT. The 30-day ORR, median PFS and median OS were 78%, 8.5 months and 12.5 months, respectively[93]. According to a phase Ib/II study (CARTITUDE-1) of ciltacabtagene autoleucel (cilta-cel), the total number of enrolled patients that received a prior autologous HSCT was 87 (90%). The ORR was 96.9%, with sCR or CR at 67%. The 12-month PFS and OS were 77% and 89%, respectively. Adverse events with grade ≥3 CRS and

immune effector cell-associated neurotoxicity syndrome occurred in 4% and 2% of the patients, respectively[92].

Adjuvant CAR T-cell therapy after stem cell transplantation

Instead of using a viral vector to generate the CAR, Kebriaei et al. studied a non-viral process using the Sleeping Beauty (SB) system, which included a mobile DNA sequence called the “transposon” and an enzyme for the cutting and insertion of the transposon,“transposase”, to generate CD19-specific CAR T-cells. The SB system modifies the genetics of target cells by utilizing a plasmid incorporated with a transposon vector containing the CAR gene. The transposase precisely binds to specific region and cuts the transposon from the plasmid, subsequently inserting it into the target DNA[94,95]. Seventeen patients with multiply relapsed B-cell ALL, with 12 out of 17 achieving CR, and 9 patients with B-cell NHL, with 4 out of 9 in CR, were enrolled in phase I study, which evaluated safety and efficacy of the SB-modified donor- or patient-derived CD19-specific CAR T-cell therapy as adjuvant therapy following stem cell transplantation. Seven autologous and 19 allogeneic transplants were enrolled. CAR T-cells were administered after stem cell infusion, on average 2 days post-transplant for autologous HSCT and at a median of 64 days post-transplant after allogeneic HSCT. In autologous HSCT group, the 30-month progression-free survival (PFS) and OS were 83.3% and 100%, respectively. For allogeneic HSCT group, the 1-year PFS and OS were 53% and 63%, respectively. Two patients developed aGVHD and 1 patient developed skin cGVHD. CAR T-cells could be identified in PB after infusion at an average of 201 days and 51 days in autologous and allogeneic HSCT, respectively. Apart from enhancing GVT effect and elimination of MRD, polyclonal nature of CAR T-cells may have stimulated immune reconstitution, as a lower rate of CMV reactivation was noted in this study[96].

A phase I study evaluating CD19 memory-enriched CAR T-cells therapy following autologous HSCT in 16 patients with B-cell NHL demonstrated favorable safety outcome, without the occurrence of CRS or graft failure[97]. Wang et al. reported results of a retrospective study comparing autologous HSCT combined with infusion of CD19 CAR T-cells on day 6 (N=21) and autologous HSCT alone (N=46) in patients diagnosed with r/r DLBCL. The group receiving autologous HSCT plus CD19 CAR T-cell therapy had superior outcomes with higher CR rate (71% vs. 33%, P=0.003), better 3-year PFS (80% vs. 44%, P=0.036), and low 3-year relapse rate (15% vs. 56%, P=0.015). Additionally, in the subgroup of patients with SD/PD or relapse, addition of CD19 CAR T-cells after autologous HSCT achieved a higher CR rate (60% vs. 21%, P=0.013). Grade 3 or 4 toxicities, including hypotension, febrile neutropenia, anemia and sepsis, were higher in the study group compared with the control group; however, only 1 patient developed ≥3 grade CRS[98].

Natural killer cell therapy after hematopoietic stem cell transplantation

NK cells are a subtype of lymphocytes, part of the innate immune system. The major functions of NK cells are to target virus-infected cells or malignant cells through direct cytotoxicity and cytokine production[99]. It is hypothesized that NK cell activity is dependent on the balance of signals from both activating and inhibitory receptors on cell surface[100]. Killer immunoglobulin-like receptors (KIRs) are NK cell receptors that can recognize self from non-self by interacting to MHC class I molecules, resulting in inhibitory of NK cell activity. The activation of NK cells occurs as a consequence of either the downregulation or absence of MHC class I proteins, as observed in tumor cells, which results from increased signals through activation receptors. [101]. In addition, NK cells may also target and mediate target cell killing by antibody-dependent cell cytotoxicity. Administration of NK cells after transplant may improve GVT effect without increasing GVHD[102-104], and has been evaluated to improve relapsed after HSCT.

Due to small numbers of NK cells in PB (15% of all circulating lymphocytes)[105], expansion process is likely required to achieve adequate cell doses for therapeutic efficacy. *Ex vivo* expansion with cytokines, mesenchymal stroma or genetically modified artificial antigen presenting cells support have been used to increase the number of NK cells[106-111]. It has been suggested that stimulation of NK cells with IL-2 may increase levels of various cytokines/chemokines, which may result in enhancing therapeutic effect[112]. Most of the studies using adoptive NK cell administration are case series or phase I/II studies, focused on haplo-SCT patients with high-risk disease features. No lymphodepletion therapy or tapering off immunosuppressive agents is required prior to NK cell transfusion.

Single-arm studies of natural killer cell therapy after stem cell transplantation

Yoon et al. studied the feasibility of adoptive donor-derived NK cell therapy after HLA-mismatched related donor transplantation in 14 patients with myeloid malignancies; six out of the 14 patients were in active disease before allogeneic HSCT. NK cells were infused approximately 6–7 weeks after stem cell infusion. No graft failure was reported. Grade II aGVHD developed in 1 patient. Moderate to severe cGVHD developed in 2 patients. 9 out of which 14 patients had disease progression after NK cell infusion[113]. Two subsequent studies from the same group also showed safety and potential efficacy of donor-derived NK cells after transplantation. Cytokine-related toxicities were associated with early infusion of NK cell within 2 weeks compared with infusion in second and third week. Higher NKp30 expression on donor NK cells associated with higher CR and less disease progression[114,115]. Rizzieri and colleagues reported clinical outcomes of NK cell-enriched donor lymphocyte infusions after nonmyeloablative transplantation in 30 patients (MSD 14 cases, mismatched related donor 16 cases). Overall grade II-IV aGVHD was reported in 8 patients. 1-year OS for MSD and mismatch related donor were 43% and 42%, respectively[116]. Lee et al. evaluated safety and clinical outcomes of haploidentical NK cell enriched product infusion at 8 days before HLA-matched stem cell infusion in 21 patients with high-risk myeloid malignancies, and 67% of the patients had active disease before allogeneic HSCT. Overall grade II/III aGVHD were reported in 7 patients. Median RFS and OS were 102 days (range 8-2,251) and 233 days (range 8-2,251), respectively[117]. Our group reported the safety

results from phase I study of membrane-bound (mb) IL-21 *ex vivo* expanded donor-derived NK cells infused after haplo-SCT in 13 patients with high-risk myeloid malignancies. The study revealed no occurrences of infusion reactions, dose-limiting toxicities, or graft failure. Additionally, none of the patients developed grade III/IV aGVHD or cGVHD[118]. A very low relapse rate was observed in this study and suggested that further investigation of this approach is warranted. In a prospective phase II study Stern and colleagues also showed safety of NK cell administration after haplo-SCT in 16 high-risk patients, with 1-year OS at 44±12%. Severity of aGVHD was not associated with the number of infused NK cells; however, it was related with dose of infused T-cells[119].

Comparative studies of natural killer cell therapy after stem cell transplantation

Due to the low number of patients and lack of a comparative group, a matched pair analysis has been applied to analyze treatment outcomes. Uharek and colleagues reported the outcomes of transfer purified CD56+CD3- NK cells at day +2 after haplo-SCT in 25 patients. The subgroup of high-risk AML patients (*N*=16) was matched with the patients from the EBMT database. Adoptive NK cell transfer group had superior 2-year OS at 40%, compared with matched control group at 11% (P=0.02)[120]. Choi et al. compared outcomes of 41 patients with NK cells infusion after haplo-SCT with 31 haplo-SCT patients with a historical control. A lower cumulative incidence of leukemic progression was shown in study group when compared with control group (46% vs. 74%, P=0.038). Engraftment rate, incidence of GVHD and TRM were not different between the 2 groups[114]. Jaiswal and colleagues evaluated early immune reconstitution of T-cells, regulatory T-cells and NK cells in a group of 10 patients with NK cell infusion after haplo-SCT compared with 20 patients with post haplo-SCT as a control group. The incidence of aGVHD was lower in the NK cell group (0% vs. 50%, P=0.01).No differences in incidence of cGVHD, NRM, relapse and OS were observed[121].

Our group reported long-term follow-up of safety and efficacy from a phase I/II study of administration of high doses of mb-IL21 and 41BB *ex-vivo* expanded donor-derived NK cells in 25 patients with myeloid malignancies receiving haplo-SCT. Median follow-up was 24 months (all patients were beyond 1-year post-transplant). Grade II-IV aGVHD occurred in 10 patients and no cGVHD occurred. Compared with case-matched cohort from CIBMTR database, the 2-year relapse rate was 4% vs. 38% (P=0.014), DFS was 66% vs. 44% (P=0.1) and OS was 70% vs. 58% (P=0.34) in study group and control group, respectively, with no significant differences in TRM. DFS was significantly better when patients with donor-specific anti-HLA antibodies were excluded from analysis suggesting that transplant outcomes can be improved by administration of donor-derived NK cell administration post-transplant. Patients who received NK cells had increased production of IFN-gamma and tumor necrosis factor-alpha, and higher doses of NK cells was correlated higher number of NK cell detected early post-transplant, which may result in higher number of functional NK cells early post-transplant and presumed better anti-tumor effect[118,122].

Recently, a phase II randomized clinical trial was reported in patients with high-risk AML and MDS receiving haplo-SCT. Patients were randomized to receive donor-

derived *ex-vivo* expanded, IL-15 and IL-21-activated NK cells after transplantation at day+13 and +20 (*N*=40) or not (*N*=36). The cumulative incidence of disease progression at 30-month in study group was significantly lower than control group (35% vs. 61%, P=0.040). PFS and OS, although better with NK cells were not statistically significantly different, presumably because the study was not adequately powered. No significant differences in engraftment rate, incidence of GVHD or viral reactivation were demonstrated between study patients and control. In a subgroup of active AML or MDS patients, CR rate post haplo-SCT with NK cell therapy was 77% (23/30)[123]. Other clinical studies also showed safety and efficacy of NK cell therapy after transplantation[124-127], including for pediatric solid tumors[128] (Table 2).

Conclusions

ACT after transplantation is a promising treatment strategy to prevent disease relapse for patients with high-risk hematological malignancies, including those with detectable disease at the time of transplantation, which usually have a poor prognosis. However, major limitations of ACT include the complexity in manufacturing processes as well as high expense associated with cell production, which can be applied only in a limited number of institutions, leading to accessibility problems. It is expected, however, that, as more companies are entering this space, the costs assoayed with these therapies will be progressively decrease.

Most of the TAA-T and TCR-edited T-cell therapy studies are phase I/II studies with a small number of participants to date, which may not be sufficiently powered to detect significant differences in survival between patients who received treatment with this type of ACT after transplant versus not. Expansion and persistence of modified T-cells in PB are important factors to achieve long-term remission. The standardization of dosing and infusion timing, and accessibility are current limitations that could potentially be improved over time.

Despite the fact that it has been applied to a category of patients with advanced disease CAR T- cell therapy demonstrated high response rates with manageable toxicities for patients with B-cell malignancies. Application of CAR T-cell therapy post-transplant in patients adequately cytoreduced may prevent disease relapse in patients with B-cell malignancies, possible with limited toxicities due to lower disease burden post-transplant. Therefore, the pre-emptive administration of CAR T-cell therapy for patients with high-risk for relapse should be considered in the future.

Adoptive NK cell therapy is still an investigational treatment, with high potential for improving post-transplant outcomes in patients with high-risk myeloid malignancies, without adverse effects or increased incidence of GVHD. The heterogeneity of NK cell expansion procedures, cell doses, number of infusions and timing of administration are important factors to consider, which may impact overall efficacy of this approach, and should be carefully evaluated in future clinical studies.

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Table 1. Investigational CAR T-cell therapy post allogeneic stem cell transplantation

	Diagnosis	Total *N*, (post HSCT patients)	CAR-T cell	Response, (post HSCT patients)	Toxicities
Davila ML, et al.[70]	B-ALL	16, (4)	CD19/28z CAR-T cells	CR 88%	severe CRS 7, neurotoxicity 4
Lee DW, et al.[71]	B-ALL, NHL	21, (8)	CD19/28z CAR-T cells	CR 66.7%, (50%)	grade 3/4 CRS 28.6%, neurotoxicity 28.6%
Dai H, et al.[72]	B-ALL	9, (3)	CD19/4-1BB CAR-T cells	CR 5/9, (2/3)	CRS 4/9, neurotoxicity 2/9
Curran KJ, et al.[73]	B-ALL	25, (5)	CD19/28z CAR-T cells	CR 75%	grade 3/4 CRS 16%, grade 3/4 neurotoxicity 28%,
Zhang X, et al.[74]	B-ALL	110, (16)	CD19/28z or 4-1BB CAR-T cells	CR 92.7%	grade 3/4 CRS 16%, grade 2/3 neurotoxicity 14%
Liu P, et al.[75]	B-ALL	15, (15)	CD19/4-1BB CAR-T cells	CR 86.7%	grade 3/4 CRS 33.3%, neurotoxicity 33.3%

Abbreviation: B-ALL: B-cell lineage acute lymphocytic leukemia, CAR: chimeric antigen receptor, CR: complete remission, CRS: cytokine release syndrome, HSCT: hematopoietic stem cell transplantation

Table 2. Natural killer cell therapy after stem cell transplantation studies

	Diagnosis	*N*	NK cell	Treatment	Outcome	Toxicities
Ciurea S, et al.[118]	AML, MDS, CML	13	mbIL21 ex vivo–expanded donor-derived NK cells	augmented therapy with transplantation	1 yr DFS & OS: 85% & 92%	No grade 3/4 aGVHD or cGVHD
Ciurea S, et al.[122]	AML, MDS, CML	25	mbIL21 ex vivo–expanded donor-derived NK cells	augmented therapy with transplantation	2 yr RR & DFS: 4% & 66%	Grade II aGVHD 9/24, Grade IV aGVHD 1/24
Devillier R, et	AML, MDS, ALL,	16	donor-derived IL-	prophylaxis	2 yr PFS & OS:	cGVHD 4/16

al.[124]	PMF, NHL, HL, MM		2 -activated NK cells		75% & 88%	
Berrien-Elliott MM, et al.[125]	high-risk AML not in CR	15	donor-derived memory-like NK cells	augmented therapy after transplantation	CR day 28 87% (13/15)	grade I/II aGVHD 10, cGVHD 2, PGF 1
Shaffer BC, et al.[126]	AML, MDS	8	donor-derived NK cells	relapsed or persistent	CR 3/8	no GVHD
Slavin S , et al.[127]	AML, MDS, ALL, MPAL, HL, NHL	8	IL-2 activated purified NK cells	treatment for relapsed after HSCT	CR 3/8	no GVHD
Shah NN, et al.[128]	ultra-high-risk pediatric sarcomas	9	donor-derived IL-15/4-1BBL–activated NK cells	augmented therapy after transplantation	CR 7/9, long-term remission 3/9	Grade II-IV aGVHD 5/9

Abbreviation: aGVHD: acute graft-versus-host disease, ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, cGVHD: chronic graft-versus-host disease, CR: complete remission, DFS: disease-free survival, HL: Hodgkin lymphoma, IL: interleukin, mb: membrane-bound, MPAL: mixed phenotype acute leukemia, MDS: myelodysplastic syndromes, MM: multiple myeloma, NHL: non-Hodgkin lymphoma, PGF: primary graft failure, PMF: primary myelofibrosis, RR: relapse rate

Figure 1