Haploidentical hematopoietic transplantation without T-cell depletion: current status and future perspectives

Lei Gao, Xi Zhang

Department of Hematology, Xinqiao Hospital, Third Military Medical University, Chongqing 40037, China
Correspondence to: Xi Zhang. Department of Hematology, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, China. Email: zhangxxi@sina.com.

Abstract: Human leukocyte antigen (HLA)-haploidentical hematopoietic stem cell transplantation (HLA-haplo HSCT) without T-cell depletion has tremendously progressed over the past 20 years and has become a feasible treatment option for leukemia patients without an HLA-identical sibling donor. Advances in conditioning regimens, graft manipulation, and pharmacological graft-versus-host disease (GVHD) prophylaxis have reduced the risk of fatal graft failure and severe GVHD, two of the most serious complications of traversing the HLA barrier. According to clinical observations, killer immunoglobulin-like receptor (KIR) mismatch and donor-specific anti-HLA (DSA) antibodies—negative status play potential roles in reducing the risk of GVHD and graft failure following HLA-haploidentical SCT. New strategies to improve transplant outcomes include donor lymphocyte, NK cell and selected T-cell subset infusion, mesenchymal stem cell (MSC) co-transplantation and interleukin-2 (IL-2) application. Future challenges remain in improving post-transplant immune reconstitution and finding the best approach to reduce the incidence and severity of GVHD while simultaneously preserving the graft-versus leukemia effect to prevent the recurrence of underlying malignancy.

Keywords: Hematopoietic stem cell transplantation; haploidentical; graft-versus-host disease (GVHD); relapse

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Human leukocyte antigen (HLA)-haploidentical hematopoietic stem cell transplantation (HLA-haplo HSCT) is an alternative transplant option for the majority of patients with hematological disease and is available without search or acquisition costs to the patient (1-7). Over the past 2 decades, many haploidentical transplantation protocols, including T cell-replete and T cell-depleted (TCD) haplotype HSCT, depending on whether the allografts have been engineered in vitro, have demonstrated promising clinical outcomes (8-10). Several transplant centers have reported success with the transplantation of TCD peripheral blood stem cells (PBSCs) with a low rate of graft-versus-host disease (GVHD); however, serious infections and disease relapses resulting from delayed immune reconstitution remain the 2 most frequent causes of mortality after allogeneic HSCT, particularly in patients who receive extensive TCD CD34+ cell megadose allografts (5,11-14). Therefore, many centers actively pursue bone marrow transplantation without T-cell depletion using unmanipulated haploidentical transplant protocols (6-9,15-17). The approaches used include anti-thymocyte globulin (ATG) preparative regimens for partial in vivo T-cell depletion, granulocyte colony-stimulating factor (G-CSF)—primed grafts to polarize the T-cell response to a Th2-type pattern, and high-dose post-transplant cyclophosphamide (Cy) to preferentially deplete alloreactive T cells (17-21). In this review, we summarize advances in the development of new conditioning regimens, improvements in GVHD prophylaxis, the incidences of invasive fungal disease (IFD) and cytomegalovirus (CMV) infection after transplantation, and strategies to improve transplant outcomes. In addition, we discuss the future
directions of unmanipulated HLA-haplo HSCT.

**Conditioning regimen of unmanipulated haploidential transplantation**

**Transplant procedures using a myeloablative conditioning regimen**

Peking University in China investigated the combination of G-CSF-primed bone marrow and peripheral blood with intensive immunosuppression using ATG for *in vivo* T-cell depletion. Other drugs in the conditioning regimen included cytosine arabinoside (Ara-C), busulfan (Bu), Cy, and semustine, and GVHD prophylaxis included cyclosporine (CsA), mycophenolate mofetil (MMF), and short-course methotrexate (MTX) (18). In the first report, the authors compared the clinical outcomes of HLA-haploidential transplantation with those of HLA-matched sibling transplantation without ATG administration. The cumulative incidence of grade II to IV acute GVHD (aGVHD) was 32% and 40% in matched sibling and haploidential transplants, respectively (P=0.13). Surprisingly, treatment-related mortality (TRM) was similar (14% vs 22%), as were the relapse rate and overall survival (OS) (13% vs 18% and 72% vs 71%, respectively). In an updated report, Huang et al. reported encouraging clinical outcomes in 145 Ph+ acute lymphoblastic leukemia patients and 450 acute myeloid leukemia patients who underwent unmanipulated HLA-haplo HSCT with the following conditioning regimen: total body irradiation (TBI) + methyl-N-(2-chloroethyl)-N-cyclohexyl-N-nitrosourea (Me-CCNU) + Ara-C + Cy + ATG and Bu + Me-CCNU + Ara-C + Cy + ATG. The 3-year probability of leukemia-free survival (LFS) in AML patients was 74%, and the 5-year probability of LFS in Ph+ ALL patients was 65.8% (15,22). The results indicate that unmanipulated HLA-haplo HSCT produces outcomes similar to those of identical sibling donor HSCT.

A multicenter randomized controlled trial in southwest China studied the outcomes of unmanipulated HLA-haplo HSCT in high-risk AML patients using a combination of G-CSF priming during the chemotherapy conditioning regimen. G-CSF at 5 mg/kg daily was administered subcutaneously on days -10 to -7 of the chemotherapy-based conditioning regimen, which comprised CCNU 200 mg/m² orally on day -9, high-dose Ara-C (4 g/m²) daily on days -8 to -7, Bu 3.2 mg/kg daily on days -6 to -4, and Cy 1.8 g/m² daily on days -3 to -2 (23). Based on the known activities of G-CSF (24,25), the use of G-CSF-mobilized PBSCs and enhanced leukemic chemosensitization with the combination of high-dose Ara-C plus G-CSF priming in the conditioning regimen (25-28) is expected to decrease GVHD and leukemia relapse.

**Transplant procedures using non-myeloablative (NMA) conditioning regimens**

Although the incidences of graft failure and GVHD have been reduced through the use of myeloablative conditioning regimens, these procedures remain associated with high regimen-related toxicity and TRM, mainly due to infectious complications, thereby limiting the applicability of haploidential transplantations to the majority of patients. Therefore, the use of NMA conditioning regimens has been tested in multiple studies, with encouraging results (29).

Most NMA conditioning regimens incorporate the highly immunosuppressive drug fludarabine (Flu) (28). Studies from Tubingen, Germany and from Duke University in the United States have combined Flu-based conditioning with *in vivo* TCD using OKT3 (30) or CAMPATH (31) to enable the engraftment of HLA-haploidential stem cells. These regimens were associated with acceptable non-hematologic toxicities and sustained donor cell engraftment in patients up to 66 years of age. OS at 1 year after transplantation ranged from 31% to 37% (32,33), establishing the feasibility of HLA-haploidential HSCT after NMA conditioning.

Recently, a prospective, multicenter phase I/II study of unmanipulated, reduced-intensity HLA-haplo HSCT using a low dose of ATG and steroid was conducted in 5 institutions in Japan (34). The study enrolled 34 patients with hematologic malignancies who exhibited advanced stage disease or who were at a high risk of relapse at the time of transplantation. The conditioning regimen comprised Flu, Bu, and ATG (Fresenius, 8 mg/kg), and GVHD prophylaxis comprised tacrolimus (Tac) and methylprednisolone (1 mg/kg). Thirty-three patients achieved donor-type engraftment. The cumulative incidences of grade II to IV aGVHD and extensive cGVHD were 30.7% and 20%, respectively. Fourteen patients (41.2%) exhibited relapse. The cumulative incidence of TRM at 1 year after transplantation was 26.5%. The survival rates at 1 year for patients with complete remission (CR)/chronic phase (n=8) and non-CR (n=26) statuses before transplantation were 62.5% and 42.3%, respectively. This transplantation protocol is safe and feasible if a suitable
donor is not available in a timely manner.

**GVHD prevention of unmanipulated haploidentical transplantation**

**G-CSF-primed bone marrow (G-BM) and peripheral blood stem cells (G-PB)**

G-CSF can induce T-cell hyporesponsiveness and a skewing toward a Th2 phenotype through an increase in plasmacytoid dendritic cells and a decrease in CD28-CD80/86 signaling (35-38). Based on these findings, the Chinese researchers Huang et al. developed a HLA-haplo HSCT protocol using myeloablative conditioning, intensified immunologic suppression with ATG, and a donor graft comprising G-CSF-primed bone marrow and PBSCs (17,18,23,35,38-40). Their most recent update included 450 acute leukemia patients (15), 231 (51.3%) of whom were assigned to undergo unmanipulated HLA-haplo HSCT. In this group, donors were treated with G-CSF 5 mg/kg/day subcutaneously; BM cells were harvested on the fourth day of G-CSF, and PBSCs were collected on the fifth day. GVHD prophylaxis included CsA, MMF, ATG and methotrexate. The cumulative incidence of grades II to IV and III to IV aGVHD were 36% and 10%, respectively. The cumulative incidence of cGVHD was 42% at 1 year, and the 3-year disease-free survival (DFS) and OS rates were 74% and 79%, respectively (15).

**Short-term Tac**

The calcineurin inhibitor Tac possesses a 100-fold higher in vitro inhibitory activity against T cells compared with CsA and has been used for GVHD prophylaxis both alone and in combination with other immunosuppressive agents in patients undergoing HLA-matched HSCT (41,42). A low dose of Tac has been shown to induce functional regulatory T cells (Tregs) (43). Both Tac and MMF dampen Th1-related gene transcription and preserve Treg/Th2 phenotypes (44). Our previous retrospective single-arm studies demonstrated the feasibility of the decreasing stepwise addition of Tac in GVHD prophylaxis in patients undergoing HSCT with HLA-haplo donors. However, the long-term use of Tac led to an increased incidence of infection, especially CMV infection (45). Based on our previous study, we tested a short-term Tac protocol combined with MTX and MMF compared with a classical CsA + MTX + MMF for GVHD prophylaxis in patients undergoing HSCT from HLA-haplo donors. The 100-day cumulative incidences of grade III to IV aGVHD in patients receiving the short-term Tac regimen vs. the CsA regimen were 29.1% vs. 50.0% (P=0.005) and 3.6% vs. 13.5% (P=0.027), respectively. No significant differences were found between the two groups in the incidences of cGVHD, relapse, and CMV infection or in DFS and OS. Lymphocyte subset analysis revealed that the number of T cells decreased to a lesser extent in the short-term Tac regimen within 3 months of transplantation (unpublished data). Thus, the short-term addition of Tac for GVHD prophylaxis in patients undergoing HLA-haplo HSCT is associated with a low incidence and decreased severity of aGVHD and does not increase the incidences of relapse and CMV infection.

**Post-transplantation cyclophosphamide (PT/Cy)**

PT/Cy is an attractive approach for crossing the HLA barrier in allo-HSCT because the treatment is cheap and strikingly effective and requires no special expertise beyond intravenous (IV) chemotherapy administration. A number of mechanisms likely contribute to the establishment of bi-directional tolerance by PT/Cy, and these multistep process likely proceeds through several distinct and sequential phases. The first step includes the selective killing of proliferating alloantigen-stimulated T cells. Several lines of evidence support the differential sensitivity of naive T cells versus effector (Teff)/memory T cells to Cy-mediated killing. The relative resistance of donor Teff/memory T cells to PT/Cy, as demonstrated in mice, may contribute to the overall long-term reconstitution of peripheral T-cell pools and immune competence (46). These processes are important, given the slow recovery of thymic and T-cell functions after transplantation. The second step in the process of PT/Cy-induced tolerance includes the central deletion of donor HSC-derived anti-host T cells in the thymus. This mechanism, which is advantageous because it cannot be broken with TLR ligation and/or infections, is essential for maintaining lifelong tolerance after allografting. The existence of intrathymic clonal deletion after PT/Cy was also confirmed using superantigen-disparate murine allo-combinations, which is a well-studied system used to explain self-tolerance (46). In the final key step of PT/Cy tolerance, a late breakdown of clonal deletion and an emergence of regulatory or suppressive T cells occur (20). The notion that CD4+ Tregs may also contribute to Cy-induced tolerance is consistent with recent observations that
Foxp3+Tregs are critical for tolerance induction in MHC-matched and MHC-mismatched models using anti-T-cell abs and co-stimulatory blockade (47).

Based on promising preclinical results, clinical trials of HLA-haplo HSCT using PT/Cy have been performed at many transplant centers. Luznik et al. (48) administered 100 mg/kg PT/Cy over days +3 and +4 after a reduced-intensity conditioning (RIC) regimen. The cumulative incidences of grades II–IV and grades III–IV aGVHD by day 200 were 34% and 6%, respectively, and the cumulative incidence of extensive cGVHD was 5%. Actuarial OS and event-free survival (EFS) at 2 years after transplantation were 36% and 26%, respectively. PT/Cy as GVHD prophylaxis was initially developed for haploidentical BMT after RIC, but several recent small studies have extended the approach to myeloablative conditioning and to the use of PBSCs as the graft source. Recently, Bacigalupo et al. (49) reported 148 patients with hematologic malignancies who received an unmanipulated HLA-haplo HSCT followed by PT/Cy. All patients underwent myeloablative conditioning comprising thiotepa + Bu + Flu or TBI + Flu. GVHD prophylaxis comprised PT/Cy on days +3 and +5, CsA (from day 0), and MMF (from day +1). The cumulative incidences of grades II–IV and III–IV aGVHD were 24% and 10%, respectively, and the incidence of moderate to severe cGVHD was 12%. The actuarial 22 months OS was 77% for CR1 patients, 49% for CR2 patients and 38% for patients grafted in relapse (P<0.001). The study suggests that a myeloablative conditioning regimen followed by unmanipulated HLA-haplo HSCT with PT/Cy results in a low risk of acute and chronic GVHD and in encouraging TRM and overall survival rates.

**IFD and CMV infection after transplantation**

**IFD incidence after transplantation**

Due to the poor post-transplant immune reconstitution for HLA-haplo HSCT with ex vivo TCD, IFD is an important cause of morbidity and infection-related mortality (50). According to a study of 205 patients from Perugia (51), the risk of invasive aspergillosis (IA) after haploidentical transplantation with TCD was 2.7-fold higher than that after HLA-matched transplantation. Unmanipulated HLA-haplo HSCT included ATG preparative regimens for partial in vivo T-cell depletion, G-CSF-primed grafts to polarize the T-cell response to a Th2-type pattern, post-transplantation rapamycin to favor regulatory T-cell population development, or high-dose post-transplant Cy to preferentially deplete allo-reactive T cells (15,18,22,45,48,49). Huang et al. (52) reported a head-to-head comparative study performed at a single center to assess whether the above-described strategies helped to reduce the IDF incidence. Of the 1,042 consecutive patients enrolled, 390 received the HLA-matched HSCT, and 652 received unmanipulated HLA-haplo HSCT. IFD was evaluated according to the revised EORTC/MSG criteria, and only proven and probable cases were included. A total of 61 (5.8%) patients had IFD, including 15 proven cases and 46 probable cases. The IFD incidence after unmanipulated HLA-haplo HSCT was significantly higher than that after HLA-matched transplantation (7.1% vs. 3.3%, respectively; P<0.007). IFD occurred later in patients receiving HLA-matched transplantation compared with patients receiving unmanipulated HLA-haplo HSCT (141.5 vs. 23 days, respectively; P=0.04). In multivariate analysis, aGVHD grades III to IV, extensive cGVHD and haploidentical transplantation were identified as significant risk factors associated with IFD. The prognosis of IFD was not associated with the type of transplantation. These results demonstrate that more active IFD prophylactic strategies should be adopted in the setting of unmanipulated HLA-haplo HSCT.

**CMV infection after transplantation**

CMV infection after HLA-haplo HSCT continues to adversely affect transplant outcomes (53-55) despite the use of prophylactic or preemptive treatment (56). Peking University researchers developed the GIAC protocol for HLA-haplo HSCT and observed that patients undergoing HLA-haplo HSCT had a higher 100-day cumulative CMV antigenemia incidence compared with a matched group (65% versus 39%), whereas the CMV-associated interstitial pneumonia incidence was the same in both groups (17% in both) (18). In Japan, Kurokawa et al. (57) conducted HLA-haplo HSCT on 66 adults with hematologic malignancies using RIC without TCD. CMV antigenemia occurred in 45 of 57 evaluable patients at a median of 19 days after transplantation. CMV-related diseases were diagnosed in 3 patients, and one patient died of CMV colitis.

Immune reconstitution of the immune subsets likely has the greatest impact on clinical outcomes after HLA-haplo HSCT. In healthy CMV-seropositive individuals, high frequencies of CMV-specific CD4+ and CD8+ T cells that mediate the control of viral reactivation can be detected (58).
Both the quantity and quality of CMV-specific T cell recovery are essential for the immune control of CMV infection following HSCT. A strategy of deferred antiviral therapy based on the presence of a detectable functional CMV-specific T cell response at the time of CMV DNAemia documentation was clinically applied, allowing for the sparing of antiviral treatment in transplant patients (59). The process of immune reconstitution is influenced by patient- and transplant-related factors, including donor and patient ages, primary disease, transplant type, conditioning regimen, stem cell source, HLA disparity, GVHD, and infection (60). A recent study indicates that the selection of a young donor, the use of stem cells derived from PBSC or G-BM/PB, the occurrence of subclinical CMV reactivation while on antiviral therapy, the avoidance of GVHD, and the use of a decreased steroid dose can improve CMV-specific immune reconstitution (61).

### Strategies to improve transplant outcomes

#### Donor selection

Most patients have more than 1 potential haploidentical donor, and various factors have been implicated in selecting the most suitable donor for HLA-haplo HSCT. Among these factors, killer immunoglobulin-like receptor (KIR) mismatch and donor-specific anti-HLA (DSA) antibodies are the main factors to be considered.

KIR mismatch between recipients and donors has been associated with improved outcomes after HLA-haplo HSCT in several studies (62,63). Ruggieri et al. (62) reported improved graft rejection, GVHD, and disease relapse rates among AML patients who received stem cells from donors with KIR mismatches in the GVH direction compared with those who did not. More recently, Symons et al. (63) reported similar results in a cohort of 86 patients with various hematologic malignancies who underwent unmanipulated HLA-haplo HSCT with non-myeloablative conditioning and PT/Cy with improved NRM, OS, and EFS among those transplanted with KIR-mismatch donors compared with those without KIR-mismatch donors (63). Although NK cell alloreactivity likely plays a role in the success of HLA-haplo HSCT, further studies are required to better define the role of KIR mismatch in donor selection and to exploit NK alloreactivity to improve post-transplantation outcomes.

The presence of DSA by the cross-matching technique is considered an absolute contraindication to the use of that donor due to the indicated increased risk of graft failure (64). Three assays are available for measuring the presence of antibodies against donor HLA molecules: (I) lymphocytotoxic cross-matching; (II) flow cytometric crossmatching; and (III) a solid-phase immunoassay (SPI) using fluorochrome-conjugated beads coated with single HLA molecules. The SPI is the most sensitive test for DSA (65). Recently, Ciurea et al. (66) analyzed 122 haploidentical transplant recipients prospectively tested for DSA. Retrospective analysis to detect C1q binding DSA (C1q + DSA) was performed on 22 allo-sensitized recipients. The presence of C1q+DSA was labeled as C1q positive, and the absence of C1q+DSA was labeled as C1q negative. Of the 122 patients, 22 (18%) had DSA, 19 of whom were women (86%). Seven patients with DSA (32%) rejected the graft. The median DSA level at the time of transplant for patients who failed to engraft was 10,055 mean fluorescence intensity (MFI) vs. 2,065 MFI for those who engrafted (P=0.007). According to this study, patients with high DSA levels (>5,000 MFI) appear to be at a much higher risk of primary graft failure. The presence of C1q + DSA should be assessed in allo-sensitized patients before HSCT, as reducing C1q + DSA levels might prevent engraftment failure in HSCT.

#### Donor lymphocyte infusion (DLI)

A few studies have investigated DLI after HLA-haplo HSCT. Lewalle et al. (67) proposed that 10⁵ cells/kg should be the starting dose for DLI in patients undergoing HLA-haplo HSCT. In a study conducted in Israel, 28 patients received prophylactic (n=6) or therapeutic DLI (n=22) in doses ranging from 1x10⁵ to 1.5x10⁶ T cells/kg (68). A clinical response to therapeutic DLI was observed in 6 of 22 (27.3%) patients; a greater tumor burden was correlated with a lower response. Huang et al. (69) administered G-CSF-primed DLI to prevent disease recurrence. The authors analyzed the data of 88 patients with advanced-stage acute leukemia after unmanipulated HLA-haplo HSCT whose treatment did (n=61) or did not (n=27) include G-CSF-primed DLI. The 2-year cumulative incidences of relapse in patients receiving prophylactic DLI vs. those not receiving prophylactic DLI was 36% and 55% (P=0.017), respectively. Estimated OS and EFS at 3 years for patients receiving or not receiving prophylactic DLI were 31% vs. 11% and 22% vs. 11%, respectively (P=0.001 and 0.003). According to multivariate analysis, the use of prophylactic DLI after transplantation was an
independent prognostic factor for relapse. Subsequently, the
authors retrospectively compared the anti-leukemic effects
of chemotherapy alone and chemotherapy followed by
modified DLI in patients with relapsed acute leukemia after
unmanipulated HLA-haplo HSCT. In patients receiving
chemotherapy followed by modified DLI, the complete
remission rate was significantly higher (64.0% vs. 12.5%,
P=0.000), the incidence of relapse was significantly lower
(50.0% vs. 100.0%, P=0.000), and DFS was significantly
improved (36.0% vs. 0.0%, P=0.000) compared with
patients receiving chemotherapy alone (70). Zhou et al. (71)
reported the long-term follow-up of 10 HLA-haplo HSCT
patients infused with inducible human caspase 9-modified T
(iC9-T) cells in vivo. These patients displayed immediate
and sustained protection from major pathogens, including
CMV, adenovirus, BK virus, and Epstein-Barr virus in
the absence of acute or chronic GVHD, supporting the
beneficial effects of this approach to immune reconstitution
after haplo-HSCT.

Donor NK cell infusion

NK cell allo-reactivity may be exploited to improve the
efficacy and safety of HLA-haplo HSCT. NK cells are
thought to recognize their targets through both inhibitory
and activating receptors. At Duke University Medical
Center, 14 matched and 16 mismatched transplanted
patients received a total of 51 NK cell-enriched DLIs.
Long-term responders with multiple NK cell-enriched
infusions and improved T cell phenotypic recovery
exhibited improved durations of response and OS (72).
Based on this exciting result, several studies evaluated the
feasibility of NK cell infusions after HLA-haplo HSCT
to utilize innate immunity against different tumors.
Recently, the success of clinical-grade NK cell purification
demonstrated that NK cell infusion is a promising method
for prophylaxis and/or therapy for relapse after HLA-haplo
HSCT (73). Yoon et al. (74) reported a series of 14 patients
with acute leukemia or myelodysplastic syndromes who
were infused with donor NK cells derived from CD34+
 hematopoietic cells 6 to 7 weeks after TCR HLA-haplo
HSCT. No acute side effects occurred, and 4 patients
developed cGVHD. Four patients were alive and disease-
free 18 to 21 months post-transplantation. Two patients
with active leukemia who received an NK cell infusion
did not respond. Recently, Choi et al. (75) reported a
series of 41 patients with hematologic malignancies who
underwent HLA-haplo HSCT after reduced-intensity
conditioning. The NK cells were infused into patients
twice at 2 and 3 weeks after HSCT at an escalating dose
from 2 to $10^7$ cells/kg of body weight or available cells.
At all dose levels, no acute toxicity was observed after NK
cell infusion. No significant differences were found in the
cumulative incidences of major HSCT outcomes, including
engraftment, grade II to IV aGVHD, moderate to severe
cGVHD, and TRM, in patients who received HLA-haplo
HSCT and subsequent donor NK cell infusion compared
with the same conditioning regimen but without high-
dose NK cell infusion. However, a significant reduction
was observed in leukemia progression (46% to 74%), and
post-transplantation NK cell infusion was identified as an
independent predictor of decreased leukemia progression
(hazard ratio, 0.527). Prospective studies are required to
explore the use of NK cells post-HLA-haplo HSCT.

Selected T-cell subset infusions

As an alternative approach to unmodified donor T cell
infusions, several groups have tested the feasibility of donor
T-cell infusions that were depleted of allo-reactive T cells
(76-79) or that were introduced with a herpes simplex
thymidine kinase suicide gene, allowing the allo-reactive
T cells to be killed in the case of severe GVHD (80). In
the haploidentical setting, Amrolia et al. (81) used an anti-
CD25 immunotoxin to deplete allo-reactive lymphocytes
and infused allo-depleted donor T cells after ex vivo TCD
haploidentical transplantation. Viral-specific responses
were observed in 4 of 6 evaluable patients receiving higher
doses of T cells with a low incidence of severe GVHD.
Interestingly, loss of the HLA haplotype that differed
from the donor’s haplotype in leukemic cells was recently
reported in patients who relapsed after haploidentical
transplantation and donor T cells infusion, indicating that
escape from donor alloreactive T-cell killing represents one
mechanism underlying leukemia relapse (82). Therefore, the
status of mismatched HLA on relapsed leukemic cells may
require examination before the utility of additional donor
T-cell infusions is explored. In a study from Perugia (83),
28 patients with high-risk hematologic malignancies
received myeloablative conditioning followed by $2\times10^7$/kg
freshly isolated donor Tregs. Four days later, patients
received $1\times10^6$ conventional T lymphocytes (Tcons)
and $10\times10^6$ highly purified CD34+ cells from full
haplootype donors. Although no post-transplantation
immunosuppression was administered, the incidences of
aGVHD and cGVHD were extremely low. Interestingly, the pattern of post-transplantation immune reconstitution markedly differed from that of standard TCD HLA-haplo HSCT, with the rapid recovery of T-cell subpopulations, the development of a wide T-cell repertoire, and high frequencies of antigen-specific CD4+ and CD8+ lymphocytes. Significantly fewer CMV reactivation episodes and no CMV disease-related deaths occurred. Their innovative method of infusing regulatory T cells enabled the administration of larger amounts of mature T cells, which may lead to earlier immune reconstitution and improved outcomes.

**Application of mesenchymal stem cells (MSCs)**

Numerous studies have demonstrated that MSCs exhibit profound immune-modulatory functions both in vitro and in vivo (84). MSCs modulate the proliferation, activation, and maturation of T and B lymphocytes in vitro in a dose-dependent and time-limited manner (85,86). In adult patients undergoing transplantation from an HLA-identical sibling, MSC infusion is safe and possibly accelerates hematopoietic recovery and reduces the incidences of both acute and chronic GVHD. Lazarus et al. (87) previously demonstrated that the co-transplantation of MSCs with HSC is feasible and appears to be safe, without immediate or late MSC-associated transfusion toxicities. The sustained donor engraftment observed in patients treated with MSCs compared favorably to the risk of rejection observed in HLA-haplo HSCT recipients. Ball et al. (88) co-transplanted donor-derived MSCs in 14 children undergoing TCD haploidentical transplantation. None of the patients who received MSCs experienced either an adverse reaction or a graft failure. Additionally, Zhou et al. (89) and Weng et al. (90) also suggested that the transfusion of in vitro-expanded MSCs is a safe and effective salvage therapy for patients with steroid-resistant cGVHD. Our groups evaluated the safety and cGVHD prophylaxis efficacy of discontinuous MSC infusion in patients following unmanipulated HLA-haplo HSCT. We found decreased 2-year cumulative incidences of both cGVHD and severe lung cGVHD. After MSC transfusion, the number of NK cells decreased, but the number of memory B lymphocytes and the ratio of Th1:Th2 increased (unpublished data).

**Future directions**

Over the past several years, unmanipulated HLA-haplo HSCT has been adopted by increasing numbers of transplant centers worldwide (9,15,23,33,94-96). Unmanipulated HLA-haplo HSCT provides an opportunity for patients to benefit from HSCT when an HLA-matched donor is not available. The final goal of HLA-haplo HSCT is to successfully overcome the HLA barrier and capture an optimal GVL effect with moderate GVHD. Several novel approaches exist that may be promising in the future: (I) selective but effective allo-depletion, which facilitates successful donor engraftment and improved post-transplant immune reconstitution while reducing the incidence of GVHD; (II) improved DLI to achieve a GVL effect without or with limited GVHD; (III) adoptive cellular immunotherapy with cells such as Tregs, NK/Tregs, MSCs and donor-derived NK cells as well as third-party cell infusion; and (IV) pathogen- or leukemia-specific donor-derived T cell infusion, which could represent an additional approach for preventing opportunistic infection and reducing the leukemia relapse rate after HLA-haplo HSCT.

**Application of interleukin-2 (IL-2)**

IL-2, a pleiotropic cytokine, plays a central role in immune responses. The administration of IL-2 early after HSCT during minimal residual disease might reduce the relapse rate and increase the immunocompetence of these patients (91). This effect could be due to a lymphoid orientation of primitive CD34+CD105+ cells expressing high-affinity IL-2 receptors. Thus exogenous IL-2 might lead to an enhancement of the graft-versus leukemia (GVL) effect (92). Liu et al. (93) studied 19 patients with acute lymphoblastic malignancy, including 6 patients receiving allografts from haploidentical donors, who underwent IL-2 treatment for a high probability of disease recurrence after allo-HSCT. After a median follow-up of 6 months (range, 3-19 months), 14 of 15 evaluable patients in the cohort were disease free (93.33%), whereas one patient in the ‘high-risk’ pre-transplantation category relapsed. The toxicities from IL-2 mainly included fever, pain, redness and swelling at the injection site. The authors concluded that the subcutaneous administration of low-dose IL-2 for 100 days or more could represent a safe and effective strategy for preventing relapse in acute lymphoblastic malignancy patients with a high risk of recurrence after unmanipulated allo-HSCT.

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**Footnote**

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