Advances and Controversies in
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Haploidentical Transplantation

Concepts & Clinical Application

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I would like to dedicate this book to my wife, Ana and my daughter, Stefania, who have always been supportive of my academic endeavors and have sacrifice time together to help me advance the science in hematopoietic stem cell transplantation.

—Stefan O. Ciurea

Preface

The resurgence of haploidentical transplantation over the past decade is one of the most important advances in the field of hematopoietic stem cell transplantation and a major step forward in our quest to offer this procedure to all patients in need.

Extensive research in this field has led to the development of several approaches to perform haploidentical transplants. This has allowed application of this type of transplant to an ever-increasing number of patients, with an increasing number of diseases and different ages, from the children to more than 70 years old, and with outcomes similar to HLA matched transplants.

Although much improved, much more needs to be done. Future directions will explore further improvements in treatment related toxicity, control of viral reactivation, as well as decrease in rate of disease relapse, which is now the most important cause of treatment failure.

We believe that, the next 10 years will bring a safer procedure with a higher likelihood of success, extended to patients with nonmalignant diseases and possibly to solid tumors, which will be performed routinely world wide.

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Massimo F. Martelli, Yair Reisner, and Richard E. Champlin

1.1 Introduction

Since Edward Donnall Thomas first performed his seminal studies in the mid-1970s [1, 2], allogeneic hematopoietic cell transplantation (HCT) has developed from a last resort into a routine procedure that cures numerous cancers. Clinical observations [3, 4] and experimental models [5, 6] established that the allogeneic immune system played a crucial role in eradicating malignancy, i.e., the graft-*versus*-leukemia effect (G*v*L), across all genomic subsets with similar relative potency. Over the years clinical approaches to allogeneic HCT have improved, thanks to insights from basic science research. Major progress was made in conditioning regimens, prevention of graft-*versus*-host disease (G*v*HD), and diagnosis and treatment of infectious complications, all of which reduced transplant-related mortality (TRM). The greatest step forward was arguably the use of an alternative source of hematopoietic $CD34^+$ cells. Unrelated cord blood (CB) and HLA-haploidentical with two- or three-loci-mismatched HCTs are feasible options that have emerged for patients without a HLA-matched donor and/or in urgent need of transplantation. International registries include approximately 25 million adult hematopoietic graft donors and with high-resolution typing MUDs are selected on the basis of matching

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for HLA-A, -B, -C, -DRB1, and/or -DQB1 at the molecular level. The probability of finding an eight of eight HLA-matched donor in the registries varies, however, with ethnic group, ranging from 75% for Caucasians to 16–19% for Africans [7]. Although seven of eight or six of eight HLA-matched donors can be found for most patients, even one mismatched allele can compromise transplant outcomes [8, 9]. Furthermore, the time lapse to donor identification and obtaining the graft may lead to relapse and failure of intention to treat patients who urgently need a transplant.

The obvious advantage of HLA-haploidentical HCT (haplo-HCT) is prompt donor availability of one or more family members. The best donor can be selected on the basis of age, cytomegalovirus (CMV) status, and natural killer (NK) cell alloreactivity. If a patient rejects the graft, a second transplant from the same donor or another donor within the family circle is immediately feasible, and, if the patient needs donor-derived cellular therapies, they are easily accessed. Looking back over the years since the haplo-HCT first became a clinical reality, what principally assisted was the nonstop cross talk between clinicians and biologists to resolve several clinical problems that hindered early use of haplo-HCT. Until the 1990s, the high frequency of T-cells that recognized major class I or II HLA disparities between donor and recipient meant that T-cell-replete haplo-HCTs were associated with a high incidence of severe (≥III) G*v*HD and graft rejection [10], while T-cell-depleted (TCD) haplo-HCTs were linked with graft rejection [11].

To overcome such problems of relapse and G*v*HD, two approaches were developed in haplo-HCT setting:

1. The TCD graft with a megadose of hematopoietic progenitor cells (HPC), i.e., $CD34^+$, without any posttransplant immunosuppression.
2. The unmanipulated, i.e., T-cell-replete, graft with innovative posttransplant pharmacological immunosuppression for G*v*HD prophylaxis.

Thanks to the latter approach (#2), T-cell-replete haplo-HCT is enjoying popularity worldwide. At the same time, progress in research on TCD (#1) haplo-HCT has led to "second-generation" grafts, namely, α-/β-TCD grafts, or grafts which contain an established number of regulatory and conventional T-lymphocytes (T_{reg} and T_{con}, respectively).

1.2 T-Cell-Depleted HLA-Haploidentical Transplants: Biologic and Clinical Studies

1.2.1 Strengths

In the early 1980s, extensive 3-log TCD of the graft by means of soybean lectin and E-rosetting prevented acute and chronic G*v*HD even after haplo-HCT. The first clinical evidence was derived from patients with severe combined immune deficiency (SCID) [12, 13], which encouraged extending the haplo-HCT to leukemia patients. Although graft rejection was reasoned not be a major problem if patients were conditioned to

transplant with a myeloablative conditioning (MAC) regimen, its incidence rose dramatically because of residual recipient T-cytotoxic lymphocyte precursors with anti-donor specificity that survived supralethal conditioning [14]. Adding agents like anti-T-cell antibodies, cytosine arabinoside [15] and thiotepa to enhance immunosuppression and myeloablation was unsuccessful. Pioneering studies by Reisner and coworkers showed that full-donor engraftment without G*v*HD was achieved by escalating doses of $CD34^+$ cells in TCD bone marrow (BM) in mouse models for T-cell-mediated allograft rejection [16, 17]. These results provided the biological basis for overcoming the HLA barrier in clinical transplantation. The first clinical trial included 17 patients with high-risk acute leukemia who received grafts containing a "megadose" (on average 10×10^6) of $CD34^+$ cells/kg of recipient body weight [18]. BM and G-CSF-mobilized peripheral blood (PB) progenitor cells were depleted of T-cells by soybean agglutination and E-rosetting. The conditioning included total body irradiation (TBI), thiotepa, cyclophosphamide (Cy), and rabbit ATG. In subsequent reports [19, 20] peripheral blood progenitor cell processing became less time-consuming with $CD34^+$ cell-positive immunoselection, while fludarabine replaced Cy to reduce the extra-hematologic toxicity of the conditioning [21]. Primary sustained full-donor-type engraftment was achieved in 95% of a large series of acute leukemia patients. The incidence of acute and chronic G*v*HD was extremely low, even though no posttransplant immunosuppression was given. With a very long follow-up, leukemia-free survival (LFS) was 50% for acute myeloid leukemia (AML) in CR1 and 35% for AML CR ≥2. A retrospective study, analyzing the outcome of "megadose" TCD haplo-HCT in several European centers, reported LFS of 48% for AML patients in CR1 [22]. Handgretinger and coworkers [23, 24] described encouraging results in children using similar protocols. A European multicenter analysis of 127 children with high-risk ALL reported a 5-year LFS of 27% for patients in CR. Multivariate analysis detected a trend toward an institution-related effect with better LFS in institutions doing greater number of transplants (39% *vs.* 15%) and with higher $CD34^+$ cell doses [25]. These studies laid the foundation for the TCD haplo-HCT platform. In providing a high engraftment rate and a low incidence of G*v*HD in the absence of posttransplant immunosuppression, this strategy also led to the discovery of the following biological principles that impacted strongly on clinical outcomes:

1. To prevent G*v*HD in leukemia patients, the threshold dose of T-lymphocytes was 3×10^4/kg of recipient body weight in the setting of a conditioning regimen with anti-T-cell antibodies (anti-thymoglobulin [ATG] or OKT3) which exerted additional *in vivo* TCD.
2. A megadose of purified $CD34^+$ cells was a crucial factor as it reduced the frequencies of *in vivo* recipient anti-donor cytotoxic T-lymphocyte precursors (CTL-ps). *In vitro* studies showed that in bulk mixed lymphocyte reactions, cells within the $CD34^+$ cell population neutralized specific CTL-ps directed against their antigens but not against a third party, i.e., "veto" activity [26, 27]. Furthermore, early myeloid $CD33^+$ cells were, like $CD34^+$ cells, also endowed with marked "veto" activity, which was not found in late myeloid cells expressing CD14 or CD11b [28].

3. The haplo-HCT platform in the absence of posttransplant immunosuppression favored the emergence of donor-*versus*-recipient NK cell alloreactivity that induced a powerful graft-*versus*-AML effect [29–32]. Interestingly, in T-cell-replete unmanipulated HLA-haploidentical and unrelated donor transplantation, donor-*versus*-recipient NK cell alloreactivity was not associated with clinical benefits [33, 34].

Combined evidence from *in vitro* studies, murine models, and clinical trials indicated donor-*versus*-recipient alloreactivity killed AML cells, but not B-cell precursor acute lymphoblastic leukemia (ALL). An updated analysis of patients with AML in any CR showed allogeneic HCT from NK-alloreactive donors was associated with 12% relapse rate which translated into a remarkable 60% LFS. These findings were confirmed in T-cell-depleted haplo-HCT in children with acute leukemia [34]. Consequently, NK cell alloreactivity, which is potentially available for almost 50% of patients, should become a major criterion for donor selection in TCD haplo-HCT for high-risk AML patients (see Chaps. 2, 10, and 19). Unfortunately for patients who could not benefit from NK cell alloreactivity, i.e., ALL and AML, without an NK-alloreactive donor, clinical outcomes were faced with 30–35% relapse rates.

1.2.2 Drawbacks

The weak point of TCD haplo-HCT was clearly poor posttransplant immunological recovery due to the paucity of T-cells in the graft and additional *in vivo* TCD by ATG. The key challenge was to infuse sufficient T-cells without causing G*v*HD. Attempts focused on the adoptive transfer of pathogen-specific T-lymphocytes against CMV, aspergillus adenovirus, and Epstein-Barr virus (EBV) [35–38] or broad repertoire T-cells that were photodynamically depleted of alloreactive T-cells by dibromorhodamine (see Chap. 6) [39]. Another strategy was to insert a herpes simplex thymidine kinase (TK) suicide gene into T-cells to achieve *in vivo* susceptibility to ganciclovir (see Chaps. 19 and 20) [40, 41]. The TK-engineered T-cell add-back facilitated broad immune reconstitution with T-lymphocytes that did not express the TK gene [42]. This T-cell population was enriched by recent thymic emigrants, suggesting that immune reconstitution after TK-cell infusion was supported by a thymic-dependent pathway.

1.3 Today's World: State of the Art in T-Cell-Depleted HLA-Haploidentical Transplants

An encouraging step forward in improving posttransplant immunological reconstitution came with the use of *good manufacturing practices* (GMP)-grade magnetic beads to remove T-cell receptor (TcR) $\alpha\beta^+$ cells [43, 44]. Instead of undergoing $CD34^+$ selection, the leukapheresis product was depleted of only TcR $\alpha\beta^+$ cells,

thus retaining large numbers of effector cells like NK cells and TcRγδ⁺ T-cells. The TcRγδ⁺ T-cells combine conventional adaptive features with direct, rapid responses against sterile stresses and a variety of pathogens. They do not initiate G*v*HD since they do not recognize specific processed peptide antigens as presented on major histocompatibility complex molecules [45]. Indeed, following a chemotherapy- or TBI-based conditioning in children with acute leukemias [46, 47] and nonmalignant disorders [48], TcRαβ⁺-/CD19⁺-depleted grafts were found to be associated with a low incidence of G*v*HD, rapid immunological reconstitution, and low TRM (see Chap. 3).

Another innovative strategy of TCD in haplo-HCT setting is the so-called "designed" graft, an approach to counteract relapse and poor immunological reconstitution [49]. It consists of adoptive immunotherapy with Tcons and thymic-derived CD4+CD25+FoxP3+ Tregs (Treg/Tcon strategy). HLA-mismatched allogeneic HCT murine models showed that, when co-infused with Tcons, donor Tregs prevented lethal G*v*HD while allowing immune system reconstitution [50–54]. Furthermore, they did not impair Tcon control of neoplastic cell line expansion [55, 56] and eradicated human myeloid primary leukemia in humanized mouse models [57]. As expected from preclinical data, early adoptive transfer of naturally occurring donor Tregs made administration of a high dose of mature Tcons feasible and kept the incidence of grade II–IV acute G*v*HD to a low 14% with no posttransplant pharmacological immunosuppression in TCD haplo-HCT [57, 58] (see Chap. 4). The most remarkable outcome was the very low cumulative incidence of posttransplant relapse ($P = 0.09$), considering patients were at high risk of relapse [57]. Interestingly, AML patients who were transplanted from NK-alloreactive donors benefit from both anti-leukemic mechanisms, and indeed, so far, no relapse has occurred in this setting. These results provide the first real *in vivo* evidence that the G*v*L effect can be separated from G*v*HD in the clinical setting. A feasible hypothesis is that infused Tregs with low CXCR4 expression do not home to BM and do not inhibit Tcon activity. Thus, alloreactive Tcons lyse leukemic stem cells in BM.

1.4 T-Cell-Replete HLA-Haploidentical Transplants

Today, *ex vivo* TCD is no longer essential for crossing the histocompatibility barrier in haplo-HCT. One effective method for G*v*HD prevention is to administer high-dose Cy after transplant (PTCy) in a narrow time window. By counteracting alloreactive T-cells, Cy reduced the risks of G*v*HD and graft rejection. This transplant modality was first applied clinically in the setting of a non-myeloablative (NMA) conditioning regimen which included low-dose TBI, Cy, and fludarabine [59] (see Chap. 7). Easy to administer, it was well tolerated, with a 4% incidence of G*v*HD and a low TRM (15% at 2 years). Since then, several single and multicenter phase II trials have confirmed these findings with favorable outcomes. For instance, Bashey and coworkers [60] reported that outcomes were similar after haplo-HCT with PTCy, HLA-matched sibling donor (MSD), and HLA-matched unrelated

donor (MUD) transplants (see Chap. 19). At 6 months cumulative incidences of grade III–IV acute G*v*HD were 11%, 8%, and 11%, respectively; extensive chronic G*v*HD occurred in 38%, 54%, and 54% of patients, respectively. The 2-year cumulative incidences of TRM were 7%, 13%, and 16%, respectively, and relapse rates were 33%, 34%, and 34%. Probabilities of LFS were 60%, 53%, and 52%, respectively.

Unfortunately, the relapse rate was high in all studies. Although switching to MAC regimens reduced the risk of relapse, the overall survival (OS) rate remained unchanged as TRM rose. A large retrospective study [61)] reported 44% relapse, 14% TRM, and 45% OS for MAC haplo-HCT and 58% relapse, 9% TRM, and 46% OS for non-MAC regimen-based haplo-HCT. These outcomes were similar to MUD-HCT with myeloablative or reduced intensity conditioning regimens.

Another way to overcome the HLA barrier is to use "G-CSF-primed" bone marrow and/or peripheral blood. Transplant protocols included a MAC and intensive posttransplant immunosuppression [62] (see Chap. 5). Experience with this technique is limited to China, except for reports from one Italian group [63]. A recent multicenter Chinese study [64] reported 74% LFS at 3 years in a large series of AML patients in CR1. The incidence of grade II–IV acute G*v*HD was 36%, with 42% chronic G*v*HD. The relapse rate was 15% and TRM 13%. Similar LFS was observed after HLA-matched sibling donor transplants. An updated of Italian study [65] reported a high engraftment rate with 5-year outcomes as follows: 34%TRM, 28% relapse, and 48% LFS. Outcomes in the study by Wang and coworkers [64] clearly diverged greatly from other transplantation strategies and even from the Italian group who employed a similar protocol. Although it is always arduous to compare outcomes of nonrandomized studies, it is clear that the different outcomes may be due to various factors, such as median age (21 years) and a low percentage of patients with high-risk features in the Chinese study.

1.5 Expert Point of View

1.5.1 The Way Forward for HLA-Haploidentical Transplant: T-Cell-Depleted or T-Cell-Replete?

Today's dilemma in the haplo-HCT setting reechoes the discussion about T-cell-depleted or T-cell-replete grafts that featured in HLA-matched sibling HCT from the mid-1980s onward. T-cell-replete and T-cell-depleted haplo-HCT outcomes have never been compared in randomized studies. Extrapolating meaningful data from observational registries is difficult because TCD haplo-HCT has been performed in many different modalities with diverse conditioning regimens, graft processing techniques, adoptive immunotherapies, and presence or absence of posttransplant pharmacological immunosuppression. *A commitment to conducting well-planned, prospective randomized studies appears ever more crucial*. There is no doubt a T-cell-replete haplo-HCT with PTCy is easy to perform, is associated with low TRM, and does not require particularly well-skilled laboratory staff. Thus

it is potentially accessible in all transplant institutions worldwide at a relatively low cost. Its use continues to rise worldwide, unlike the CB transplant which has plateaued in Europe and the USA [66]. Despite these successes, the major challenge is the high rate of posttransplant relapse, especially in high-risk acute leukemias and in the NMA-conditioning setting. The crucial factor underlying the high relapse rates is post-HCT pharmacological G*v*HD prophylaxis, which is immunologically nonspecific, thus weakening the G*v*L effect. The same effect is observed also in T-cell-replete HCT from other sources (MSD, MUD, or cord blood transplants [CBT]).

The historical challenge in allogeneic HCT has always been on how best to preserve the G*v*L effect without G*v*HD, especially in the poor-risk genetic subtypes of AML. The added value of TCD haplo-HCT lies in its providing a unique setting that responds to this challenge. Since no posttransplant pharmacologic immunosuppression is administered, it is an ideal platform for adoptive T-cell immunotherapy aimed at strengthening the G*v*L effect as exemplified by the T_{reg}/T_{con} strategy (see Chap. 4). It could also be used for posttransplant infusion of specific donor-derived antineoplastic cells, considering that prerequisites for this treatment are absence of G*v*HD and posttransplantation immunosuppression. For tomorrow's world, experiments in animal models showed that adding donor-type "veto cells" to the megadose graft eradicated recipient anti-donor T-cell clones, thus inducing donor-specific tolerance, while sparing the polyclonal T-cell population [67]. This protocol with its NMA conditioning regimen is now in translation to humans. Hopefully, it will promote durable T-cell chimerism resulting in transplantation tolerance to donor-derived allografts, in the absence of G*v*HD prophylaxis. This strategy might potentially be applied to elderly patients with acute leukemias or in patients with nonmalignant diseases which require allogeneic HCT as well as for solid organ transplantation.

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Part I

Graft Manipulation and Methods to Control T-cell Alloreactivity

2 Toward Safer CD34+ Megadose T-Cell-Depleted Transplants Following Reduced Intensity and Nonmyeloablative Conditioning Regimens

Noga Or-Geva and Yair Reisner

2.1 Introduction

Haploidentical hematopoietic cell transplantation (haplo-HCT) offers a curative procedure for patients with malignant and nonmalignant hematological diseases, as well as an expanding number of inherited disorders. Haplo-HCT is likely the best HLA-related unmatched source of hematopoietic cell transplantation (HCT), on account of immediate availability and willingness of family members to donate, possibility of secondary grafting or posttransplant T-cell therapy, and increased graft-*versus*-tumor (G*v*T) reactivity. However, haplo-HCT has traditionally been associated with higher transplant-related mortality (TRM) rates as compared to transplants from HLA-matched donors, thereby limiting its application. High TRM was a direct result of increased frequency and severity of graft-*versus*-host disease (G*v*HD) caused by the HLA discrepancy between recipient and donor. Over the past decade, haplo-HCT has emerged as an important clinical option in the treatment of neoplastic hematologic diseases especially for patients who lack HLA-matched sibling donor. The risk of G*v*HD and graft rejection associated with such transplants has been markedly reduced by extensive T-cell depletion (TCD) for G*v*HD prevention and escalated doses of $CD34^+$ progenitors (i.e., megadose) to overcome graft rejection.

Haplo-HCT has traditionally been applied in the context of myeloablative conditioning, due to the high relapse risks in leukemia patients. However, new developments enabling haplo-HCT transplantation following nonmyeloablative (NMA) conditioning have increased its safety and made it a viable alternative option, expanding its application as a platform for tolerance induction in organ transplantation and

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for subsequent T-cell therapy with immune cells from the same donor. Despite the fact that NMA conditioning prior to HCT is routinely practiced worldwide, it is still associated with increased risk of G*v*HD, due to utilization of T-cell-replete grafts, where donor T-cells are meant to promote engraftment. Such unmanipulated grafts are conventionally coupled with aggressive pre- and posttransplant immunosuppressive protocols, utilizing extensive G*v*HD prophylaxis and sometimes accompanied by *in vivo* TCD [1]. Although many improved protocols have been tested, including the current leading approach of early posttransplant administration of high-dose cyclophosphamide (Cy) [2–4], such protocols are still associated with high relapse rates and substantial G*v*HD.

Haplo-HCT in the context of TCD and NMA conditioning is associated with minimal risk for G*v*HD but with risk of higher rates of graft rejection. Thus, over coming this challenge could offer a highly attractive and safer treatment modality for patients with different hematological diseases or a platform for organ transplantation and cell therapy by addition of $CD34^+$ cell megadose. Despite early demonstrations [5] that TCD-megadose transplants under sublethal irradiation can lead to chimerism and induce tolerance to skin grafts in animal models, application in humans has been impeded by the inability to collect sufficient $CD34^+$ cell numbers using current technologies. In this chapter, we describe novel approaches for chimerism induction with $CD34^+$ megadose in the settings of TCD and NMA conditioning regimens, based on insights regarding the mechanism by which $CD34^+$ megadose transplants overcome graft rejection.

2.2 Overcoming HLA Barriers with T-Cell-Depleted Transplantation

The first successful TCD transplant in humans was performed in severe combined immune deficiency (SCID) patients who received three-loci-mismatched haploidentical bone marrow (BM) using T-cell-selective agglutination with the soybean lectin (SBA), followed by E-rosetting with sheep red blood cells. The remaining SBA-E-cell fraction afforded over a thousand-fold depletion ($<5 \times 10^4$ T-cells per kg) [6, 7], defining the extent of depletion required for effectively preventing G*v*HD. This method has been successfully used for the treatment of hundreds of SCID patients in multiple centers worldwide [8–10] with low mortality.

The success in SCID patients led to TCD HLA-mismatched transplant trials in acute leukemia patients conditioned with supralethal radiotherapy regimens, in which recipient immunity is dramatically reduced. Surprisingly, though G*v*HD onset was prevented, the prevalence of graft rejection sharply rose [11, 12]. The increased graft rejection was later found to be mediated by recipient anti-donor cytotoxic T-lymphocyte precursors (CTLp) that survive the conditioning regimen [13, 14].

The clinical breakthrough that allowed successful haplo-HCT for acute leukemia patients for the first time was the application of a megadose of TCD hematopoietic $CD34^+$ progenitor cells. This concept, of escalating $CD34^+$ cell doses to overcome rejection, was first successfully demonstrated in animal models in the early 1990s

[15–18]. In humans, higher numbers of CD34+ cells from the BM donors was afforded by the advent of granulocyte colony-stimulating factor (G-CSF) [19]. Beginning in 1995, TCD methodology was replaced by positive selection of CD34+ cells using magnetic beads. Such methodology was first applied by the Perugia group, on high-risk leukemia patients, and resulted in primary engraftment of the haploidentical CD34+ megadose transplants with low rates of G*v*HD in more than 93% of the patients, in the absence of posttransplantation G*v*HD prophylaxis [20]. Comparable results were achieved in other centers in both adults [21, 22] and children [23, 24].

2.3 Megadose of CD34+ Cells: Regulatory Activity and Overcoming the HLA Barrier

The impact of cell dose escalation on the engraftment of TCD HLA-mismatched transplants in both humans and mice hinted to an immunoregulatory capacity of CD34+ cells. This was confirmed upon the discovery that CD34+ cells are endowed with "veto activity" [25, 26].

The veto phenomenon was first defined [27] as the capacity to specifically suppress CTLp, directed against antigens (Ags) expressed by the veto cells themselves, but not against third-party Ags [28]. Suppression of effector T-cells directed against the veto cells is both Ag-specific and major histocompatibility complex (MHC) restricted, resulting from the unique manner by which the veto cell deletes its target. (Hence, the veto activity results from the unidirectional recognition of the veto cell by the responding T-cell, but not vice versa.) Thus, the recognizing CTLp binds via its T-cell receptor (TcR), directed against the MHC of the veto cell, but instead of becoming activated is killed upon binding to its veto target [29]. Importantly, this activity is not linked to any single cell phenotype but is rather a functional hallmark of a cell; therefore, veto activity has been attributed to various cell types including T-lymphocytes, natural killer (NK) cells, and dendritic cells (DCs). Accordingly, different veto cells exert their veto effect via diverse pathways (Table 2.1).

This specificity of veto cells can be harnessed as an effective modality for the induction of donor-specific immune tolerance in transplantation settings, by the

Table 2.1 A variety of veto cells carry out veto activity via different molecules/pathways

Veto cell type	Molecules involved in veto activity
CD34+ hematopoietic stem cells [26, 30]	TNFα
CD8+ cytotoxic T-cells [28, 31]	Perforin
CD8+ cytotoxic T-cells [32, 33]	FAS-L
CD8+ central memory T-cells [34]	FAS-L
Immature dendritic cells [35, 36]	Perforin
Activated BM cells (ABM) [37] or LAK cells [38]	Veto cell degranulation
CD3-CD8+CD16+ BM cells [39]	TGF-β1

addition of donor veto cells, adept in eliminating solely the host anti-donor T-cell clones that mediate the transplant rejection, while sparing all additional T-cells that can persist and fight infectious pathogens.

The finding that $CD34^+$ cells were equipped with veto capability, mediated via tumor necrosis factor-α (TNFα)-mediated deletion [26, 30], was soon followed by the discovery that early myeloid $CD33^+$ cells, which rapidly expand after engraftment, are also endowed with marked veto activity, which is absent from late myeloid cells that express CD14 or CD11b [25, 30].

BM-derived immature DCs, formerly described as competent inducers of immune tolerance, were also found to possess veto activity directed against $CD8^+$ T-cells using a distinct MHC-dependent, perforin-based killing mechanism involving activation of Toll-like receptor 7 (TLR7) and signaling through triggering receptor-1 expressed on myeloid cells (TREM-1) [35, 36]. NK cells were also found to exhibit veto activity upon activation with IL-2 and develop and appear early during the post-transplant period [32, 40].

Hence, the ability of $CD34^+$ cell megadose to overcome anti-donor activity probably occurs in steps; first the host anti-donor CTLp are dwindled by the veto exerted by the infused $CD34^+$ cells, this activity is then reinforced by the $CD33^+$ progeny expansion, and finally the tolerance could potentially be maintained by BM-derived $CD11c^+$ immature DCs and NK cells which come into play after donor's BM graft has seeded.

2.4 Megadose $CD34^+$ Cells Coupled with TCD and Reduced Intensity Conditioning (RIC)

Although the number of HLA-mismatched allogeneic transplants has increased steadily over the past few decades, TCD HCT remains a high-risk procedure on account of conditioning-related toxicities and slow immune reconstitution and is therefore reserved for patients with life-threatening diseases. To this end, reduced intensity, i.e., RIC or NMA, conditioning regimens are continuously being perfected, expanding the range of patients who can benefit from the procedure (i.e., elderly patients, patients with comorbidities, and patients with nonmalignant disorders). These regimens reduce TRM and also promote mixed donor chimerism (i.e., a state in which the lympho-hematopoietic system of the recipient of allo-HCT consists of a mixture of host and donor cells) which has been associated with improved immunity and central tolerance [41–45]. The major advantage of RIC is also its main caveat; while sparing much of the host immunity improves immune reconstitution, it also allows a robust host-*versus*-graft response which increases the chances of graft rejection.

The ability of HCT megadose to induce mixed chimerism under RIC was first demonstrated in sublethally (7Gy) irradiated mice in 1999 [5]. This mixed chimerism induced specific tolerance to donor-type skin grafts facilitated by BM cells within the $Sca\text{-}1^+Lin^+$ cell fraction capable of specifically deleting anti-donor CTLp both *in vitro* and *in vivo* [5, 15]. However, the high numbers of $CD34^+$ cells required to overcome graft rejection under RIC conditions suitable for elderly or

nonmalignant patients cannot presently be attained with state-of-the-art technologies (Gan et al. unpublished results). One approach to overcome this hurdle was to seek alternative immunoregulatory cells such as other types of veto cells that could be adoptively transferred with the allogeneic TCD-megadose graft to allow its application under RIC regimens.

2.5 Combining HLA-Mismatched TCD Megadose BM with Adoptive Transfer of Veto Cells Under RIC

Based on insights regarding the mechanism by which megadose transplants overcome rejection, we have suggested the addition of veto cells to the megadose transplant, in an effort to overcome the problem of graft rejection under RIC. This antigen-specific MHC-restricted activity results in the suppression of effector T-cells directed (via TCR specificity) against the veto cells. Utilizing this attribute, we have shown that addition of donor-type veto cells along with the graft can induce donor-specific immune tolerance, by mediating specific eradication of host anti-donor T-cell clones, while host-mediated immunity is maintained by the remaining polyclonal T-cell population.

Of the various types of *veto cells* described over the years (Table 2.1) (for review, see Or-Geva and Reisner [46]), none are more potent than $CD8^+$ cytotoxic T-cell (CTL) lines or clones [32, 47–50]. We have recently substantiated the ability of *veto central memory-like CD8+ T-cells* (veto T_{cm}) to facilitate engraftment of HLA-mismatched megadose TCD HCT in murine models under RIC [34]. To eliminate the inert ability of such cells to instigate G*v*H immunity in allogeneic settings, we developed a new approach for the generation of host-nonreactive veto T_{cm}, by stimulation of donor $CD8^+$ T-cells against third-party stimulators under cytokine deprivation (i.e., "anti-third-party activation") [46], followed by further *ex vivo* expansion using third-party stimulators and IL-15 for the generation of donor-type anti-third-party veto T_{cm}. These T_{cm} are then co-administered with a megadose TCD completely HLA-mismatched graft under sublethal TBI and support engraftment resulting in mixed chimerism that supports central immune tolerance to donor-derived skin grafts. The importance of administering veto $CD8^+$ T-cells that express the central memory phenotype lies in the ability of these cells to migrate to the lymph nodes of the host where they can eliminate anti-donor CTLp in the lymph nodes of the host, soon after their activation and prior to their expansion in the periphery. Furthermore, these cells were shown to specifically and efficiently delete host TcR-transgenic T-cells carrying a TcR transgene with anti-donor specificity, via apoptosis [51] as determined by FACS and immunohistochemistry.

Thus, anti-third-party veto $CD8^+$ T_{cm}, devoid of G*v*H reactivity by virtue of their activation and expansion against a third-party MHC that is not cross-reactive with the host, can facilitate the engraftment of TCD megadoses in the absence of G*v*HD in murine models. Mixed chimerism was supported under RIC, in the absence of G*v*HD, and allowed subsequent donor-skin engraftment, whereas third-party skin was promptly rejected, demonstrating the specificity of the immune tolerance is

achieved [34]. Using a novel *in vivo* bioluminescence imaging model for tracking host anti-donor CTLp, we showed that veto T_{cm} cause accumulation of host CTLp in the lymph nodes and thereby drive CTLp away from the BM where the donor $CD34^+$ cells reside. Furthermore, two-photon microscopy revealed formation of conjugates between host CTLp and veto T_{cm} accompanied by decelerated and confined host CTLp movement within the lymph nodes, thereby leading to the demise of these anti-donor host CTLp via apoptosis in an antigen-specific manner.

This protocol, now in translation to human settings, may promote durable chimerism resulting in transplantation tolerance to donor-derived allografts, without G*v*HD in the absence of G*v*HD prophylaxis. Taken together, this approach can potentially serve as a safe modality for nonmyeloablative haplo-HCT, and also as a platform for organ transplantation and cell therapies.

2.6 Expert Point of View

Induction of hematopoietic mixed chimerism by means of BMT offers a promising approach by which to achieve immune tolerance. Allo-HCT is the curative option for multiple nonmalignant diseases including immunodeficiency syndromes [52, 53], autoimmune disease [54, 55], inherited metabolic disorders [56], and hemoglobinopathies such as thalassemia and sickle cell disease (SCD) [57, 58]. Moreover, allo-HCT can also serve as a platform for the subsequent engraftment of organ transplants without the need for continuous immunosuppressive therapy. The main barriers today remain the transplant-related complications and the lack of HLA-matched donors. The use of HLA-mismatched transplants, such as transplants from a haploidentical donor, boasts the advantage of high availability. Safe transplantation of haplo-HCT has been achieved in TCD-megadose $CD34^+$ cell transplants under RIC, affording safe induction of stable long-term mixed chimerism, in the absence of G*v*HD. However, insights gained from the use of megadose $CD34^+$ cell transplants on immune tolerance exhibited by hematopoietic stem and progenitor cells and their early myeloid derivatives suggest, that while these cells can effectively overcome residual host CTLs surviving the myeloablative conditioning in leukemia patients, the same $CD34^+$ cell numbers are not capable of overcoming the large number of T-cells remaining after RIC. Hence, the use of different sources of tolerizing cells, devoid of G*v*H reactivity, could enable facilitation of engraftment of hematopoietic allografts under RIC without the unwanted complications of G*v*HD-producing T-cells.

In particular, host-nonreactive anti-third-party T_{cm} have been shown to be good candidate veto cells as they facilitate engraftment of fully mismatched megadose TCD allo-HCT, thereby promoting mixed chimerism and immune tolerance in murine models. If indeed the use of these veto T_{cm} will translate well into elderly patients with hematological malignancies, their application could be further extended to treat diseases that are lethal over the course of years, but that do not present an immediate threat, such as nonmalignant hematological diseases and autoimmune disorders. Furthermore, this modality could be used as a prelude for cell therapy and as a platform for organ transplantation.

2.7 Future Directions

2.7.1 The Challenge of Chimerism Induction Following NMA Conditioning by *Ex Vivo*-Designed or *In Vivo*-Manipulated Haplo-HCT

2.7.1.1 Posttransplant Cellular Therapies for Malignancies After Megadose Haplo-HCT

The recent burst of immunotherapy treatments for cancer and specifically for hematological malignancies emphasizes the great underlying potential of using NMA allogeneic transplants as a prelude to cell therapy. Protocols that would enable stable chimerism under safe conditioning with minimal risks for lethal infections or for acute and chronic G*v*HD could serve as an attractive platform for advanced treatment with donor-type tumor-directed T-cells or NK cells. In this context, newly designed haplo-HCT grafts may soon prove to be sufficiently effective in promoting engraftment under very mild, NMA conditioning, thereby inducing a tolerant state ideal for administration of single or repeated infusions of *TCR-transgenic T-cells, genetically modified redirected NK cells, or* donor cells bearing *chimeric antigen receptor (CAR)* T-cells. Notably, unlike donor lymphocyte infusion (DLI) used in RIC transplants, such genetically modified T-cells can be generated in a manner that poses minimal risk for G*v*HD, by virtue of endogenous TcR knockout or by introducing appropriate suicide genes. Furthermore, even without the use of cell therapy after chimerism induction, the emergence of a newly formed donor-type immune system, in the absence of any posttransplant immune suppression, might allow better control of residual disease, with or without further treatment, using the new generation of immune checkpoint-directed therapies to enhance the antitumor response.

2.7.1.2 Treatment of Nonmalignant Hematopoietic Diseases Using Megadose Haplo-HCT

Hemoglobinopathies

Allogeneic transplantation remains the sole curative option for certain hemoglobinopathies such as β-thalassemia and SCD. High cure rates have been noted for pediatric SCD patients undergoing HCT following myeloablative conditioning protocols, mostly using busulfan (Bu) and Cy for myeloablation [57, 59–62], along with cyclosporine and methotrexate as G*v*HD prophylaxis. Adult SCD and thalassemia patients treated with these protocols suffered from transplant-related toxicity [58, 63] and were therefore excluded from many of these studies.

Considering that the majority of patients requiring HCT do not have a suitable HLA-matched family donor, the use of haplo-HCT has been explored as an alternative source for patients lacking a HLA-matched donor. Sodani and coworkers [64, 65] describe a trial in pediatric thalassemia patients receiving a haploidentical transplantation of a megadose of purified CD34+ cells and a highly immuno-myeloablative conditioning regimen (hydroxyurea, azathioprine, fludarabine, busulfan or busulfan, Cy,

thiotepa, and ATG) with G*v*HD prophylaxis for 2 months after transplantation. In this study 22 of 31 patients were cured; however 7 rejected the graft and 3 died of transplant-related complications. The adverse effects of the high regimen-related toxicity have prohibited the use of HCT in older patients with hemoglobinopathies.

Mixed chimerism after NMA HCT has been found beneficial for both thalassemia and SCD patients [66–69], where a limited percentage of engrafted donor cells may be sufficient to overcome disease phenotype. Thus, the use of lower intensity conditioning is attractive for these patients, as it can potentially enable mixed chimerism and tolerance induction with decreased risks for transplant-related toxicity and mortality.

Attempts to use RIC protocols have mainly focused on HLA-matched HCT for SCD, producing favorable [70–72] and unfavorable [73, 74] outcomes depending on the regimen used. Results of a phase I/II haploidentical HCT trial in adult SCD patients under RIC (ATG, Cy, Flu, 2Gy TBI, cyclosporine and tacrolimus as G*v*HD prophylaxis) showed that 17 of 31 patients accepted the graft and 11 of them were disease-free. No G*v*HD was noted [75]; however posterior reversible encephalopathy syndrome (PRES) occurred in three patients, and a high rejection rate (43%) warrants further fine-tuning of the preparatory regimen.

Our previous demonstration that combined TCD HCT and adoptive transfer of T_{cm} veto cells under NMA conditions facilitates graft acceptance and indicates that this treatment modality could potentially offer an attractive option for patients with hemoglobinopathies, without the risk for G*v*HD and transplant-related toxicities.

Autoimmunity

The possibility of employing HCT as an effective therapy for autoimmune diseases (AID) has been studied since the early 1990s. Supported by animal models of antigen-induced autoimmunity, the primary goal of this therapeutic approach is to achieve drug-free remission by elimination of the disease-mediating immune cells and reestablishment of immune regulation, thereby restoring self-tolerance. Numerous phase I and II studies have tested autologous HCT (auto-HCT) in diseases such as multiple sclerosis, systemic sclerosis, systemic lupus erythematosus, rheumatoid arthritis, and juvenile idiopathic arthritis (reviewed in Liu et al. [76], Openshaw et al. [77]). Most commonly, the graft is TCD, since lympho-depletion of disease-causing cells is vital. To date, autologous HCT has been used as a rescue strategy for patients with a poor prognosis and lack of alternative treatment options, as the side effects of this procedure still pose a major risk for patients. These risks include increased organ sensitivity to conditioning, opportunistic infections, de novo autoimmunity, and increased frequency of secondary malignancies. Allo-HCT for patients with AID, aiming to induce tolerance through the transfer of a genetically diverse, healthy immune system, has been less fully explored. The effectiveness of such treatment is predicted from a combination of data from animal models [78] and data from AID patients who received allo-HCT for a conventional hematological indication. Allo-HCT may be superior to autologous transplantation for AID patients, since it may be able to replace the diseased cells which are thought to be the root of many AIDs [79]. Nevertheless, G*v*HD and increased TRM owing to conditioning toxicity and poor immune reconstitution previously associated with

allo-HCT have hampered trials for patients with severe AID. With the advent of RIC regimens and TCD methods to prevent G*v*HD, the interest in testing the efficacy of allo-HCT in these disorders has been revived.

2.7.1.3 Induction of Tolerance in Organ Recipients by Combining Veto T_{cm} and Haplo-HCT

Recent improvements in NMA and RIC protocols have re-sparked the interest in establishing mixed chimerism via HCT as an attractive platform for tolerance induction in solid organ transplantation. This approach is particularly advanced for protocols combining HCT and kidney transplantation [80]. These protocols utilize TCD HCT under RIC, since the risk of G*v*HD is unacceptable in such patients and because TCD favors the induction of mixed chimerism. Several approaches have demonstrated the efficacy of this treatment in both HLA-matched and haploidentical transplants. In patients receiving megadose of matched-related donor CD34+ selected cells, following a relatively mild conditioning protocol, it has been shown that tolerance for a subsequent kidney transplant from the same donor enables immune suppression [81] to be gradually discontinued. However, hematopoietic chimerism and immune tolerance could not be attained by this approach in haploidentical recipients. Alternative protocols for haploidentical donors resorted to T-cell-replete transplants which enable chimerism induction at the expense of G*v*HD risk. This was partially addressed either by *in vivo* TCD [82] or posttransplant Cy [83]. However, considering that any risk for G*v*HD in organ transplantation is unacceptable, this approach might be improved by implementation of some of the advanced techniques described above, such as addition of veto cells or genetically modified cells, to facilitate engraftment without the risk of G*v*HD.

Conclusion

Over the past three decades, TCD has proven as the most efficient tool for preventing onset of G*v*HD after allogeneic transplantation. Since the initial finding that depletion of T-cells from the graft effectively eliminates G*v*HD, the field has evolved through the research of multiple groups, and many innovative TCD protocols have been tested with varying degrees of success. After the problem of TCD graft rejection was overcome by the use of CD34+ cell megadose transplants, the field has focused on solving the issue of slow immune reconstitution, mostly through adoptive transfer of non-alloreactive T-cells, either genetically modified or specifically selected. These advances, along with the development of NMA and RIC protocols, have made TCD safer, thereby encouraging initiation of prospective trials comparing it to conventional T-cell-replete transplants in leukemia patients.

Preclinical studies strongly suggest that using a new cell composition, comprising T-cell-depleted CD34+ cell megadose HCT combined with anti-third-party veto T_{cm}, will likely offer prompt engraftment without G*v*HD, thereby overcoming the hurdle of graft rejection of TCD haplo-HCT following RIC. Clinical translation of this approach is in progress and will hopefully provide a safe modality for the treatment of a wide range of diseases, such as nonmalignant hematological

diseases and autoimmune disorders, for which the risk of fully myeloablative conditioning is not justified. Importantly, this protocol could also offer a method for achieving mixed chimerism as a prelude for cell therapy and as a platform for organ transplantation. It is hoped that this new generation of "designed grafts," which have paved the way for safer allogeneic HCT, will soon enable its extension to the treatment of autoimmune diseases and as a platform for tolerance induction in solid organ transplantation and cancer immunotherapy.

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Selective Allo-depletion: TcRαβ and CD19⁺ T-Cell Depletion

3

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3.1 Introduction

Depletion of T-lymphocytes from allogeneic bone marrow (BM) or mobilized peripheral blood (PB) grafts has been employed in HLA-matched sibling donor (MSD), in HLA-matched unrelated donors (MUD), and especially in haploidentical transplantation (haplo-HCT) in order to avoid graft-*versus*-host disease (G*v*HD). Various methods have been described and used in clinical studies such as soybean agglutination, E-rosetting, antibodies plus complement, and immunoaffinity columns. Most of these techniques were very time-consuming and not suitable for the processing of large numbers of cells. In addition, these methods of negative depletion of T-cells from BM grafts were not very efficient with only low T-cell depletion (TCD) efficacies [1] and were not suitable for the PB CD34⁺ grafts. With the availability of indirect TCD techniques by positive selection of highly purified CD34⁺ cells using magnetic cell sorting (CliniMACS), a much more efficient TCD from PB grafts was achieved in the range of 3.5–5 log [2]. Subsequently, negative depletion strategies of CD3⁺ and TcRαβ⁺ T-lymphocytes from PB grafts have been developed and used in patients undergoing HLA-matched unrelated and haploidentical transplantation.

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3.2 The Technique and Efficacy of TcRαβ T-Cell Depletion

The first large-scale TCD methods using the semiautomated CliniMACS device included the positive selection of $CD34^+$ hematopoietic cells [3], the CD3-negative depletion method [4], and the additional depletion of $CD19^+$ B-cells together with the CD3 depletion [5].

Chaleff and coworkers have first described the method of large-scale TcRαβ depletion using the semiautomated CliniMACS device in a preclinical setting [6] which has later been extended to clinical settings [7]. While the $CD34^+$ selection and the CD3-negative depletion technique was based on an anti-CD34 or anti-CD3 antibody directly conjugated to magnetic microbeads, the TcRαβ depletion is based on the use of an biotinylated anti-TcRαβ antibody and a second anti-biotin antibody conjugated to the microbeads. This indirect labeling results in a robust staining of the target cells with sufficient binding of magnetic particles to the surface of the cells. Therefore, the cells coated with the microbeads are effectively retained in the magnetic field. In Fig. 3.1,

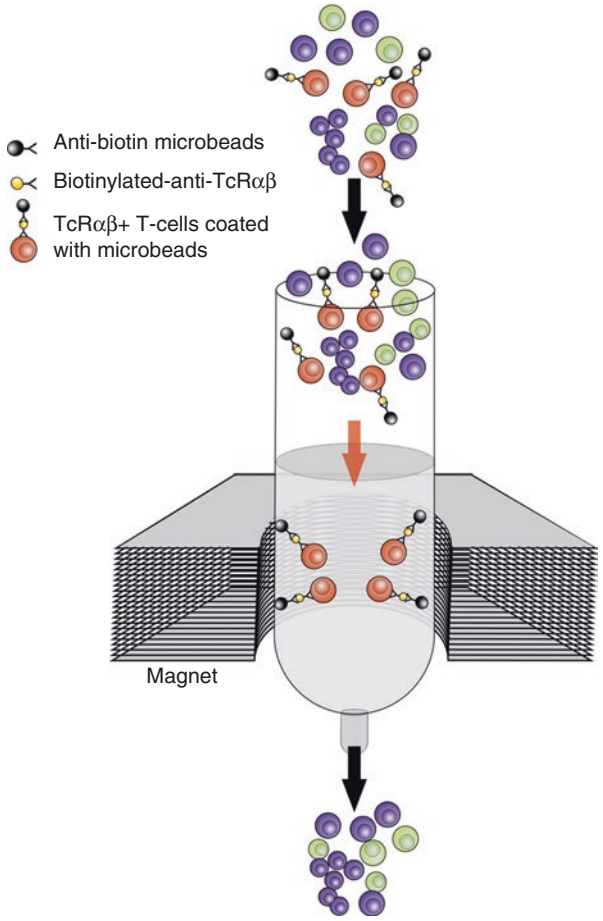


Fig. 3.1 The TcRαβ depletion is based on the use of a biotinylated anti-TcRαβ antibody and a second anti-biotin antibody conjugated to the magnetic microbeads

the basic principle of this approach is shown. An additional B-depletion is possible by adding an anti-CD19 antibody directly conjugated to the magnetic particle. While $CD34^+$ selection from 139 mobilized peripheral stem cell grafts resulted in a high log^{10} TCD of 4.6 log and a recovery of 73% of the $CD34^+$ cells, the CD3-negative depletion of 125 stem cell grafts was less effective with a log^{10} depletion of 4.0 log, while the recovery of $CD34^+$ cells of 69% was not different. The TcRαβ depletion resulted in log^{10} depletion of 4.7 logs which is very similar to the $CD34^+$ selection and a recovery of $CD34^+$ cells of 73%. More recently, the completely closed and fully automated Prodigy device was introduced by the Miltenyi Biotec company (Bergisch-Gladbach, Germany), and the first large-scale depletion of TcRαβ T-cells showed an effective log^{10} TCD and an excellent recovery of $CD34^+$ cells of 86%.

One of the major reasons to switch from positive selection to CD3-negative depletion was the preservation of natural killer (NK) cells and from CD3 depletion to TcRαβ depletion the additional retention of γδ T-cells in the graft. In the report by Schumm and coworkers [7], the recovery of $CD56^+$ NK cells and γδ T-cells was 80% and 83%, respectively. Moreover, these grafts contain a large number of myeloid cells due to the mobilization with G-CSF, mainly comprised of monocytes and other myeloid-derived cells. In Fig. 3.2, an example of the graft composition for a series of 17 pediatric patients is shown, and the numbers of infused cells based on the recipient body weight are shown. All grafts were obtained from adult haploidentical donors mobilized with G-CSF. In this series of patients, the mean number of infused TcRαβ T-cells was low with 14,000 TcRαβ T-cells/kg, whereas the mean numbers of infused NK cells and γδ T-cells were 100×10^6/kg and 15×10^6/kg, respectively. The patients received a megadose of $CD34^+$ cells of 16.2×10^6/kg.

The effective depletion of TcRαβ/$CD19^+$ T-cells and the good recovery of NK and γδ T-cells have recently been corroborated by Li Pira and coworkers, who reported their experience with 170 procedures in 165 donors [8]. The mean log^{10}

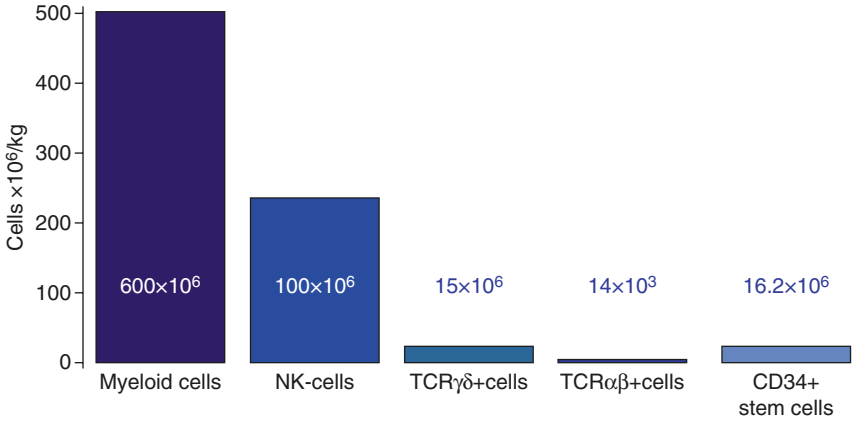


Fig. 3.2 An example of the graft composition for a series of 17 pediatric patients is depicted, and the numbers of infused cells based on the body weight of the patients are shown. All grafts were obtained from adult haploidentical donors mobilized with G-CSF

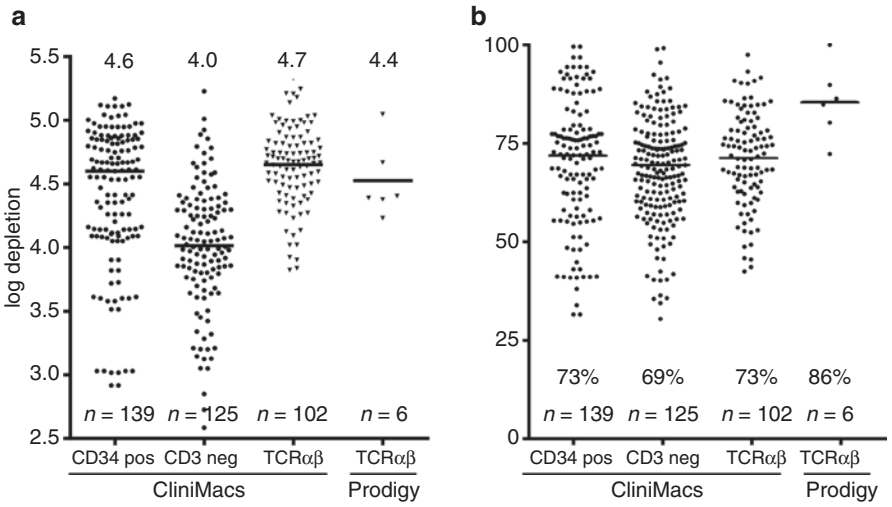


Fig. 3.3 A comparative analysis of the different methods of T-cell depletion based on the use of the semiautomated CliniMACS device and the fully automated and closed Prodigy system is shown. It should be appreciated that, in contrast to the CD34-positive selection and CD3-negative depletion, there were no failures of the TcRαβ depletion in the 102 procedures and almost all procedures resulted in a $\log^{10}$ depletion of >4 log

depletion of TcRαβ$^+$ T-cells and CD19$^+$ B-cells was 4.18 log (±0.48) and 3.5 log (±0.49), respectively. The median yield of NK cells and γδ T-cells was 90.7% (range 33–111) and 88.3% (range 34–112), respectively.

In Fig. 3.3, a comparative analysis of the different methods of TCD based on the use of CliniMACS and the Prodigy system is shown (own unpublished results). It can be appreciated in Fig. 3.3 that, in contrast to the CD34-positive selection and CD3-negative depletion, there were no failures of the TcRαβ depletion in the 102 procedures and almost all procedures resulted in a $\log^{10}$ depletion of >4 log. Lower depletions in the range of <3.5 log, as observed with CD34-positive selection and CD3-negative depletion, are associated with higher residual T-cell numbers in the graft and are considered by us as a failure of the procedure. This finding further indicates the robustness of the method, which is due to the indirect labeling step of the target cells.

3.3 Quality Control of the Negatively Depleted Grafts by Flow Cytometry

In contrast to CD34$^+$ selection, where the determination of residual T-cells in the graft is easier due the lower number of cells in the graft (in the range of several hundreds of millions), the number of cells in a negatively depleted graft is much higher in the range of 10–100 billions. Therefore, extreme care has to be taken in the determination of residual TcRαβ T-cells. Errors or miscalculations can lead to severe and even lethal G*v*HD. Schumm and coworkers have described a method

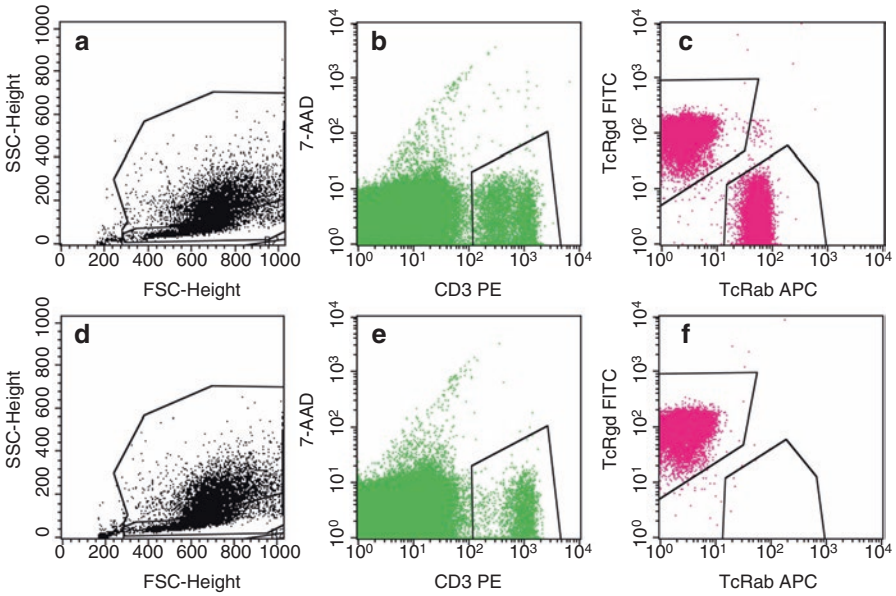


Fig. 3.4 Determination of residual αβ⁺ T-cells in the graft. After TcRαβ depletion, the graft is spiked with 3% of TcRαβ-positive T-cells obtained from the magnetic column, and gates are set on lymphoid cells (**a**), viable and CD3⁺ T-cells (**b**), and TcRαβ and γδ T-cells (**c**). These gates are then used for the determination of the residual T-cells in the depleted graft (**d**–**f**)

which allows the accurate determination of residual T-cells [9]. In Fig. 3.4, an example is shown. After TcRαβ depletion, the graft is spiked with 3% of TcRαβ-positive T-cells obtained from the magnetic column, and gates are set on lymphoid cells (a), viable and CD3⁺ T-cells (b), and TcRαβ and γδ T-cells (c). These gates are then used for the determination of the residual T-cells in the depleted graft (d–f). A more recently described method is the use of 10-color quality control panel [10]. However, in this analysis in a limited number of donors, the $\log^{10}$ depletion was only 3.9 log, and a head-to-head comparison between the two methods is warranted.

3.4 Clinical Experience in Haploidentical Transplantation Using TcRαβ-Depleted Peripheral Mobilized Grafts

Since the introduction of this method and the availability of clinical grade reagents, a number of smaller and larger clinical studies and case reports were published mainly in pediatric patients and few studies in adult patients.

3.4.1 Clinical Experience in Children

A series of 41 patients with mainly malignant diseases and few nonmalignant diagnosis was reported by Lang and coworkers [11]. The conditioning was based on

fludarabine or clofarabine, thiotepa, and melphalan. The first seven patients received the anti-CD3 antibody murononab for rejection prophylaxis, and the remaining patients received ATG-Fresenius after withdrawal of murononab from the market. A short course of mycophenolate mofetil (MMF) was given from day –1 to day +30. The median number of graft-containing TcRαβ T-cells was 16.9×10^3/kg of recipient weight. Primary engraftment was seen in 88% of the patients with a median time to neutrophils $>0.5 \times 10^9$/L of 10 days (range 7–21 days). Five patients rejected the graft and were successfully rescued by transplantation of TcRαβ-depleted grafts from another haploidentical donor. Acute G*v*HD (aG*v*HD) grade 0–I was seen in 31 patients (76%), 4 patients had grade II aG*v*HD, and 6 patients (15%) experienced grade III or IV aG*v*HD. Eighteen and nine percent of the patients showed a limited and extensive chronic G*v*HD, respectively.

The analysis of the immune reconstitution showed a rapid early expansion of γδ T-cells starting already at day +7 after graft infusion followed by the expansion of TcRαβ$^+$ T-cells several weeks later. There was also a rapid expansion of CD56$^+$ NK cells. A retrospective comparison with patients who obtained a CD34 positively selected graft showed a faster recovery of the CD3$^+$ T-cells at day +30 and +90, a faster recovery of CD4$^+$ T-cells at day +30 and faster recovery of CD56$^+$ NK cells at day +30 in the TcRαβ-depleted group. Due to the concomitant depletion of CD19$^+$ B-cells, the B-cell recovery was delayed and started around day +30.

At the European Bone Marrow Transplantation (EBMT) meeting 2016 in Valencia, Spain, Bertaina and coworkers presented data on 80 children with acute leukemia who received TcRαβ-depleted grafts after a myeloablative conditioning (MAC) regimen [12] and compared the outcome with a group of HLA-matched sibling ($n = 41$) and HLA-matched unrelated ($n = 51$) transplants performed at the same center. No G*v*HD prophylaxis was given. Two patients experienced primary graft failure. The cumulative incidence (CI) of aG*v*HD was 30%, but none of the patient had gut or liver involvement, whereas the CI of visceral aG*v*HD was 17% for the HLA-matched sibling and 16.3% for the HLA-matched unrelated group. The CI of limited chronic G*v*HD was 5.4%. With a median follow-up of 30 months, the 3-year probability of event-free survival (EFS) was 73.1% for the haplo-HCT, 66.1% for the HLA-matched sibling group, and 65.4% for the HLA-matched unrelated group.

The same author treated 23 children with nonmalignant disorders using TcRαβ-/CD19-depleted haploidentical grafts [13]. The patients received a busulfan- or treosulfan-based preparative regimen. In addition to the CD19$^+$ B-cell depletion, a single dose of rituximab (200 mg/m^2) was applied at day –1. Remarkably, no post-transplant immunosuppression was given, and the number of infused TcRαβ$^+$ T-cells was 40×10^3/kg of recipient weight. All but four patients were engrafted and the four non-engrafted patients were rescued by a second allograft. Three patients had skin-only grade I–II aG*v*HD, and no patient developed visceral acute or chronic G*v*HD. The cumulative incidence of transplant-related mortality (TRM) was 9.3%. With a median follow-up of 18 months, 21 of the 23 children are alive and disease-free with a 2-year probability of disease-free survival (DFS) of 91.1%. The analysis of the immune reconstitution showed a rapid expansion of γδ T-cells and CD56$^+$ NK

cells and a subsequent slower recovery of $\alpha\beta^+$ T-cells. The B-cell recovery was delayed and started at 6–9 months posttransplant.

The same group analyzed the γδ T-cell recovery in detail in 27 children transplanted for malignant and nonmalignant diseases [14]. They found that γδ T-cells are the early predominant T-cell population comprised of central-memory Vδ1 and Vδ2 subsets. Interestingly, the Vδ1 subset was specifically predominant in patients who experienced a cytomegalovirus (CMV) reactivation. They could also show that these subsets of T-cells displayed a cytotoxic phenotype and degranulated when challenged with primary acute myeloid and lymphatic blasts and that Vδ2 cells expanded after *in vitro* exposure to zoledronic acid and effectively killed primary lymphoid and myeloid blasts.

The same group also analyzed the impact of CMV infection on the development of NK cells in 27 patients with malignant diseases who received TcRαβ-depleted haploidentical grafts [15]. Most children showed a progressive expansion of memory-like NK cells expressing NKG2C, a putative receptor for human CMV and CD57, which is a marker for terminal NK cell differentiation. $NKG2C^+CD57^+$ NK cells were detectable at month 3 and expanded until at least month 12 after transplant. The cells were further characterized by the expression of killer Ig-like receptors (KIRs), leukocyte inhibitory receptors 1 (LIR-1), low Siglec-7 and NKG2A, and cytotoxicity against tumor targets.

Another study by Im and coworkers reported a series of 42 children and adolescents who received TcRαβ-depleted grafts repleted with an add-back of $\alpha\beta^+$ T-cells at $1–5 \times 10^5$/kg of recipient weight after a uniform-reduced intensity conditioning regimen comprised of fludarabine, cyclophosphamide, ATG, and low-dose total body irradiation (TBI) and posttransplant immunosuppression with tacrolimus and MMF [16]. All 42 patients achieved neutrophil engraftment at a median of 10 days (range 9–17 days). The CI of ≥grade II and ≥grade III aG*v*HD was 31% and 12%, respectively, and the 1-year CI of chronic G*v*HD was 15%. Only one patient died of CMV pneumonia, resulting in a TRM of 2.6%. Sixteen patients relapsed, and 11 died of disease. The estimated 2-year event-free survival (EFS) for patients with nonmalignant and hematological malignancies was 88% and 50%, respectively.

The group of Maschan and coworkers reported on 33 patients with acute myeloid leukemia, who received TcRαβ-depleted grafts from HLA-matched unrelated (20 patients) and haploidentical (13 patients) donors [17]. All patients achieved primary engraftment. The TRM was 10% with an EFS and overall survival (OS) of 60% and 67%, respectively. Also at the EBMT meeting in 2016, Karakukcu and coworkers presented data on 30 children with mostly malignant disorders [18]. Engraftment was rapid with 12 days (range 9–28 days) for myeloid and 12 days (range 9–33 days) for platelets. Grade II skin G*v*HD was observed in five patients and grade II–III gastrointestinal and liver G*v*HD in four and two patients, respectively. A short course of MMF was given only if the graft contained $>25 \times 10^3$/kg of recipient weight $TcR\alpha\beta^+$ T-cells. The TRM was 16.6%, and the OS is 63.6% with a median follow-up of 18 months. At the same meeting, Park and coworkers [19] reported on 41 patients with mainly acute leukemia and other disorders. At day 14, the predominant T-cell population was γδ T-cells, which then gradually decreased, while the percentage of αβ

T-cells gradually increased. Grade II–IV G*v*HD was seen in 11 patients. Patients with a higher percentage of γδ T-cells at day 30 had no grade II–IV G*v*HD, whereas patients with lower numbers had a higher incidence of grade II–IV G*v*HD (40.9 ± 10%, $p = 0.05$). The patients who relapsed had a lower percentage of γδT-cells compared to patients who did not relapse (median 33.3% *vs.* 51.6%, $p = 0.05$).

In addition to these larger studies, various successful case reports have been reported using haploidentical TcRαβ-depleted grafts in a patient with severe aplastic anemia (SAA) and aspergillosis [20], in a patient with Wiskott-Aldrich syndrome [21], in a patient with DOCK8 deficiency and severe pretransplant viremia [22], and in a patient with severe combined immunodeficiency with additional donor lymphocyte infusions with CD45RA-depleted lymphocytes [23] (see Chap. 12).

3.4.2 Clinical Experience in Adult Patients

Up to date, there are only few data available regarding haploidentical transplantation of TcRαβ-depleted grafts in adult population. To determine whether the post-transplant immunological reconstitution can be improved in adult patients, this approach has been recently tested in 25 patients, median age 45 years (range 19–71), with AML ($n = 19$), ALL ($n = 5$), MDS ($n = 1$) (Aversa F et al, unpublished results). Nine patients were in CR1, 5 in CR2, and 11 in advanced-stage disease at transplant. The chemotherapy alone-based conditioning consisted of ATG 1.5 mg/kg from day –13 to day –10, treosulfan 12 g/m² from –9 to –7, fludarabine 30 mg/m² from –6 to –2, and thiotepa 5 mg/kg on days –5 and –4. No additional pharmacologic prophylaxis of G*v*HD was given after transplantation. G-CSF (10 μg/kg of donor's weight) was used to mobilize PB $CD34^+$ cells. One-haplotype mismatched donors were as following: three mothers, five brothers, two sisters, five sons, five daughters, and five cousins. Depletion was performed using the CliniMACS device. Grafts contained a median of 11.6×10^6/kg of recipient weight (range 5–19) $CD34^+$ cells, 4×10^6 $CD3^+$ T-cells/kg of recipient weight (range 1–35), 4.4×10^4/kg (range 0.4–62) $\alpha\beta^+$T-cells/kg of recipient weight, 3.85×10^6 $\gamma\delta^+$ T-cells/kg of recipient weight (range1–34), 4.9×10^4 B-cells/kg of recipient weight (range 1.8–32), and 23.40×10^6 $CD56^+$ NK cells/kg of recipient weight (range 8–91). All but one patient, who required a second graft from the same donor to boost hematopoietic reconstitution, achieved a full donor sustained engraftment. Median time to reach 0.5×10^9/L neutrophils and 20×10^9/L platelets was 12 (range 10–18) and 11 days (range 6–16), respectively. Overall, aG*v*HD occurred in four patients. Only one patient, who had received the highest dose of $\alpha\beta^+$ T-cells (37×10^4/kg), developed and died from grade III–IV aG*v*HD. Skin limited aG*v*HD occurred in the remaining three patients (two recovered after short course of steroids, one after extracorporeal photopheresis); no patient has so far developed liver and/or intestinal G*v*HD. Only one patient progressed to chronic G*v*HD that was successfully treated with steroids and MMF. Tending to confirm the working hypothesis, there was a rapid, sustained increase in peripheral blood T-cell subpopulations. The $CD4^+$ and $CD8^+$ cell counts reached 0.2×10^9/L on days 45

(range, 19–98) and 38 (range, 13–69), respectively. Naïve and memory T-cell subsets increased significantly during first year after transplantation. The B-cell reconstitution was rapid and sustained, and immunoglobulin serum levels normalized within 3 months after transplantation. The CMV reactivation occurred in five cases (in one, donor/recipient CMV serology was unfavorable); no patient has so far developed CMV disease. In two patients, CMV reactivation was associated with a significant expansion of pathogen-specific $CD8^+$ T-cells, and both cleared viral load spontaneously. No patient had EBV-related PTLD, and no invasive fungal disease occurred. Two patients died from non-leukemia causes (one heart failure and one severe aG*v*HD). The 10% probability of transplant-related mortality was extremely low despite the median age of these 25 patients with 10 of them in the upper age for transplantation (between 55 and 71 years). Relapse was the main cause of failure (8 of 11) for patients transplanted with active disease. At a median follow-up of 12 months (range 2–32), 12 of 25 patients survive and are disease-free (10 of 14 in CR and 2 of 11 in relapse at transplant).

Kaynar and coworkers reported on the immune recovery in adult patients [24]. Thirty-four patients with acute leukemia with a median age of 28 years (range 18–60) were conditioned with fludarabine, thiotepa, and melphalan. ATG-Fresenius was given upfront at day –12 to –9 and MMF was given if the graft contained more than 25×10^3/kg of recipient weight $\alpha\beta^+$ T-cells. All but three patients were engrafted, and one of them died from bacterial infection. The other two patients were successfully retransplanted. The median time to reach neutrophils $>0.5 \times 10^9$/L and platelets $>20 \times 10^9$/L was 12 days (range 10–15) and 11 days (range 10–12), respectively. Eleven patients experienced aG*v*HD, and two patients had gut and liver grade IV aG*v*HD. Two patients developed chronic G*v*HD. Four patients died from disease and seven from TRM with a short follow-up of 191 days (range 35–933).

The results of an ongoing company-sponsored (Miltenyi Biotec) multicenter trial in Germany (six pediatric and six adult centers) and one center in the Netherlands registered at EUDRACT No. 2011-005562-38 with 30 pediatric and 30 adult patients are eagerly awaited in full publication [25] with longer follow-up.

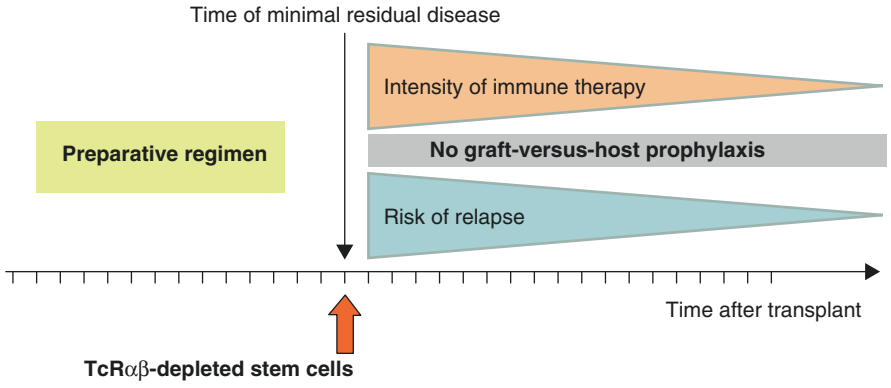
3.5 Expert Point of View

In contrast to T-replete haploidentical transplants, *ex vivo* T-cell-depleted grafts offer the reduction or even the complete omission of pharmacological posttransplant immunosuppression. The negative depletion of $TcR\alpha\beta^+$ T-cells retains potent and potentially important effector cells in the graft, such as NK cells and $\gamma\delta^+$ T-cells. Both effector populations have been shown to have anti-infectious and antitumor activities, while both cell types do not cause an alloreactive immune response leading to G*v*HD. After infusion, these effector cells are rapidly expanding which leads to a faster immune recovery compared to previously used TCD methods. Especially alloreactive NK cells play an important role in the prevention of relapse in adult patients with AML [26] and pediatric patients with ALL [27], and the transplantation of TcRαβ-depleted grafts is associated with the adoptive transfer of large

numbers of donor NK and γδ⁺ T-cells. The NK cells can further be activated via the induction of the antibody-dependent cellular cytotoxicity (ADCC) via their Fc receptors using antibodies directed against target antigens expressed on the malignant cells, which even overcomes the inhibition induced by the killer Ig-like inhibitory receptors (KIRs) expressed on NK cells [28]. NK cells also seem to be able to mount a memory-like response to CMV, which might concomitantly increase their cytotoxicity against leukemic blasts [29, 30]. In addition, the cytotoxicity of NK cells can further be increase by the application of cytokines, such as interleukin-2 (IL-2) [31] or more recently interleukin-15 (IL-15) [32]. Besides the NK cells, the adoptive transfer of γδ⁺ T-cells and their early rapid expansion might also have some beneficial effect on the immune recovery with a better protection against viral infection or against relapses.

It has been shown that γδ⁺ T-cells from G-CSF-mobilized donors retain strong tumoricidal activity and produce immunomodulatory cytokines [33] and the adoptive transfer of these cells together with the graft might also contribute to the acceleration of the immune recovery of other cell population posttransplant.

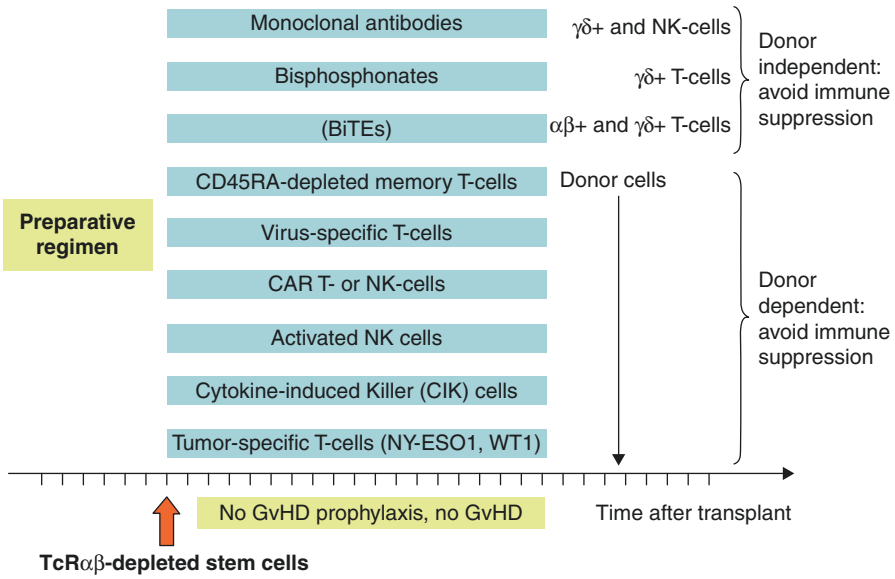
Similar to NK cells, γδ⁺ T-cells can also perform ADCC and can be activated *in vivo* early after transplantation by antibodies [34]. Moreover, cell proliferation and cytotoxic function including the ADCC of γδ⁺ T-cells can be induced by zoledronic acid [35] or the synthetic nonpeptide phosphoantigen bromohydrin pyrophosphate [36], which could be given early after transplantation at the time of the recovery of γδ⁺ T-cells at around days 7–14. A combination of zoledronic acid together with monoclonal antibodies could therefore result in a strong antileukemic effect early after transplantation. Especially in patients not in remission at time of transplantation, early immunological intervention strategies starting directly with the infusion of the TcRαβ-depleted grafts and at the timepoint where the patient has the lowest tumor burden might be necessary to prevent relapses and improve the outcome (Schema 3.1).



Schema 3.1 Early immunological intervention strategies starting directly with the infusion of the TcRαβ-depleted grafts and at the timepoint where the patient has the lowest tumor burden are feasible due to the absence of immune suppression for the prevention of G*v*HD

3.6 Future Directions

The omission of posttransplant immune suppression and the continuous availability of the donor after transplantation offer unique opportunities for further posttransplant immunotherapeutic strategies. In Schema 3.2, such strategies are shown and include early intervention with antibodies to stimulate the ADCC of NK- and γδ T-cells, the use of bispecific T-cell engagers (BiTes) to activate αβ- and γδ T-cells without inducing G*v*HD [37], and the additional use of aminobisphosphonates to stimulate the expansion and cytotoxic function of γδ T-cells [38]. While these strategies do not require the availability of the donor, they do require the absence of immune suppression. For further cellular therapeutic strategies, the continuous availability of the donor is necessary. These strategies include the adoptive transfer of virus-specific T-cells in case of therapy-refractory viral infections [39–41]; the adoptive transfer of chimeric antigen receptor (CAR) T-cells [42] or CAR NK cells [43]; the adoptive transfer of NK cells ex vivo activated with cytokines, such as IL-15 [44]; or the infusion of cytokine-induced killer (CIK) cells [45]. More recently, methods have been developed to generate donor-derived tumor-specific T-cells directed against NY-ESO-1 or other antigens expressed on tumor cells [46]. Furthermore, it might be possible in the future to vaccinate the donor with peptides derived from mutations specific for the patient's tumor [47] and transfer this donor immunity to the patient. With the future development of closed and completely automated systems such



Schema 3.2 Haploidentical transplantation with TcRαβ-depleted grafts can be a platform for further posttransplant immunotherapeutic strategies. All the depicted approaches are already or can readily be applied in clinical settings

as the Prodigy device [48], these strategies can become a reality in the treatment of patients with otherwise non-curable diseases.

Conflict of Interest R.H. is a co-patent holder of TcRαβ depletion.

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4 Adoptive Immunotherapy with Regulatory and Conventional T-cells in Haploidentical T-cell Depleted Transplantation Protects from GvHD and Exerts GvL Effect

Massimo F. Martelli, Mauro Di Ianni, and Loredana Ruggeri

4.1 Introduction

After conventional ("unmanipulated") allogeneic hematopoietic cell transplantation, eradication of residual disease, i.e., the so-called graft-*versus*-leukemia (G*v*L) effect, depends on donor T-lymphocytes which recognize host histocompatibility antigens on leukemic cells. Crossing the histoincompatibility barrier is feasible without *ex vivo* T-cell depletion (TCD). However, such transplant is far from optimal because it is associated with high incidence of relapse and risk of graft-*versus*-host disease (G*v*HD). Recent clinical trials suggest that adoptive immunotherapy with regulatory and conventional T-lymphocytes prevents G*v*HD while allowing a G*v*L effect in acute leukemia patients undergoing T-cell-depleted haplo-transplantation. We discuss the clinical relevance of this new immunotherapeutic strategy and the mechanisms underlying the separation of G*v*L effect from G*v*HD.

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4.2 Allogeneic Transplantation as Immunotherapy for Acute Leukemia

In patients with acute leukemia at high risk of relapse because of unfavorable cytogenetics, molecular markers, and disease status, the most powerful post-remission therapy is allogeneic hematopoietic cell transplantation (allo-HCT) from an HLA-matched sibling (MSD) or unrelated donor (MUD). When patients do not have an HLA-MSD or HLA-MUD, unrelated cord blood (UCB) and HLA-haploidentical-donor HCTs have emerged as alternatives.

In eradicating residual disease, clinical observations [1, 2] and experimental models [3–5] established that donor T-lymphocytes in the graft play a crucial role. This so-called G*v*L depends on recognition of host alloantigens on leukemic cells, even though hematopoietic-specific and leukemia-specific responses may also occur [6]. On the other hand, alloreactive donor T-cells also mediate immune destruction of host tissues, i.e., G*v*HD, a major cause of morbidity and mortality after allo-HCT. G*v*HD prevention has, until today, mainly relied on pharmacological immunosuppression to functionally inactivate donor T-cells after T-cell replete grafts. This immunologically non-specific strategy is only partially efficacious and, above all, weakens the G*v*L effect. Indeed post-transplant relapse is still the major cause of treatment failure in high-risk acute leukemia patients, independently of graft type.

For instance, Gupta and coworkers (2010) reported 37% cumulative incidence of relapse and 42% 3-year leukemia-free survival (LFS) in 226 acute myeloid leukemia (AML) patients with unfavorable cytogenetics who underwent HLA-MSD transplants. Similar outcomes (40% relapse and 34% LFS) were observed in 254 HLA-MUD transplant recipients. The cumulative incidence of grade II to IV acute G*v*HD at 100 days was 38% and 54% in patients undergoing HLA-MSD and HLA-MUD transplantation, respectively. The 3-year cumulative incidence of transplant-related mortality (TRM) was, respectively, 21% and 26% after HLA-MSD and HLA-MUD transplantation [7].

In adults with acute leukemia who received transplant with 1 (n = 106) or 2 (n = 303) UCB units, the 2-year cumulative incidence of relapse was 32% with 38% TRM after single and 36% relapse with 32% TRM after double UCB transplantation (dUCBT). The probability of LFS after single or double UCB transplantation was around 32%. The day-100 probabilities of grade II–IV acute G*v*HD were, respectively, 27% and 31% [8].

Other studies reported lower relapse risks after dUCBT compared with single UCB transplantation for patients in remission. However, neither report demonstrated differences in LFS [9, 10].

In haplo-HCT the infusion of a megadose (on average 10×10^6 $CD34^+$ cells/kg of recipient body weight) of TCD G-CSF-mobilized, peripheral blood (PB) hematopoietic progenitor cells was associated with a high rate of engraftment, with a low incidence of G*v*HD, even though no post-transplant immunosuppression was given ([11–13]; see Chap. 2). In a large series of acute leukemia patients, LFS was 50% for AML in complete remission (CR) 1 and 35% for AML CR ≥2 at 18 years. Similar results with 48% LFS in CR1 AML patients were reported by a

retrospective study, analyzing the outcome of "megadose" TCD haplo-HCT in several European centers [14].

These clinical studies led to the discovery that post-transplant generation of donor-*vs.*-recipient alloreactive NK cells induced a powerful graft-*versus*-AML effect in the absence of G*v*HD [15–17]. Indeed, an updated analysis of 32 patients with AML in any CR showed that transplants from NK-alloreactive donors were associated with a low 12% relapse rate *vs.* 30–35% for patients with acute lymphoblastic leukemia (ALL) or with AML who did not have NK-alloreactive donors. Since LFS was an encouraging 60%, NK cell alloreactivity which is potentially available for almost 50% of patients should become a major criterion for donor selection in haplo-HCT for high-risk AML patients (see Chaps. 5 and 10).

Crossing the histoincompatibility barrier is feasible without *ex vivo* TCD. The most commonly used strategy is based on administration of high doses of cyclophosphamide (Cy) in a narrow time window after transplantation [18]. Post-transplant Cy (PTCy) selectively counteracts both donor-derived and recipient proliferating alloreactive T-cells, thus reducing the risk of both G*v*HD and graft rejection (see Chaps. 7 and 8). Tacrolimus and mycophenolate mofetil (MMF) were also given as additional G*v*HD prophylaxis. Pre-transplant non-myeloablative (NMA) conditioning included Cy, fludarabine, and low-dose TBI. This type of transplant appeared well-tolerated, with a 4% incidence of G*v*HD and low NRM (15% at 2 years). However, the relapse rate was above 40%. As in other NMA transplant trials, the conditioning regimen might not have been intense enough to achieve sufficient leukemia debulking. Switching to myeloablative conditioning regimens (MAC) (i.e., busulfan- or TBI-based) reduced the risk of relapse but increased TRM. Consequently, overall survival remained unchanged. A large study compared haplo-HCT patients ($n = 192$) with HLA-MUD patients ($n = 1982$) who underwent transplant for AML [19]. Separate analyses were performed for MAC and NMA conditioning regimens. Three-year outcome data after MAC haplo-HCT and HLA-MUD HCT showed that relapse was 44% *vs.* 39% ($p = 0.37$) and TRM was 14% *vs.* 20% ($p = 0.14$), while OS was 45% *vs.* 50% ($p = 0.38$). In NMA cohorts, haplo-HCT was associated with more relapse (58% *vs.* 42%, $p = 0.0001$) and better TRM (9% *vs.* 23%, $p = 0.002$), but again OS was not significantly different (46% *vs.* 44%, $p = 0.71$) [19].

These data clearly showed the predominant cause of treatment failure, whatever the protocol and whoever the donor, was post-transplant relapse.

A different approach to overcoming the HLA barrier consisted of using "G-CSF-primed" bone marrow and peripheral blood [20] or bone marrow alone graft(s) [21]. Both approaches included a MAC and intensive post-transplant immunosuppression, i.e., ATG, cyclosporine, methotrexate, MMF, and basiliximab [21].

A multicenter study from China included 231 AML patients in CR1 (see Chap. 5). Engraftment was achieved in all patients. The cumulative incidences of grade II–IV and grade III–IV acute G*v*HD were 36% and 10%, respectively, with 42% chronic G*v*HD, which was severe in 12%. The relapse rate of 15% was associated with 13% TRM. The 3-year LFS was 74% [22]. Except for one Italian group, experience is limited with this technique outside China. An update of the initial Italian

study included 60 acute leukemia patients in first or second CR (early phase). The cumulative incidence of engraftment was 94 ± 3%. Grade II–IV and grade III–IV acute G*v*HD at 100 days were 24% and 5%, respectively. The 5-year TRM was 34%, relapse was 28%, and LFS was 48% [23] (see Chap. 5).

Outcomes in the study by Wang and coworkers (2015) were clearly much better than the great majority of other transplantation strategies, including the Italian group who employed a similar protocol to the Chinese group. Although it is always arduous to compare outcomes of non-randomized studies, the divergent outcomes may be due to several factors: Chinese patients were younger (median age 21 years) and fewer had very high-risk features such as poor cytogenetics and delayed CR.

In conclusion, whatever the transplantation strategy and whoever the donor, all these diverse forms of allo-HCTs do not have a strong enough antileukemic effect, showing conventional allo-HCT being far from the optimal form of immunotherapy. Preserving and exploiting the G*v*L effect without G*v*HD remains the central challenge in the allo-HCT field.

4.3 Overcoming Leukemia Relapse with Next-Generation Immunotherapy

4.3.1 Learning from Animal Models

Manipulation of alloreactive donor conventional T-cells (T_{cons}) so as to prevent G*v*HD while potentiating the G*v*L effect and rapid immune reconstitution focused attention on T-regulatory cells (T_{regs}), a thymic-derived $CD4^+CD25^+$ $FoxP3^+$ T-cell subpopulation which under physiologic conditions helps maintain immunological self-tolerance and immune homeostasis [24]. HLA-mismatched HCT murine models showed that freshly isolated [25–27], *ex vivo*-expanded polyclonal [28], or recipient-type, T_{regs} [29], when coinfused with T_{cons}, prevented lethal G*v*HD. T_{reg} infusion was followed by activation, expansion of alloantigen-specific T_{regs} in lymph nodes, and migration to G*v*HD target tissues (skin, gut, liver, lung) [26]. During their effector phase, T_{regs} specifically suppressed early alloreactive T-cell proliferation in lymph nodes and in nonlymphoid tissues by interacting with antigen-presenting cells (APCs) in priming sites. Conversely, T_{regs} did not cross-inhibit pathogen-specific T_{con} expansion and responses, thus allowing functional immune system reconstitution [27, 30, 31]. Indeed, they prevented G*v*HD-induced damage to the thymus and secondary lymphoid microenvironment, accelerated donor lymphoid expansion of a diverse T-cell receptor (TcR) V (variable) β-repertoire, and protected mice from lethal CMV infection. Supporting evidence was reported by Gaidot and coworkers (2011), using an HCT murine model that excluded thymic output, thus mimicking human adult post-transplant immune reconstitution which, for several months, derives only from mature T-cells within the graft [32].

In further investigations into T_{reg}/T_{con} reciprocal interference after allo-HCT, Trenado and coworkers (2003) [30], and Edinger and coworkers (2003) [33],

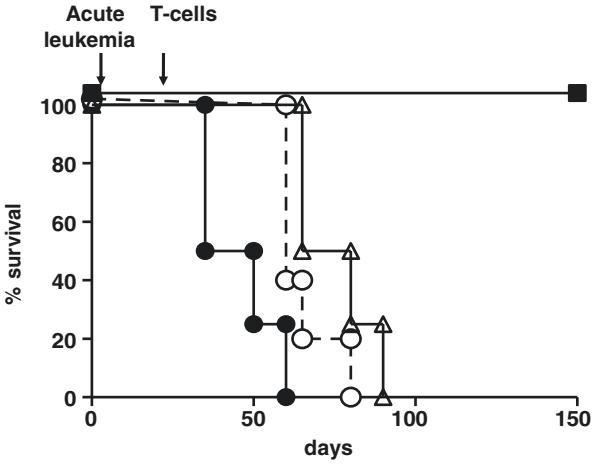

Fig. 4.1 Coinfused human T_{cons} and T_{regs} exert a G*v*L effect without G*v*HD in NSG mice with human acute leukemia. Mice engrafted with human acute leukemia died of leukemia if left untreated (open circle) or infused with T_{cons} (open triangle). T_{con}-treated mice (filled circle) died of G*v*HD; mice coinfused with T_{cons} and T_{regs} (filled square) survived without G*v*HD

showed that T_{regs} did not impair T_{con} control of neoplastic cell line expansion, such as A20 leukemic cells of BALB/c origin, BCL1 lymphoma, and P815 mastocytoma. Similarly, in humanized mouse models, i.e., mice engrafted with human myeloid primary leukemia or AML or ALL cell lines, adoptive transfer of human T_{regs} and T_{cons} eradicated leukemia without G*v*HD [34]. In contrast all mice that received only T_{regs} died of leukemia within 60–75 days. Mice that received only T_{cons} developed severe G*v*HD and died within 60 days (Fig. 4.1).

4.4 Adoptive Immunotherapy: Translating Lessons to the Clinical Setting in Haploidentical Transplants

Insights from animal models prompted us to incorporate adoptive immunotherapy with freshly isolated T_{regs} and broad repertoire T_{cons} into our original HCT platform [34, 35]. Indeed TCD haplo-HCT is an ideal platform for exploiting the G*v*L effect of adoptive cellular immunotherapy because of its high engraftment rates, ability to prevent G*v*HD, and no need for post-transplantation pharmacologic immunosuppression (see Chapter 2).

The updated T_{reg}/T_{con} trial included 59 consecutive acute leukemia patients: 41 with AML (22 CR1, 19 ≥ CR2) and 18 ALL (12 CR1; 6 ≥ CR2) patients. The median age was 40 years (range 20–59). All patients transplanted in CR1 were at high risk of relapse. The first 25 patients were conditioned to transplant with TBI (8 Gy in a single fraction), thiotepa (4 mg/kg/day for 2 days), fludarabine (40 mg/m^2/day for 5 days), and Cy (35 mg/kg/day for 2 days). To reduce non-hematological toxicity, the following 34 patients received either a lower dose (30 mg/kg) of Cy or anti-T antibodies (alemtuzumab or thymoglobulin) 21 days before T_{regs}. On day −4, all patients received freshly isolated T_{regs} ($2.5 \pm 1 \times 10^6$/kg) followed by $CD34^+$ cells ($9 \pm 3.2 \times 10^6$/kg of recipient body weight) and T_{cons} ($1.1 \pm 0.6 \times 10^6$/kg of recipient body weight) on day 0 (Fig. 4.2). Murine models had shown the 4-day gap between

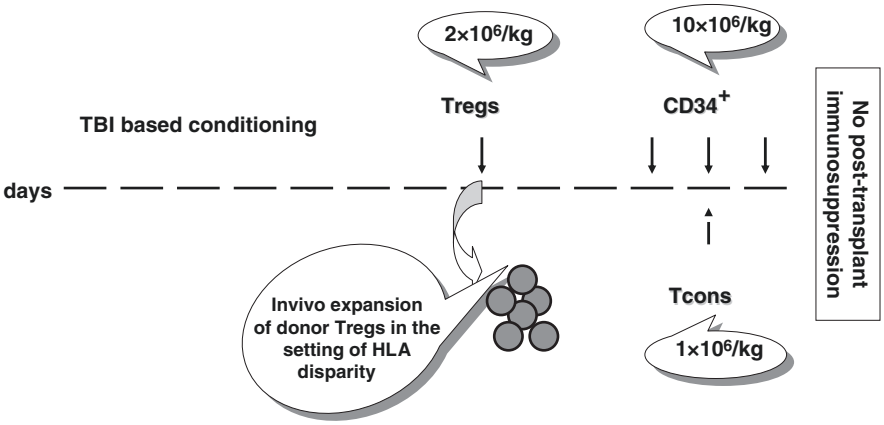

Fig. 4.2 Timing of T_{reg}, T_{con}, and $CD34^+$ cell infusion. Naturally occurring $CD4^+CD25^+$ $FoxP3^+$ T_{regs} were selected by means of a fully automated immunomagnetic procedure (Miltenyi) by depleting the leukapheresis product of $CD8^+/CD19^+$ cells and then positively immunoselecting $CD25^+$ cells. T_{cons} were separated from peripheral blood mononuclear cells by negative selection using Miltenyi CliniMACS CD19 reagent. $CD34^+$ cells were positively immunoselected using the using the CliniMacs (Miltenyi Biotec) one-step procedure

T_{reg} and T_{con} infusions, and the 2:1 T_{reg}/T_{con} ratio provided best protection against G*v*HD. No pharmacological post-transplant G*v*HD prophylaxis was given.

Rapid, full-donor-type engraftment occurred in 57 of 59 patients. As in animal models, early adoptive transfer of naturally occurring donor T_{regs} made administration of a high dose of mature T_{cons} feasible and kept the incidence of G*v*HD very low in the absence of any post-transplant pharmacological immunosuppression. Indeed only 8 of 57 patients (14%) developed grade II to IV acute G*v*HD. Two patients developed chronic G*v*HD. Even though 1.1–0.6 × 10^6 T_{cons}/kg of recipient body weight were infused, which is about 2 log more than the threshold dose for G*v*HD in haplo-HCT, the incidence of G*v*HD was similar ($P = 0.2$) to the 11% in T-cell-depleted historical controls. Brunstein and coworkers (2016) also reported that T_{reg} adoptive immunotherapy was an effective prophylaxis for G*v*HD in a dUCBT. In this study, T_{regs} were isolated from a third UCB unit 4–6 of 6 HLA-matched to the patient and expanded in cultures with K562 stimulatory cells that had been modified to express the high-affinity Fc receptor (CD64) and CD86, the natural ligand of CD28 (KT64/86). This methodology yielded a targeted T_{reg}-to-T_{con} cell ratio (≥1:1) for clinical purposes, which was similar to what had been employed in animal models. Eleven patients were treated with T_{reg} doses from 3 to 100 × 10^6 T_{regs}/kg of recipient body weight. In the presence of post-transplant immunosuppression with sirolimus and MMF, the cumulative incidence of grade II–IV acute G*v*HD at 100 days was 9% *vs.* 45% in controls. Chronic G*v*HD at 1 year was zero. This incidence of acute G*v*HD was also lower than in a previous study, reporting 43% in recipients of bead-stimulated UCB T_{regs} and 61% in historical controls [37].

In our transplant, recipients T_{regs}/T_{con} adoptive immunotherapy ensured a good immune recovery which was stronger and faster than in the historical controls. The

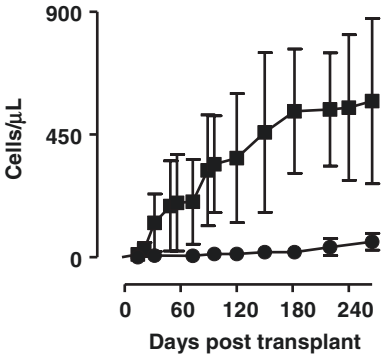

Fig. 4.3 Pattern of immune reconstitution. Recovery of $CD4^+$ T-cells in T_{reg}/T_{con} (filled square)-based haplo-transplants and in standard T-cell-depleted haplo-transplants (filled circle)

PB T-cell subpopulations increased rapidly (Fig. 4.3) displaying relatively high frequencies of pathogen-specific $CD4^+$ and $CD8^+$ T-lymphocytes against aspergillus, candida, CMV, and other opportunistic pathogens. As expected from experimental models, the presence of T_{regs} during early immune reconstitution induced not only large-scale peripheral T-cell pool expansion but also expression of a wider TcR repertoire. There were no CMV-related deaths, which had been one of the major causes of mortality in the historical control group. Patients were immunologically competent as vaccination against pandemic influenza with two doses of MF59-H1N1 induced strong protection in four of seven subjects within 2 months. Furthermore, adoptive transfer of T_{regs} did not impair NK cell post-transplant regeneration/maturation which was faster with enhanced donor-*versus*-recipient alloreactive NK cell repertoires against KIR-ligand mismatched targets.

In the pilot study testing T_{reg}/T_{con} adoptive immunotherapy for the first time, disease-free survival was 50%, because of a high TRM rate which needs to be viewed in light of the patients' clinical characteristics. Most patients had been heavily pretreated and some had active fungal disease before transplant [35]. In the next cohort of 34 patients, who received anti-T antibodies or low dose of Cy (30 mg/kg) in the conditioning, TRM fell to 22% and the probability of DFS rose to 61%.

One major concern about the use of T_{regs} in HCT for acute leukemia patients is their potential to suppress immune-mediated G*v*L responses, in light of reports indicating that T_{regs} may contribute to a defective immune response against solid tumors [38, 39] and hematological malignancies [40, 41]. However our clinical study showed that such worries and fears were totally unfounded. Indeed, the cumulative incidence of post-transplant relapse was 0.09 at a median follow-up of 44 months, which is extremely low considering these patients were at high risk of relapse according to cytogenetics, molecular markers, and disease stage at transplant. In the T_{reg}/T_{con} cohort, the cumulative incidence of relapse was significantly lower than in historical controls (0.09 *vs.* 0.21; $P = 0.04$) (Fig. 4.4). Multivariate analysis identified T_{reg}/T_{con} adoptive immunotherapy as the only predictive factor associated with a reduced risk of relapse (relative risk 0.06; 95% CI, 0.03–0.4; $P = 0.02$).

The mechanisms underlying T_{reg} suppression of G*v*HD with no loss of G*v*L can be explained by findings from several studies on the T_{reg}/T_{con} interrelationship and

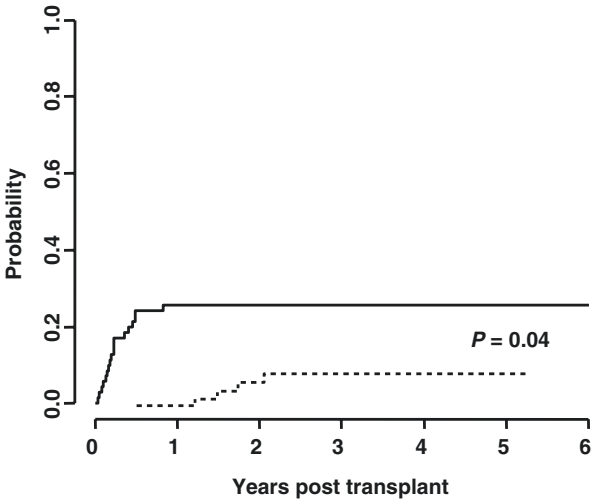

Fig. 4.4 Cumulative incidence of relapse in T_{reg}/T_{con}-based haploidentical transplantation (solid line) and in historical T-cell-depleted haplo-transplants (dotted line)

T_{reg} migration upon infusion into the recipient. In murine models, Edinger and coworkers (2003) showed that alloantigen-specific T_{regs} blocked early alloreactive T_{con} expansion in lymph nodes and consequently, their G*v*HD-inducing capacity. However, as they did not inhibit activation, allo-stimulated T_{cons} conserved their capacity to kill tumor cells *in vitro* and to eradicate established tumors.

In our humanized mouse models, human bone marrow T-cells killed human leukemia cells and autologous PHA blasts *in vitro*, showing their cytotoxicity was preserved [34]. In contrast purified $CD8^+$ T-cells from the spleen and liver displayed no alloreactivity against targets. Furthermore infused $CD4^+$ T-cells which retained their regulatory function were found in spleen and liver but not in bone marrow. Consequently T_{cons} were free to manifest their alloreactivity against leukemic cells in the bone marrow [42].

Pertinent are the following observations on the role of CXCR4 in T_{reg} homing and the function of T_{regs} in the hematopoietic stem cell niche. In humans, Booth and coworkers (2010) showed $CD45RO^+$ T_{regs} with low CXCR4 expression homed to the skin, whereas $CD45RA^+$ T_{regs} with high CXCR4 expression localized in bone marrow [43]. In non-irradiated recipient mice, Fujisaki and coworkers (2011) reported recipient $FoxP3^+$ T_{reg} cells accumulated on the endosteal surface, forming clusters around transplanted hematopoietic stem and progenitor cells (HSPCs). Bone marrow T_{reg} cells were critical in suppressing rejection of allo-HSPCs that survived for more than 30 days. Indeed T_{reg} depletion led to loss of these hematopoietic cells [44].

These findings lead to the following working hypothesis in clinical haplo-transplantation: (1) After infusion alloantigen-specific 100% $CD45RO^+$ donor T_{regs} are activated and expanded in lymph nodes. Depending on expression of homing molecules, they then migrate to the skin, gut, liver, lungs, etc. where they act as a second checkpoint for controlling T_{con} alloreactivity. G*v*HD prevention thus occurs not only in lymph nodes but also in peripheral tissues. (2) With low CXCR4

expression, T_{regs} do not home to the bone marrow and do not check T_{con} activity in bone marrow environment. Thus, alloreactive T_{cons} are free to lyse leukemic stem cells in the bone marrow.

In conclusion T_{reg}/T_{con} adoptive immunotherapy confines graft-*versus*-host reaction only to the hematopoietic system, consequently separating the G*v*HD from the G*v*L effect. Thus, the crucial dilemma in HCT—"if both G*v*HD and G*v*L result from alloreactivity, how can alloreactive T-cells spare normal cells (no G*v*HD) and kill leukemic cells (G*v*L)?"—may finally have its unconventional solution. Innovative T_{reg}/T_{con} immunotherapy could well constitute a major advance on "conventional" T-cell replete HCT which today is still only partially efficacious against patients with acute leukemia and is associated with a significant risk of acute and chronic G*v*HD.

4.5 Expert Point of View

Decades of research have led to "designed" grafts which contain not only a "mega-dose" of purified hematopoietic progenitor cells but also appropriate numbers of T_{regs} and T_{cons}. T_{reg}/T_{con} adoptive immunotherapy eliminates the need for pharmacological prophylaxis against G*v*HD which, as was shown above, inhibits T_{con} activity and so risks impairing the G*v*L effect. Thus, infusion of 1×10^6 T_{cons}/kg of recipient body weight is sufficient to eradicate minimal residual disease (MRD), markedly reducing the 30–40% incidence of relapse which is generally observed in T-cell replete HCTs for high-risk acute leukemia patients, independently of whether the donor is an HLA-matched sibling, HLA-matched unrelated volunteer, unrelated cord blood unit, or haplo-family member. Finally, it is worth noting that another, independent immunological mechanism, donor-*vs.*-recipient NK cell alloreactivity, strengthens the T_{con}-related G*v*L effect in AML patients who are transplanted from NK-alloreactive donors. Indeed so far, no relapse has occurred in this setting. These exciting results are reflected in the 3-year DFS which rose to an encouraging 61% in high-risk patients who were transplanted under the current protocol.

Achieving a suitable number of freshly isolated T_{regs} was a major obstacle to their clinical use in haplo-transplant, bearing in mind that ≥2:1 is the optimal T_{reg}/T_{con} ratio to prevent G*v*HD. Since an adult donor can usually provide around $2–3 \times 10^6$ T_{regs}/kg recipient body weight, infused T_{cons} cannot rise above 1×10^6/kg of recipient weight. These limits are easily surpassed through the use of *ex vivo*-expanded T_{regs}. For instance, Brunstein and coworkers (2016) managed to infuse up to 100×10^6 T_{regs}/kg of recipient body weight together with double cord blood units containing a large number of T_{cons}. It remains to be established whether more T_{regs}/T_{cons} are always better for the patients [36].

Our study, which included 59 acute leukemia patients with a long follow-up, suggests that adoptive immunotherapy with T_{regs} does not require *ex vivo* T_{reg} expansion systems. Indeed infusion of 1×10^6 T_{cons}/kg of recipient body weight under the protective umbrella of 2×10^6 T_{regs}/kg ensures rapid immunological reconstitution and powerful G*v*L effect. Better results might perhaps be achieved with carefully "designed grafts" including, for instance, one log more of *ex vivo*-expanded T_{regs} and

T_{cons} together with a megadose of $CD34^+$ cells. *Ex vivo* T_{reg} expansion systems require, however, GMP manufacture which is expensive and not always available and requires expert, dedicated laboratory staff. Furthermore, a certain percentage of products not infrequently fail to meet the target cell dose.

Whether freshly isolated or *ex vivo*-expanded T_{regs} are employed, several crucial points need to be emphasized:

1. To prevent G*v*HD, the T_{reg}/T_{con} ratio must be 2:1.
2. T_{regs} should be infused 3–4 days before the T_{cons}.
3. No pharmacological prophylaxis for G*v*HD should be administered post-transplant so as to ensure a strong G*v*L effect and optimal immune reconstitution.

4.6 Future Directions

The next step forward in haplo-HCT is to expand the pool of eligible patients, thus including the elderly and unfit. To this aim, a low toxicity, myeloablative conditioning was designed, using an image-guided tomographic intensity-modulated radiation therapy delivery system (helical tomotherapy (HT)). With HT high-dose irradiation is delivered selectively to the bone marrow, major lymph node chains, and spleen (total marrow and lymphoid irradiation (TMLI)), while median doses to all major organs are substantially lower than those associated with conventional 12 Gy TBI [45]. An ongoing pilot study is testing the feasibility of TMLI treatment in high-risk acute leukemia patients over 60 years of age.

Another objective is to apply T_{reg}/T_{con} adoptive immunotherapy in TCD HLA-MSD transplant for high-risk acute leukemia patients. This would provide the opportunity to exploit the G*v*L effect of T_{cons} that recognizes minor histocompatibility antigens. Hopefully, this approach would significantly reduce the 35–40% post-transplant relapse rate which still represents a major drawback of T-cell replete HLA-MSD transplant for high-risk acute leukemia.

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5 Haploidentical Transplantation Using Unmanipulated G-CSF-Primed Blood and Marrow as Allografts: Clinical Data and Challenges

Ying-Jun Chang, Meng Lv, and Xiao-Jun Huang

5.1 Introduction

Haploidentical hematopoietic cell transplantation (haplo-HCT) is available for nearly all patients, with no search or acquisition costs to the patient [1–16]. However, successful use of haplo-HCT has been hampered by T-cell alloreactivity, due to differences in human leukocyte antigen (HLA) loci on an unshared chromosome 6 [8–14]. The first triumph over graft failure and severe graft-*versus*-host disease (G*v*HD) was attributed to infusing a mega dose of highly purified $CD34^+$ cells ($\geq 10\times10^6$/kg of recipient body weight) (see Chap. 2) [8]. Researchers from Germany found that haplo-HCT with specific $CD3^+/CD19^+$ graft depletion in the setting of reduced-intensity conditioning (RIC) could also allow a successful transplantation (see Chaps. 2 and 3) [9]. However, these approaches require expensive laboratory facilities and well-trained staff with high expertise in cell manipulation and are not widely applicable to most transplant centers [17].

Over the last 10 years, based on immune tolerance induced by granulocyte colony-stimulating factor (G-CSF) and antithymocyte globulin (ATG) [15, 18, 19], the Peking University groups established a protocol for unmanipulated haplo-HCT using myeloablative conditioning (MAC) regimen using G-CSF-primed bone marrow (G-BM) and peripheral blood (G-PB) hematopoietic $CD34^+$ cell grafts (Fig. 5.1) [20, 21]. A series of studies demonstrated that unmanipulated G-CSF-stimulated haploidentical grafts can lead to rapid immune recovery [22, 23], desirable health-related quality of life (QoL) [24], and a survival rate comparable to that following HLA-matched sibling donor hematopoietic cell transplant (MSD-HCT) or HLA-matched unrelated donor hematopoietic cell transplant (MUD-HCT) [25, 26]. This

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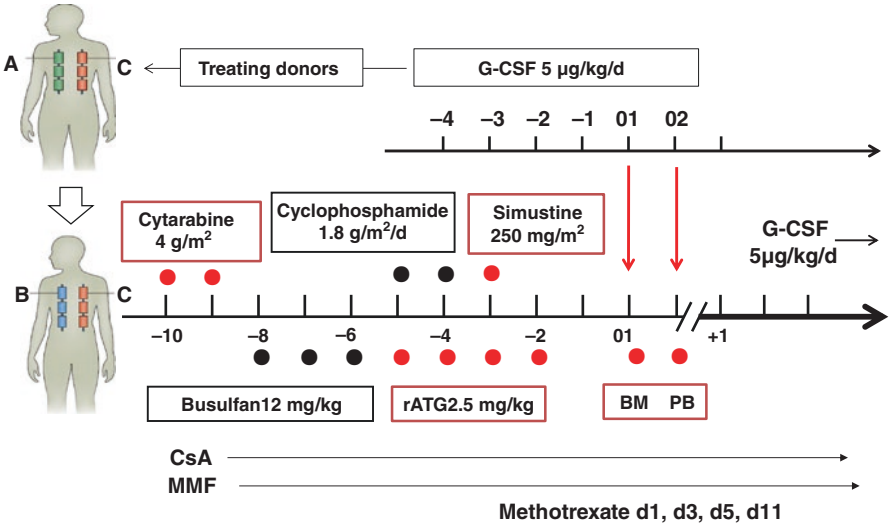

Fig. 5.1 Schema of unmanipulated haploidentical transplantation based on immune tolerance induced by G-CSF and ATG using myeloablative conditioning regimen in combination with hematopoietic myeloid growth factor (G-CSF)-primed bone marrow and peripheral blood allografts. *G-CSF* granulocyte colony-stimulating factor, *rATG* rabbit antihuman thymocyte immunoglobulin, *BM* bone marrow, *PB* peripheral blood allografts, *CsA* cyclosporine A, *MMF* mycophenolate mofetil

chapter begins with an overview of immunoregulatory effects of G-CSF, followed by a presentation of clinical results after unmanipulated haplo-HCT with ATG preparative regimen using G-BM and/or G-PB as allografts. Thereafter, strategies on improvement of the abovementioned transplant modalities are discussed.

5.2 Effects of G-CSF on Hematopoietic Graft

In humans, G-CSF treatment could significantly increase the $CD34^+$ cell numbers in BM and PB with maximum increases of 1.5- to 1.7- and 26-folds, respectively [27]. There were an approximately 50-fold increase in long-term culture-initiating cell (LTC-IC) activity and an approximately 90-fold increase in short-term repopulating cell activity in G-BM, although colony-forming cell (CFC) numbers showed very little change [27]. Presently, G-CSF has been widely known as a novel mediator of T-cell tolerance [18, 19, 28–34]. Franzke and coworkers [29] suggested a direct effect of G-CSF on human $CD4^+$ T cells through upregulating of GATA-3 and polarizing T-cell differentiation toward a Th2 type with an increase of interleukin-4 (IL-4) and decrease of interferon-γ (IFN-γ) production by suppressing the gene expression of ISGF3-γ subunit/p48 in $CD4^+$ T cells. However, the immune modulatory effect of G-CSF on T-cells is believed to be mediated exclusively through other

immunoregulatory cells [35], for example, through monocytes by downregulation of costimulatory molecules, increasing IL-10 production [36] and decreasing secretion of IL-12 and tumor necrosis factor-α [37], or selective mobilization of type 2 dendritic cells (DCs) skewing T cells toward a Th2 phenotype [28]. Furthermore, naïve $CD4^+$ T cells activated *in vitro* with regulatory or tolerogenic DCs generated after treating donors with G-CSF were hyporesponsive and acquired an $IL\text{-}10^{++}TGF\text{-}\beta$ (transform growth factor-β)$^+IL\text{-}2^{neg}IL\text{-}4^{neg}IL\text{-}5^+$ cytokine secretion profile [30]. Impressively, G-CSF-mobilized donors comprise the typical phenotype of the mononuclear and polymorphonuclear myeloid-derived suppressor cell (MDSC) subtypes [38, 39], regulatory T cells (Treg) [30], and regulatory B cells ($CD19^+CD24^{high}CD38^{high}$) [35] that have the capacity to regulate alloreactive T-cell responses. Overall, treating healthy donors with G-CSF can not only mobilize $CD34^+$ hematopoietic cells but also induce T-cell hyporesponsiveness and polarize T cells from Th1 to Th2 phenotype. Except for the direct role of G-CSF on T cells,

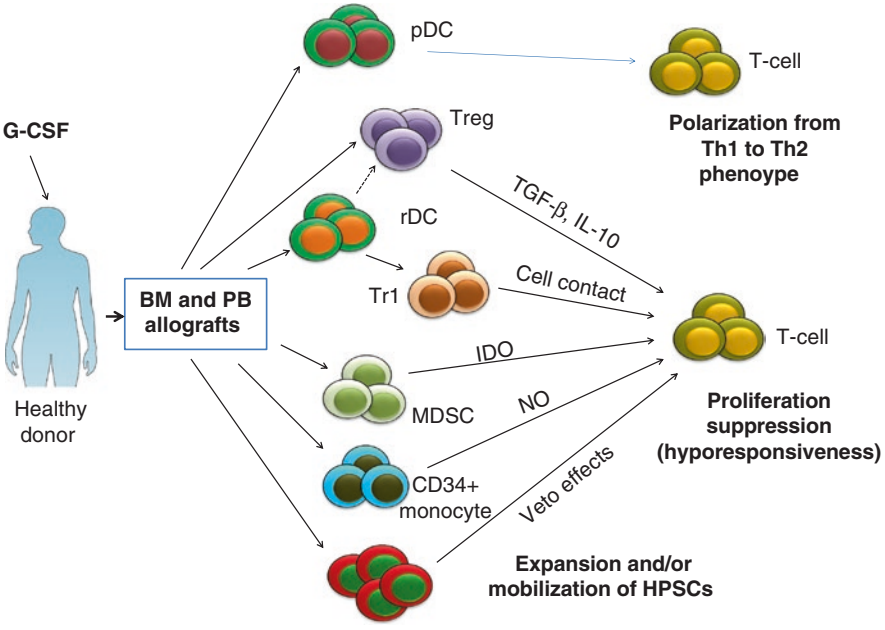

Fig. 5.2 Effects of granulocyte colony-stimulating factor (G-CSF) on hematopoietic and immune cells. G-CSF could selectively increase plasmacytoid dendritic cell subset that leads to polarization of T cell from Th1 phenotype to Th2 phenotype. Treating healthy donors with G-CSF can mobilize $CD34^+$ hematopoietic cells and expand regulatory dendritic cells, $CD4^+CD25^+Foxp3^+$T-cells, Foxp3(−) type 1 regulatory (Tr1) cells, myeloid-derived suppressor cells (MDSC), and $CD34^+$ monocytes, both of which can suppress the proliferation of T cells via different mechanisms. *TGF-β* transform growth factor-β, *IL-10* interleukin-10, *IDO* indoleamine 2,3-dioxygenase, *NO* nitric oxide

the process is involved in the complex interactions between T cells and other regulatory cells, such as MDSC, $CD34^+$ monocytes, as well as cytokines, including IL-10, TGF-β, indoleamine 2,3-dioxygenase, and nitric oxide (NO), both of which contribute to the proliferative hyporesponsiveness and suppressor activity of T cells (Fig. 5.2) [34, 39].

A number of studies showed the immunoregulatory effects of other cytokines, including granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-11, stem cell factors (SCF), and plerixafor (CXCR4 antagonist, AMD3100), and a combination of G-CSF and plerixafor. Compared to G-CSF, plerixafor-stimulated allografts contained more $CD4^+CD25^{high}CD127^{low}FoxP3^+$ T_{regs} and $CD8^+$ effector memory T cells ($CD28^-/CD95^+$ CD8 T cells), which can successfully induce early engraftment across the major histocompatibility complex (MHC)-mediated haploidentical barrier in canines [40]. Moreover, G-CSF plus plerixafor-mobilized grafts from patients with malignant disease contained a significantly higher number of plasmacytoid dendritic cells (pDCs) than those of G-CSF alone. These pDCs had a potential regulatory capacity [41]. Should these effects be confirmed in healthy donors [42–44], the combination of G-CSF and plerixafor might represent a novel approach for crossing HLA barriers [40, 41, 45].

5.3 Establishment of the Beijing Protocol for Haploidentical Transplants

5.3.1 Early Clinical Practice of the Unmanipulated G-CSF-Induced Bone Marrow and Peripheral Blood Allografts

The Peking University group began their clinical practice of unmanipulated haplo-HCT using G-CSF-stimulated BM and PB allografts in 2001 and reported the transplant outcomes of the first seven cases in 2004 [46]. In 2006, Huang and coworkers [20] reported the results of 171 patients with hematological malignancies who underwent unmanipulated haplo-HCT with grafts (BM and PB) primed with G-CSF. All patients achieved sustained, full donor chimerism. The median time for myeloid and platelet engraftment was 12 (range 9–26 days) and 15 days (range 8–151 days), respectively. The cumulative incidence of grade III–IV acute-extensive chronic G*v*HD was 23% and 47%, respectively. The 2-year probability of leukemia-free survival (LFS) for standard-risk and high-risk patients was 68% and 42%, respectively. The outcomes were not influenced by HLA disparity (Fig. 5.3) [20]. The preliminary results suggested that G-BM combined with G-PB from haploidentical donors, without *in vitro* T-cell depletion (TCD), may be used as a good source of graft for patients who lack HLA-matched donors. During the subsequent 10 years, the Peking University groups developed a number of strategies to further improve transplant outcomes of the unmanipulated haplo-HCT.

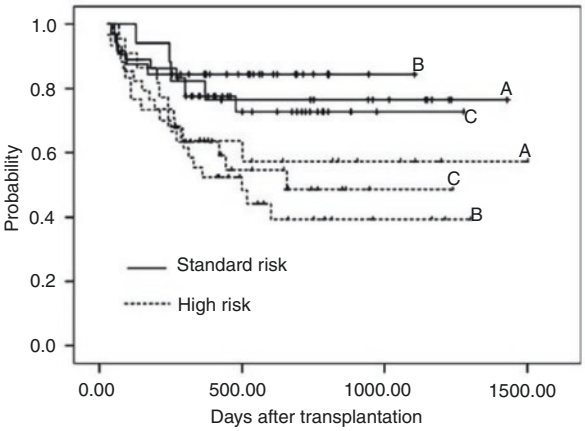

Fig. 5.3 Leukemia-free survival of 171 patients with hematological malignancies who underwent unmanipulated haploidentical blood and marrow transplantation. A, indicates patients with one mismatched human leukocyte antigen locus; B, indicates patients with two mismatched human leukocyte antigen loci; C, indicates patients with three mismatched human leukocyte antigen loci. Reprinted from Bone Marrow Transplantation. Huang XJ, Liu DH, Liu KY, et al. Haploidentical hematopoietic stem cell transplantation without *in vitro* T-cell depletion for the treatment of hematological malignancies. 2006,38:291–297. © 2006,with the permission from The Nature Publishing Group

5.3.2 Strategies to Improve Outcomes of the Unmanipulated G-CSF-Mobilized Haploidentical Transplants

Here, we provide recent progress, including the best donor selection, prophylaxis and treatment for leukemia relapse, prophylaxis for acute G*v*HD, and conditioning regimens, in the settings of the unmanipulated haplo-HCT modality [7, 47–49].

5.3.2.1 What is the Best Best Donor in Haploidentical Setting?

In the era of haplo-HCT, the candidates usually have multiple potential donors. Therefore, we reported the following results relevant to the selection of the best donor [4]. First, donor-specific anti-HLA antibodies (DSAs) should be considered, although controversy remains on cutoff value of the mean fluorescence intensity (MFI) (see Chapter 9) [50]. Second, researchers from Peking University found that patients receiving allografts from a donor younger than 30 years of age experienced superior transplant outcomes than those with a donor older than 30 years of age [4, 51]. Third, compared with female donors, male donors were associated with a lower incidence of G*v*HD, lower transplant-related mortality (TRM) rate, and higher overall survival (OS) and LFS rates. Fourth, the G*v*HD rate was not associated with the extent of HLA

disparity or any individual allele disparity [4]. Fifth, a better outcome with father donors than mother donors has been observed. Sixth, patients receiving non-inherited maternal antigen (NIMA)-mismatched donor allografts showed a lower incidence of grade II–IV acute G*v*HD [4]. Finally, killer cell immunoglobulin-like receptor (KIR) mismatch was associated with superior or inferior outcomes according to the type of transplant protocol [52, 53]. Here, we proposed an algorithm for haploidentical donor selection (Fig. 5.4) [48]. When choosing the best HLA-haploidentical donor, one should keep the following caveats in mind. (1) A single variable (such as natural killer cell alloreactivity) may have different effects on clinical outcomes in patients that receive different haplo-HCT protocols. (2) With improvements in haplo-HCT modalities, the impact of some variables (such as HLA locus mismatches) on transplant outcomes may vanish, while some new factors may emerge. For the detail of donor selection in other haplo-HCT modalities, see Chapter 10.

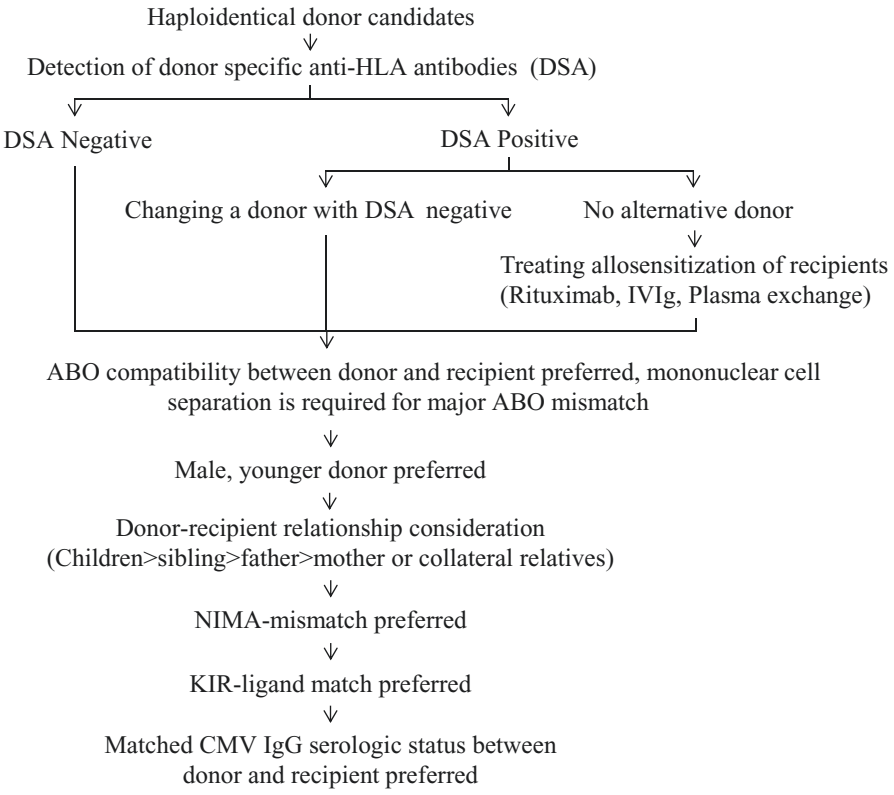

Fig. 5.4 Algorithm for haploidentical donor selection in unmanipulated haplo-HCT based on immune tolerance induced by G-CSF and ATG using myeloablative conditioning regimen in combination with hematopoietic myeloid growth factor (G-CSF)-primed bone marrow and peripheral blood allografts. *Haplo-HCT* haploidentical cell transplantation, *ATG* antithymocyte globulin, *IVIg* intravenous immunoglobulin, *CMV* cytomegalovirus, *NIMA* non-inherited maternal antigen, *KIR* inhibitory killer cell immunoglobulin-like receptor

5.3.2.2 Prophylaxis and Treatment of Relapse Disease

To decrease the leukemia relapse rate, a *modified* donor lymphocyte infusion (mDLI) protocol has been developed by Huang and coworkers [1, 2, 54, 55], which entails infusion of donor G-PB and short-term application of immunosuppressive agents with methotrexate (MTX) or cyclosporine A (CsA), after DLI for G*v*HD prophylaxis [1, 2, 56]. The 2-year probability of LFS was 40% in 20 relapsed patients after unmanipulated haplo-HCT [56]. For relapse prophylaxis, the mDLI resulted in 3-year LFS of 37.3 ± 9.6% in 29 patients with advanced leukemia after initial unmanipulated haplo-HCT [54]. The cumulative incidence of DLI-associated acute G*v*HD was 53.2% for grades II–IV and 28.4% for grades III–IV. The mDLI with G*v*HD prophylaxis for more than 6 weeks was associated with a lower incidence of grade III–IV acute G*v*HD [57]. Our results suggest that the mDLI protocol has been successfully used for relapse prophylaxis and treatment without compromising the G*v*L effects [1, 2, 54, 56]. The effects of DLI in treatment of relapse after haplo-HCT have been confirmed by several researchers, both in haplo-HCT with ATG-based regimens and in haplo-HCT with PTCy (see Chaps. 7 and 8) [58]. Early results suggest that preemptive IFN-α for minimal residual disease (MRD)-positive patients ($n = 22$) after haplo-HCT could potentially achieve disease-free survival (DFS) of 68.2% at 1 year. The regimen for IFN-α is as follows: Recombinant human IFN-α-2b injections (Anferon; Tianjin Hualida Biotechnology, Tianjin, China) were administered subcutaneously at a dosage of three million units 2–3 times per week, for a maximum of 6 months [59]. Moreover, the results from another center in China suggest that a combination of DLI and IFN-α may have synergistic effects promoting relapse prevention and treatment [60], which warrants further investigation in haplo-HCT setting.

On the basis of a previous observation of strong correlations between leukemia-associated aberrant immune phenotypes and WT1 and relapse, DFS, and survival in patients with acute leukemia receiving allo-HCT [61, 62], Yan and coworkers [1] prospectively studied the effects of risk stratification-directed interventions for MRD on relapse and DFS in 814 subjects with standard-risk acute leukemia who received allo-HCT in first or second complete remission. Patients with high relapse rate in a total of 709 subjects were MRD negative after transplantation (Group A), 105 subjects were MRD positive, 49 received low-dose IL-2 (Group B), and 56 received mDLI with or without low-dose IL-2 (Group C). Posttransplantation immunosuppression for G*v*HD was also modified based on MRD state. Group C showed significantly lower cumulative risk of relapse and higher DFS and OS than in subjects in Group B ($P = 0.001$ and $P = 0.002$, respectively), but did not differ from subjects in Group A. Multivariate analyses showed that MRD state and mDLI were significantly correlated with relapse and DFS. These data suggest that risk stratification-directed mDLI may reduce relapse and improve survival of subjects with standard-risk acute leukemia patients after allo-HCT.

5.3.2.3 Acute G*v*HD Prophylaxis After Haplo-HCT

The G*v*HD remains a common problem, with high rates, in particular, for unmanipulated haplo-HCT [4, 25, 26, 63]. More recently, we reported the results of a controlled, randomized, open-label trial to investigate whether risk-stratified corticosteroid could prevent acute G*v*HD after haplo-HCT [49]. This study included 228 recipients of

allotransplants. Based on bone marrow allograft CD4/CD8 ratios, patients were categorized as low risk ($N = 83$; Group A) or high risk ($N = 155$); patients at high risk were randomly assigned to receive ($N = 72$; Group B) or not receive ($N = 73$; Group C) low-dose corticosteroid prophylaxis. The 100-day incidence of acute G*v*HD, grades II–IV, in Group B was 21%, compared to 26% in Group A ($P = 0.43$) and 48% in Group C ($P < 0.001$). Low-dose corticosteroids were significantly associated with a relatively low risk of acute G*v*HD, grades II–IV (HR, 0.66; $P = 0.007$), and relatively rapid platelet recovery (HR, 0.30; $P < 0.0001$). The 100-day cumulative corticosteroid doses were 205 ± 111 mg in Group B, 229 ± 149 mg in Group A ($P = 0.256$), and 286.54 ± 259.67 mg in Group C ($P = 0.016$). Compared to Group C, Group B showed significantly lower incidences of femoral head necrosis ($P = 0.034$) and hypertension ($P = 0.015$) [49]. Our study suggests that stratification-directed prophylaxis of G*v*HD may represent an important future direction. Such approach [4] has an advantage to spare those patients with a low risk of developing G*v*HD from being exposed to additional immunosuppressive agents [47]. Stratification by using a biomarker allows a clinical trial to be more efficient by targeting the high-risk group, thereby reducing the total number of patients who need to be accrued to a trial.

5.3.2.4 Improvement on Conditioning Regimens

Currently, there is no standard conditioning regimen for haplo-HCT recipients. Therefore, several approaches have been adopted by our group.

Optimal ATG Dose: Do We Know the Answer?

Immune tolerance induced by ATG is an important rationale for unmanipulated haplo-HCT using G-BM and/or G-PB as allografts [25, 26, 64–70]. However, the optimal ATG dosage remains unclear across all types of allogeneic transplants. A recent randomized clinical study enrolled 224 patients with standard-risk hematological malignancies who underwent unmanipulated haplo-HCT, among whom 112 received 6 mg/kg ATG and the remaining patients received 10 mg/kg ATG [71]. The incidence of grade III–IV acute G*v*HD was higher among patients receiving the lower ATG dose (16.1% *versus* 4.5%, $P = 0.005$). On the other hand, the patients in the lower ATG dose group had a lower frequency of EBV reactivation (9.6% *versus* 25.3%, $P = 0.001$). Survival rates were comparable between patients in the two groups. These data indicate that, although 6 mg/kg ATG have decreased the incidence of Epstein-Barr virus (EBV) reactivation compared with 10 mg/kg ATG, however, such lower-dose ATG treatment cohort had higher risk of severe acute G*v*HD. Another randomized study from China showed that 7.5 mg/kg ATG might have similar efficacy in preventing acute G*v*HD after haplo-HCT compared with 10 mg/kg, although further studies are required to investigate whether patients receiving 7.5 mg/kg ATG have superior survival than those with 10 mg/kg [72]. Overall, the optimal dose of ATG in unmanipulated haplo-HCT settings remains unclear.

TBI-Based Conditioning Regimen

In a nested case-control study, Fu and coworkers [73] compared the total body irradiation (TBI, 700 cGy)/cyclophosphamide (Cy, 3.6 g/m^2)/semustine (250 mg/m^2) plus

ATG (TBI group, $n = 38$) with cytarabine (8 g/m^2)/intravenous busulfan (Bu, 9.6 mg/kg)/Cy (3.6 g/m^2)/semustine (250 mg/m^2) plus ATG (Bu group, $n = 77$) as preparative therapy for unmanipulated haploidentical graft in patients with acute leukemias. The authors found that only one graft failure occurred in the TBI group. The incidence and time of neutrophil and platelet engraftment were comparable between the two groups. Severe acute grade III and IV G*v*HD was observed in 13.4% of Bu group and only 2.6% of TBI group (P =0.083). More toxicity of the liver (37.7% *versus* 10.5%; P =0.002) and more hemorrhagic cystitis occurred in the Bu group (49.3% *versus* 23.7%, P =0.008) (see Chap. 20). Diarrhea was more common in the TBI group (44.7% *versus* 22.1%; P = 0.031). No significant differences were found in relapse, TRM, LFS, and OS between the two groups. This study indicates that the TBI/Cy plus ATG regimen seems to be feasible in T-cell-replete haplo-HCT, which promotes stable engraftment and a lower incidence of liver toxicity and hemorrhagic cystitis. However, longer follow-up is necessary to determine the late toxicity and relapse rates. In addition, prospective, multi-center, randomized clinical trials are needed to evaluated which is the best conditioning regimen, TBI-based or non-TBI based?

Conditioning Regimen for Severe Aplastic Anemia in Haploidentical Transplant Setting

At Peking University, we developed a MAC regimen to treat patients with severe aplastic anemia (SAA), which includes Bu 3.2 mg/kg on days −7 and −6, Cy 50 mg/kg/day from day −5 to day −2, and rabbit ATG 2.5 mg/kg/day or porcine ATG 20 mg/kg/day also from day −5 to day −2 [65]. All 19 patients who underwent this regimen achieved hematopoietic recovery, with a median of 12 days (range, 10–29 days) to myeloid engraftment and a median of 18 days (range, 8–180 days) to platelet engraftment. The cumulative incidences of grade II–IV acute G*v*HD and chronic G*v*HD were 42.1 ± 11.3% and 56.2 ± 12.4%, respectively. The OS was 64.6 ± 12.4%, with a median follow-up of 746 days (range, 90–1970 days) for surviving patients [65]. At another center in China, Gao and coworkers [74] treated 26 patients with SAA using a Flu/Cy/ATG-based conditioning regimen. They reported a 92.3% engraftment rate, with a median of 13 days (range, 11–19 days) to neutrophil engraftment and 13 days (range, 10–21 days) to platelet engraftment. Of 25 patients, 3 (12%) developed acute G*v*HD and 10 (40%) developed chronic G*v*HD (9 limited and 1 extensive). The OS rate was 84.6%, and the average follow-up time was 1313 days (range, 738–2005 days) for surviving patients. These results were further confirmed by other researchers [75–77]. Therefore, haplo-HCT could be a preferred treatment for SAA patients who lack an HLA-identical sibling donor and need urgent transplant. For conditioning regimen used for non-hematological malignancies in other haplo-HCT modalities, please see Chapter 11.

5.3.3 Where Do We Stand Now?

Overall, in the last decade, a haplo-HCT protocol based on immune tolerance induced by G-CSF and ATG, including donor selection algorithm, novel

conditioning regimen, prophylaxis for G*v*HD, and relapse, has been successfully established, which is also named as "the Beijing Protocol/Regimen" which has been reproduced in other centers [78]. More recently, a disease-specific prospective multicenter study compared the outcomes of 450 consecutive AML-CR1 patients undergoing unmanipulated G-CSF-primed haploidentical (BM and PB) grafts ($n = 231$) and HLA-MSD-HCT ($n = 219$) [6]. The 3-year cumulative incidences of TRM were 10% with haplo-HCT and 6% with HLA-MSD-HCT, and the respective relapse rates were 12% and 13%. Three-year probabilities of survival were 80% with haplo-HCT and 82% with HLA-MSD-HCT, and the corresponding LFS rates were 79% and 80%, with no significant between-group differences. Multivariate analysis revealed no significant differences in relapse, NRM, or survival rates between the two cohorts [6]. Additionally, LFS correlated significantly with cytogenetic risk category ($P = 0.015$), WBC count at diagnosis ($P = 0.042$), grade III and IV acute G*v*HD ($P = 0.043$), and extensive chronic G*v*HD ($P < 0.001$) [79]. In another prospective multicenter trial, 210 patients with BCR/ABL-negative high-risk ALL were assigned to undergo unmanipulated haplo-HCT ($n = 121$) or HLA-MSD-HCT ($n = 89$) according to donor availability. Wang and coworkers [80] found that the 3-year LFS did not differ between patients with transplantations from haploidentical donors and HLA-MSD (61% *vs.* 60%, $P = 0.91$), with cumulative incidences of NRM of 13% and 11% ($P = 0.84$) and relapse rates of 18% and 24% ($P = 0.30$), respectively. Wang and coworkers [81] also prospectively demonstrated that the 4-year adjusted probabilities of OS for myelodysplastic syndrome (MDS) patients who underwent transplantation with the 3 of 6 haploidentical donors ($n = 136$), 4–5 of 6 haploidentical donors ($n = 90$), and HLA-MSD were 58%, 63%, and 73%, respectively (overall, $P = 0.07$), suggesting that MDS patients receiving unmanipulated haplo-HCT can achieve comparable outcomes than those who received MSD-HCT. These data suggest that unmanipulated haplo-HCT is a valid post-remission treatment option for AML patients in CR1, for patients with ALL, and for those with MDS who lack an identical donor. Importantly, results from other trials [82, 83] in China and from Italy [84] largely reproduced these data (Tables 5.1, 5.2, and 5.3). For the detail of patients who received haplo-HCT with posttransplant cyclophosphamide (PTCy) using G-CSF-mobilized PB $CD34^+$ cells as allografts, please see Chaps. 7 and 8).

5.3.3.1 Select Questions

Haploidentical Allografts in the Setting of ATG

The allografts currently available for unmanipulated haplo-HCT include G-CSF-primed bone marrow (G-BM) harvests, G-PB, and mixture allografts of G-BM and G-PB [25, 26, 64–69]. In MSD-HCT settings, compared to PB, BM graft HCT is associated with slower hematological recovery, increased relapse rates in high-risk acute leukemias, and lower G*v*HD risk, but no significant difference in LFS or OS. Notably, a lower incidence of acute G*v*HD is associated with superior OS [85]. However, in the setting of haplo-HCT with ATG, no definite conclusion can be drawn regarding which allograft source is better. Our data suggests that haplo-HCT

Table 5.1 Association of recovered immune subsets with transplant outcomes after unmanipulated haploidentical transplantation[a]

Disease (n = patients)	Allografts	Conditioning regimen	Reconstituted immune subsets	Potential effects on clinical outcomes
HM (n = 30) Other diseases (n = 2)	Unmanipulated BM (13) and PBHC (6) Selected $CD34^+$ cells (13)	MA (32)	$CD3^+CD8^+$ T-cells	Univariate analysis demonstrated that patients whose $CD8^+CD3^+$ absolute counts rose above the fifth percentile of age-matched normal levels during the first year posttransplantation experienced superior survivor
HM (n = 43)	G-BM + G-PB	MA (43)	$CD56^{bright}$ NK cells (9.27/) and T/NK ratio at day 14 posttransplant	The patients with more $CD56^{bright}$NK cells in the recovery stage had a higher survival rate (hazard risk [HR], 0.406; P = 0.017), and the patients with a higher ratio of T/NK (>1.0) had a higher chance of getting aG*v*HD (HR, 3.436; P = 0.059) and chronic G*v*HD (HR, 3.925; P = 0.028)
HM (n = 206)	G-BM + G-PB	MA (206)	Early recovered lymphocyte	Multivariate analysis showed that patients with higher ALC30 (≧300/μL) were associated with low relapse rate and low TRM and superior survival both in adult and pediatric patients
HM (n = 60)	G-BM + G-PB	MA (60)	Early recovered lymphocyte	
HM (n = 78)	G-BM + G-PB	MA (78)	Early recovered lymphocyte	
HM (n = 21)	SS-BM	MA (21)	Early recovered lymphocyte	Patients with a day 30 absolute lymphocyte count (ALC) of more than 200 cells/μL had a markedly improved OS and EFS
HM (n = 22)	G-PB + TCD	MA (22)	iNKT	The frequency of iNKT cells significantly correlates with a remission after transplantation

(continued)

Table 5.1 (continued)

Disease (n = patients)	Allografts	Conditioning regimen	Reconstituted immune subsets	Potential effects on clinical outcomes
HM (n = 98) Nonmalignancies (n = 33)	BM (78) G-PB (42) UCB (11)	MA (131)	CMV-specific $CD8^+$ T-cells (3cyt+ cells/μL) CMV-specific $CD4^+$ T-cells (1cyt+ cells/μL)	No CMV DNAemia
Acute leukemia (n = 30)	G-PB with CD3/CD19 depletion	MA (30)	Lymphoid DC2-cells	A significant correlation between the number of lymphoid DC2-cells on day +60 with patient survival
HM (n = 89)	TCD and TCR allografts	MA (89)	CMV-specific IFN-γ ELISPOT (1000 spots/mL)	Low TRM and virus reactivation
HM (n = 47)	Unmanipulated PBHC (27), BM (20)	MA (30) NMA (17)	Treg/$CD4^+$ T-cell ratio at day 14 posttransplant	Multivariate analysis showed that a cutoff ratio value of 9% yielded the most accurate predictions of future aG*v*HD incidence. Treg/$CD4^+$ T-cell ratios of <9% predicted a significantly higher incidence of aG*v*HD than ratios of ≧9% (P = 0.0082)

HM hematological malignancies, *MA* myeloablative conditioning regimen, *TREC* TCR rearrangement excision DNA circle, *BM* bone marrow, *PBHC* peripheral blood hematopoietic cells, *G-BM* granulocyte colony-stimulating factor (G-CSF)-primed bone marrow, *G-PB* G-CSF-mobilized peripheral blood $CD34^+$ cell grafts, *NK* natural killer, *aGvHD* acute graft-*versus*-host disease, *ALC-30* absolute lymphocyte counts at day 30 posttransplantation, *TRM* transplant-related mortality, *SS-BM* steady-state bone marrow, *TCD* T-cell depleted, *OS* overall survival, *LFS* leukemia-free survival, *iNKT* invariant natural killer T cells, *UCB* umbilical cord blood, *DC* dendritic cell, *TCR* T-cell replete, *IFN-γ* interferon-γ, *ELISPOT* enzyme-linked immunosorbent spot, *NMA* non-myeloablative, *Treg* regulatory T cells

[a]Cited from Biol Blood Marrow Transplant 20(4): 440–449

using G-BM and G-PB as allografts can produce superior G*v*L effects compared to MSD-HCT in cases of high-risk acute leukemias, including both AML and ALL [3, 23, 86]. Xu and coworkers [87] compared the clinical outcomes of high-risk acute leukemia patients, including AML and ALL, who received PB grafts harvested from family members sharing at least one common haplotype to outcomes of those who received a mixture of G-BM and G-PB harvests. They showed that, compared with G-BM or G-PB transplant, haploidentical PB-HCT led to a higher incidence of 2-year TRM (62.5% *versus* 35.1%; P = 0.014), lower OS rates (26.8% *versus*

Table 5.2 Recent[a] informative trials and results of haplo-HCT with ATG preparative regimen using G-CSF-primed bone marrow and/or peripheral blood as allografts

Reference	Pts (no.)	Diagnosis	Graft	ANC	PLT	GvHD		TRM	Relapse	LFS	OS
						Acute II–IV	cGvHD				
Wang Y et al. 2014 [2]	1210	HM	G-BM + G-PB	13 (8–49)	16 (5–100)	40%	50%	17% at 3 yr	17% at 3 yr	67% at 3 yr	70% at 3 yr
Chen H et al. 2015 [29]	101	Ph^+ ALL	G-BM + G-PB	12 (7–27)	14 (9–150)	38%	68.5% at 5 yr	15.6% at 5 yr	18% at 5 yr	65.8% at 5 yr	74% at 5 yr
Mo XD et al. 2014 [42]	81	Ph^- ALL LR	G-BM + G-PB	12 (9–24)	15 (8–250)	42%	65.5% at 3 yr	16.2% at 3 yr	17.8% at 3 yr	68.2% at 3 yr	72.2% at 3 yr
	102	Ph^- ALL HR	G-BM + G-PB	13 (10–23)	16 (7–161)	36.3%	59.4% at 3 yr	18% at 3 yr	15.4% at 3 yr	67.6% at 3 yr	74.9% at 3 yr
Lin X et al. 2015 [38]	105	HM	G-PB	14 (10–25)	16 (9–38)	21.9%	24.1% at 2 yr	30.5% at 3 yr	21.9% at 3 yr	41.1% at 3 yr	50.6% at 3 yr
Shin SH et al. 2015 [21]	60	MDS	G-PB	12 (8–23)	15 (6–132)	36.7%	48.3%	23.3% at 2 yr	34.8% at 2 yr	41.9% at 2 yr	46.6% at 2 yr
Peccatori J et al. 2015 [24]	121	HM	G-PB	17 (11–61)	19 (7–154)	35%	47% at 2 yr	31% at 3 yr	48% at 3 yr	20% at 3 yr	25% at 3 yr
Luo Y et al. 2014 [4]	99	HM	G-PB	12 (8–24)	15 (6–53)	42.4%	41.4% at 2 yr	30.5% at 5 yr	14.2% at 5 yr	58.3% at 5 yr	60.8% at 5 yr
Chen J et al. 2014 [23]	50	HM	G-PB + UCB	13 (11–20)	15 (11–180)	20%	19.3% at 1 yr	16.2% at 1 yr	19.8% at 1 yr	64% at 1 yr	78.6% at 1 yr
Tang BL et al. 2015 [39]	17	HM	G-PB	12 (11–15)	17 (11–117)	35.3%	29.4%	29.4% at 180d	NA	67.4% at 1 yr	67.4% at 1 yr

(continued)

Table 5.2 (continued)

Reference	Pts (no.)	Diagnosis	Graft	ANC	PLT	GvHD		TRM	Relapse	LFS	OS
						Acute II–IV	cGvHD				
Yahng SA et al. 2015 [40]	80	AML	G-PB	11	10	47.5%	45%	12.2% at 2 yr	26.6% at 2 yr	61.1% at 2 yr	66% at 2 yr
Di Bartolomeo P et al. 2013 [45]	80	HM	G-BM	21 (12–38)	28 (14–185)	24%	17% at 2 yr	36% at 3 yr	21% at 1 yr	38% at 3 yr	45% at 3 yr
Xu LP et al. 2012 [19]	19	SAA	G-BM + G-PB	12 (10–29)	18 (8–180)	42.1%	56.2%	35.4% at 2 yr	NA	NA	64.5 at 2 yr
Gao L et al. 2014 [43]	26	SAA	G-PB + G-BM	13 (11–19)	13 (10–21)	8.0%	40%	15.4% at 2 yr	NA	NA	84.6% at 2 yr

Haplo-HCT haploidentical hematopoietic cell transplantation, *ATG* antithymocyte globulin, *Pts* patients, *no.* number, *ANC* absolute neutrophil count, *PLT* platelet, *GvHD* graft-*versus*-host disease, *cGvHD* chronic GvHD, *TRM* transplant-related mortality, *LFS* leukemia-free survival, *OS* overall survival, *HM* hematological malignancies, *G-BM* granulocyte colony-stimulating factor (G-CSF)-primed bone marrow, *G-PB* G-CSF-mobilized peripheral blood $CD34^+$ cell grafts, *MA* myeloablative, *yr* year, *AML* acute myeloid leukemia, *ALL* acute lymphoblastic leukemia, *NA* not available

[a]Published between 2013 and 2015

Table 5.3 Conditioning regimen of haploidentical hematopoietic cell transplantation with ATG preparative regimen using G-CSF-primed bone marrow and/or peripheral blood as allografts

Author, year	Conditioning regimens	Reference
Shin SH et al. 2015	Flu (180 mg/m^2), i.v. Bu (6.4 mg/kg), and rabbit ATG (12 mg/kg) (n = 37); Flu (150 mg/m^2), i.v. Bu (6.4 mg/kg), rabbit ATG (10 mg/kg), and TBI (800 cGy) (n = 23)	[91]
Peccatori J et al. 2015	Treo, 14 g/m^2 on days −6 through −4, and Flu, 30 mg/m^2 on days −6 through −2	[69]
Tang BL et al. 2015	Flu (30 mg/m^2/d i.v.) on days −6 to −3, rabbit ATG (2.5 mg/kg/d i.v.) on days −5 to −3, Cy (50 mg/kg/d i.v.) on day −2, and a single dose of 3 Gy TBI on day −1	[103]
Yahng SA et al. 2015	800 cGy TBI (fraction size of 200 cGy, twice a day, on days −9 and −8), Flu (30 mg/m^2/d i.v. on days −7 to −3), Bu (3.2 mg/kg/d, i.v. in four divided doses on days −6 and −5), and rabbit ATG (1.25 mg/kg/d on days −4 to −1)	[40]
Lin X et al. 2015	Flu, 25 mg/m^2/d, i.v. (days −9 to −5); Bu, 3.2 mg/kg/d, i.v. (days −8 to −5); Cy, 60 mg/kg/d, i.v. (days −3 to −2); rabbit ATG, 2.5 mg/kg/d, i.v. (days −5 to −1)	[101]
Wang Y et al. 2014	Cytarabine, 4 g/m^2/d, i.v. (days −10 and −9); Bu, 12 mg/kg p.o. in 12 doses (days −8 to −6); Cy, 1.8 g/m^2/d, i.v. (days −5 and −4); Me-CCNU, 250 mg/m^2, orally (day −3); rabbit ATG, 2.5 mg/kg/d i.v. (days −5 to −2)	[3, 4, 6]
Fu H et al. 2014	TBI, 700 cGy with particle shielding of the lungs (600 cGy) on day −6; Cy, 1.8 g/m^2 (days −5 to −4); Me-CCNU, 250 mg/m^2, orally (day −3); rabbit ATG, 2.5 mg/kg/d i.v. (days −5 to −2)	[73]
Luo Y et al. 2014	Cytarabine (4 g/m^2/d i.v. on days −10 to −9), Bu (3.2 mg/kg per day i.v. on days −8 to −6), Cy (1.8 g/m^2 per day i.v. on days −5 to −4), Me-CCNU (250 mg/m^2 orally on day −3), and rabbit ATG (2.5 mg/kg per day i.v. on days −5 to −2)	[68]
Gao L et al. 2014	Flu, 30 mg/m^2/d i.v. from days −5 to −2; Cy, 45 mg/kg once daily i.v. from days −3 to −2; and rabbit ATG, 2.5 mg/kg once daily i.v. from days −5 to −2	[43]
Chen J et al. 2013	Regimen A: Me-CCNU 250 mg/m^2 (day −10), Cytarabine 4 g/m^2/d (days −9 and −8), Bu 4 mg/kg/d p.o. (days −7 to −5), and Cy 1.8 g/m^2/d (days −4 and −3). Regimen B: Me-CCNU 250 mg/m^2/d (day −8), TBI 8–8.5 Gy (days −7 and −6), Cytarabine 4 g/m^2/d (days −6 and −5), and Cy 1.8 g/m^2/d (days −4 and −3)	[102]
Di Bartolomeo P et al. 2013	Regimen A: cytarabine 3 g/m^2/d i.v. for 3 days, Cy 45 mg/kg/d for 2 days, TBI 10 Gy for 2 days, or Treo 14 g/m^2/d for 3 days or oral Bu 16 mg/kg for 4 days. Regimen B: Flu 160 mg/m^2 over 4 or thiotepa 5 mg/kg/d for 1 day, Flu 150 mg/m^2 for 3 days, and Mel 140 mg/m^2 for 1 day. Regimen C: Thi 5 mg/kg/d at days −7 and −6, Bu 3.2 mg/kg/d, combined with Flu 50 mg/m^2/d i.v. at days −5 to −3. Regimen D: Thi 5 mg/kg on day −6, Bu 3.2 mg/kg/d at days −5 and −4, and Flu 50 mg/m^2/d i.v. from days −5 to −3	[84]

(continued)

Table 5.3 (continued)

Author, year	Conditioning regimens	Reference
Xu LP et al. 2012	Bu 3.2 mg/kg/d, i.v. (days −7 and −6); Cy 50 mg/kg/d, i.v. (days −5 to −2); and rabbit ATG 2.5 mg/kg/d or porcine ATG 20 mg/kg/d, i.v. (days −5 to −2)	[65]
Chen XH et al. 2009	(a) Cytarabine, 3 g/m^2/d, i.v. for 3 days; TBI for 2 consecutive days (9–9.5 Gy in total); Cy, 50 mg/kg/d, i.v. for 2 consecutive days; (b) Bu, 130 mg/m^2 for 2 consecutive days; Cy, 45 mg/m^2 for 2 consecutive days; lomustine,120 mg/m^2 for 1 day; cytarabine, 2 g/m^2 every 12 h for 2 consecutive days; all cases receive rabbit ATG, 2.5 mg/kg/d i.v. (days −5 to −2)	[107]

ATG antithymocyte globulin, *Bu* busulfan, *Cy* cyclophosphamide, *Me-CCNU* simustine, *Flu* fludarabine, *TBI* total body irradiation, *Treo* treosulfan

43.2%; $P = 0.052$), and lower LFS (26.8% *versus* 42.4; $P = 0.071$), suggesting that G-PB alone may be inferior to the combination of G-BM and G-PB as allografts in this subpopulation. This was further confirmed by our recent updated data [88].

Di Bartolomeo and coworkers [84] have recently shown a beneficial effect of using G-BM in unmanipulated haplo-HCT with ATG, reporting improvements of engraftment, G*v*HD incidence, and survival in patients with high-risk malignancies. In contrast, Wang and coworkers [89, 90] changed their haploidentical allografts from G-BM alone to G-BM and G-PB combined due to the observed slow hematopoietic recovery and weak G*v*L effects with the BM haplo-HCT. More recently, several groups reported the successful use of G-PB, achieving acceptable G*v*HD and promising outcomes [68, 69, 91, 92]. These findings may be related to the G-CSF-induced immune tolerance of T cells, improvements in the conditioning regimen, and G*v*HD prophylaxis. Therefore, the controversy regarding allograft selection (PB *vs.* BM) warrants further study. There is particularly a need for randomized studies comparing different harvests in haplo-HCT with an ATG regimen.

Immune Reconstitution

The first 90 days after unmanipulated haplo-HCT are characterized by persistent CD4$^+$ and CD4$^+$ naive T-cell and B-cell lymphopenia. However, compensatory expansion of monocytes, cytokine-producing CD56^{bright} NK cells, and cytotoxic T cells with the central memory CD45RO$^+$CD62L$^+$ cell phenotype may partly prevent leukemia relapse and infections [22, 23]. Regarding the reconstitution of T-cell function, one interesting finding is the ability of T cells to secrete interferon-γ and interleukin-4 by day +30 in patients without acute G*v*HD following unmanipulated haplo-HCT. Effects of recovered immune subsets on transplant outcomes in haplo-HCT settings are summarized in Table 5.1 [22]. In the future, these will serve as reference values for the recovered immune cell subsets after unmanipulated haplo-HCT, and de novo thymic production, including virus-specific immune recovery, as well as strategies (IL-2 and IL-7) to improve immune recovery, should be explored further.

Poor Graft Function

Primary graft failure (GF) remains a serious problem after allogeneic HCT, especially in haploidentical transplant settings. Primary GF includes graft rejection, which is

defined as a failure to achieve blood count minimum thresholds (absolute neutrophil count of ≤500/μL, platelet count of ≤20,000/μL, or hemoglobin level ≤80 g/L) beyond day 28 posttransplant, in the absence of donor hematopoiesis. Primary GF also includes poor graft function (PGF), which is the failure to achieve three adequate blood counts for GR (as described above) following allo-SCT despite complete donor hematopoiesis. In haplo-SCT with an ATG protocol, we found an approximately 1% incidence of primary GR [3]. On the other hand, poor graft function occurs with an approximately 4–5% incidence and was a severe complication leading to a higher incidence of mortality [93]. The currently available data suggest that DSAs are strongly associated with primary GF and poor graft function [50, 94–96]. Several approaches have been utilized to deal with DSA-associated primary GF, including plasma exchange, rituximab, bortezomib, and low-dose IgG [97]. Further prospective studies are needed to confirm the efficacies of these methods and are discussed in subsequent chapters. Recently, we reported that endothelial progenitor cell (EPC) impairment and increased type 1 immune response in BM microenvironment were associated with poor graft function [98]. Therefore, an increased understanding of the mechanisms underlying primary GF, especially primary poor graft function [98], will likely lead to the establishment of more effective prevention and treatment methods in the future.

5.4 Expert Point of View

Currently, the Beijing Protocol has been one of the most used haplo-HCT modalities. Here, we have listed some expert opinions, based on available data from our original data:

1. Immune tolerance induced by G-CSF and ATG may contribute to overcome HLA barriers in the Beijing Protocol.
2. Conditioning regimen, including TBI based and Bu based, can be successfully used to achieve promising transplant outcomes.
3. The algorithm to select the best haploidentical donor based on DSA, ABO compatibility, donor age, donor sex, family relationship, NIMA mismatch, and NK alloreactivity should be followed.
4. DLI is an effective approach for the treatment and prophylaxis of leukemia relapse after haplo-HCT.
5. Stratification-directed prophylaxis or treatment of G*v*HD may represent future direction in allo-HCT settings.
6. The questions include selecting the best allografts, strategy to improve immune reconstitution, and the underlying mechanism and treatment of poor graft function, which should be answered to improve transplant outcomes.

In summary, based on the data from our group and other researchers, we can draw the following conclusions. Several protocols, including ATG-based regimens and PTCy-based regimens, for haplo-HCT using hematopoietic myeloid growth factor-primed BM and PB grafts as allografts, differ in the design of the conditioning regimens and G*v*HD prophylaxis (Tables 5.2, 5.3, and 5.4) [67–69, 74, 84, 91, 99–107]. The Beijing Protocol may be a promising post-remission treatment algorithm for patients with ALL and adults with AML with unfavorable cytogenetics.

Table 5.4 G*v*HD prophylaxis of haploidentical hematopoietic cell transplantation with ATG preparative regimen using G-CSF-primed bone marrow and/or peripheral blood as allografts

Author, year	G*v*HD prophylaxis regimen	Reference
Lin X et al. 2015	CsA (3 mg/kg/d), i.v. on day −1, then shift oral and stopped from day +180 to day +270; MTX (15 mg m^{-2}, on day +1; 10 mg m^{-2} on days +3, +6, +9, and +11)	[101]
Peccatori J et al. 2015	ATG-F (10 mg/kg) on days −4 through −2; sirolimus (orally, monitored two times a week to maintain a target therapeutic plasma level of 8–15 ng/mL) from day −1 and MMF (15 mg/kg t.i.d. orally or i.v.) from day 0	[69]
Luo Y et al. 2014	CsA (2.5 mg/kg/d), from day −7 to day +300, with a target blood level of 200–300 ng/mL; MMF (500 mg/d, orally), from day −9 to day +100; MTX (15 mg m^{-2}, on day +1; 10 mg m^{-2} on days +3, +6, +9, and +11)	[68]
Chen J et al. 2013	CsA at 3 mg/kg/d was given by continuous infusion over 24 h from day −10 until patients could switch to oral intake (p.o.), with a target blood concentration ranging from 200 to 300 ng/mL. MTX was given at 15 mg/kg/d on day +1 and 10 mg/kg/d on days +3, +6, and +11. Mycophenolate mofetil 1.0 g p.o. twice a day was given from day −10 to day +30 and then gradually tapered until day +60. Rabbit ATG, 2.5 mg/kg on days −5 to −2	[102]
Di Bartolomeo P et al. 2013	CsA (1.5 mg/kg/d), from day −7 to day −2, 3 mg/kg/d from day −2 and then 5 mg/kg/d orally; ATG 5 mg/kg/d, from day −4 to day −1; MMF 15 mg/kg, from days +7 to +100; MTX (15 mg m^{-2}, on day +1; 10 mg m^{-2} on days +3, +6, and +11); basiliximab 10–20 mg/d, day 0 and day +4	[84]
Wang HX et al. 2012	CsA (1.5 mg/kg/d), from day −7 to day −2, 2.5 mg/kg/d from day −2 and then 5 mg/kg/d orally; ATG 5 mg/kg/d, from day −4 to day −1; MMF 0.25–0.5 g/d, from days +7 to +100; MTX (15 mg m^{-2}, on day +1; 10 mg m^{-2} on days +3, +6, and +11); basiliximab 20 mg/d, pretransplant and day +4	[44]
Huang WR et al. 2012	CsA (2.5 mg kg^{-1}, day −1, i.v.) from day −7 and was discontinued at 6 months. MMF (0.5 g, every 12 h, orally) from day –7 to day 28. MTX (15 mg m^{-2}, on day +1; 10 mg m^{-2} on days +3, +6, and +11)	[18]
Lee KH et al. 2011	CsA (1.5 mg/kg/d), from day −1, the blood concentrations were referenced to 100–300 ng/mL; MTX (15 mg m^{-2}, on day +1; 10 mg m^{-2} on days +3, +6, and +11)	[85]
Chen XH et al. 2009	CsA (1.5 mg/kg/d), from day −7 to day −1, 2.5 mg/kg/d from day −1 to day +365; MMF (1000 mg/d, orally), from day −7 to day +100; MTX (15 mg m^{-2}, on day +1; 10 mg m^{-2} on days +3, +6, and +11)	[107]
Ogawa H et al. 2008	FK506 0.02 mg/kg/d, i.v., with a target blood concentration of 10–15 ng/mL; PSL 1 mg/kg/d from day −4	[84]
Huang XJ et al. 2006	CsA (2.5 mg kg^{-1}, day −1, i.v.) from day –9 and was discontinued at around 9–10 months. MMF (0.5 g, every 12 h, orally) from day –9 to day 180. MTX (15 mg m^{-2}, on day +1; 10 mg m^{-2} on days +3, +6, and +11)	[3, 4, 6, 20, 46]

GvHD graft-*versus*-host disease, *ATG* antithymocyte globulin, *FK506* tacrolimus, *PSL* prednisolone, *CsA* cyclosporine A, *MTX* methotrexate, *MMF* mycophenolate mofetil

5.5 Future Directions

To date, unmanipulated haplo-HCT using hematopoietic myeloid growth factor-primed BM and PB hematopoietic progenitor cells as allografts, especially in the setting of the Beijing Protocol, has proven to be easily performed and highly effective and has become one of the most commonly applied modalities in haplo-HCT settings [7, 16, 81, 108]. Several approaches have been successfully performed to improve clinical outcomes—including donor selection, improvements of the conditioning regimen and stratification-directed G*v*HD prophylaxis, and relapse prevention and treatment. For example, data from our groups and others [1, 109] suggest that MRD-directed therapy could further reduce the incidence of relapse after allo-HCT. Therefore, identifying variables that are associated with transplant outcomes—such as poor graft function, G*v*HD, TRM, relapse, and survival—make it possible to perform stratification-directed prophylaxis and therapy [1, 49, 109]. Such progress could lead to the realization of individual therapy for patients receiving haplo-HCT using hematopoietic myeloid growth factor-primed BM and PB hematopoietic progenitor cells as allografts, although much work is still needed to achieve these goals.

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6 Photodepletion to Promote Immune Reconstitution Without Graft-*Versus*-Host Disease After HLA-Haploidentical Transplantation

Denis Claude Roy and Stephan Mielke

6.1 What You Will Learn in This Chapter

- Donor T-cells can be depleted of their alloreactive component using photodynamic *ex vivo* treatment (a product called ATIR).
- ATIR can be administered post-transplant without causing grade III–IV acute graft-*versus*-host disease (G*v*HD), even in the absence of immune suppressors.
- Infusion of photodepleted donor T-cells (ATIR) after HLA-haploidentical hematopoietic cell transplantation (haplo-HCT) decreases infections and treatment-related mortality (TRM).
- Phase I and II clinical trials of haplo-HCT with ATIR show most favorable results to date in terms of relapse and survival.

6.2 Introduction

Haploidentical hematopoietic cell transplantation (haplo-HCT) offers an immense challenge in terms of immunologic difference between donor and recipient cells. However, this major histocompatibility complex (MHC) disparity also represents a

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powerful tool for the *ex vivo* removal of alloreactive cells. Indeed, we found that it promotes donor T-cell activation upon exposure to host T-cells and leads to their selective elimination after exposure to a rhodamine-derived photosensitizer (TH9402) and visible light. In this chapter, we highlight the road to development of selectively depleted good manufacturing practice (GMP)-grade T-lymphocyte products (ATIR™, Kiadis Pharma, NL), from mechanistic determinants to bedside. This strategy enables haplo-HCT without the use of immune suppressors. A phase I clinical trial was able to identify a therapeutic ATIR cell dose associated with a decrease in infectious complications and treatment-related mortality (TRM). Long-term follow-up of these patients shows sustained remission in high-risk patients, suggesting preservation of graft-*versus*-leukemia activity in ATIR cells. Results from an ongoing phase II clinical trial in 23 acute leukemia patients using a single dose of ATIR at two million $CD3^+$ cells/kg are in line with phase I outcomes. ATIR infusion also harbors the potential to promote immune reconstitution in different types of haplo-HCT.

6.3 Selective Allo-depletion

The therapeutic efficacy of allogeneic hematopoietic cell transplantation (allo-HCT) largely depends on subpopulations of donor-derived T-lymphocytes carrying the ability to recognize antigens present on malignant cells, thereby promoting powerful graft-*versus*-leukemia (G*v*L) activity. However, nonmalignant host cell recognition by different T-cell subpopulations may also cause severe forms of graft-*versus*-host disease (G*v*HD) and contribute to transplant-related mortality and reduced quality of life (QoL). Alloreactive T-lymphocytes can be removed using selective depletion procedures that have the potential to spare those subpopulations responsible for G*v*L activity. Their administration thus offers a curative option to patients with high-risk hematologic malignancies in need of an allo-HCT [1]. Methods used for *ex vivo* and *in vivo* selective depletion of alloreactive T-cells include immunotoxins, suicide genes, photodynamic procedures, and post-transplantation cyclophosphamide (PTCy) (see Chaps. 2–5, 7 and 8). They are not only interesting laboratory developments but are part of the clinically tested transplant armamentarium. They aim primarily at improving the outcome of mismatched transplants where feasibility largely depends on sufficient *in vivo* and *ex vivo* T-lymphocyte depletion strategies or enhanced immunosuppression to ensure engraftment and prevention of severe G*v*HD [2–12].

6.4 TH9402-Based Photodepletion

The photodepletion technique employs a rhodamine-derived dye (TH9402) making these host-activated cells susceptible to elimination by visible light in an *ex vivo* co-culture system. As a first step, patient-derived irradiated, leukemia-free peripheral blood mononuclear blood cells (PBMCs) are co-cultured with donor-derived lymphocytes in a one-way mixed lymphocyte reaction [13]. This step is crucial in activating donor cells with the ability to identify host antigens. During the coloration phase, TH9402 is taken up by both activated and non-activated lymphocytes.

However, activated cells accumulate greater amounts of TH9402 than resting cells. When placed in medium without TH9402, the resting cells also extrude TH9402 to very low levels, while the photosensitizer remains at cytotoxic levels in activated cells. This is due to the fact that activated T-lymphocytes extrude TH9402 significantly less than non-activated lymphocytes because their multidrug resistance (MDR) pump becomes deactivated. Exposure to visible light then stimulates the phototoxic dye and induces apoptosis in activated lymphocytes due to mitochondrial damage. The resulting cellular product thereby loses its ability to cause G*v*HD, while desired features such as graft-*versus*-infection and G*v*L remain preserved (this product was named ATIR™, Kiadis Pharma, NL). Indeed, in view of their high level of immunogenicity, major histocompatibility complex (MHC) differences between donor and host cells are most likely responsible for donor cell activation. In this context, T-cells recognizing less immunogenic antigens, such as minor histocompatibility and tumor-associated antigens, should not be activated during this *ex vivo* culture period and therefore spared to exert G*v*L effects. Cells are then kept frozen until needed to accelerate T-cell reconstitution after haploidentical stem cell transplantation. TH9402-based photodepletion of alloreactive T-cells has proven feasible and efficient in preclinical studies performed in both mice and man [7, 14]. The process is illustrated in Fig. 6.1.

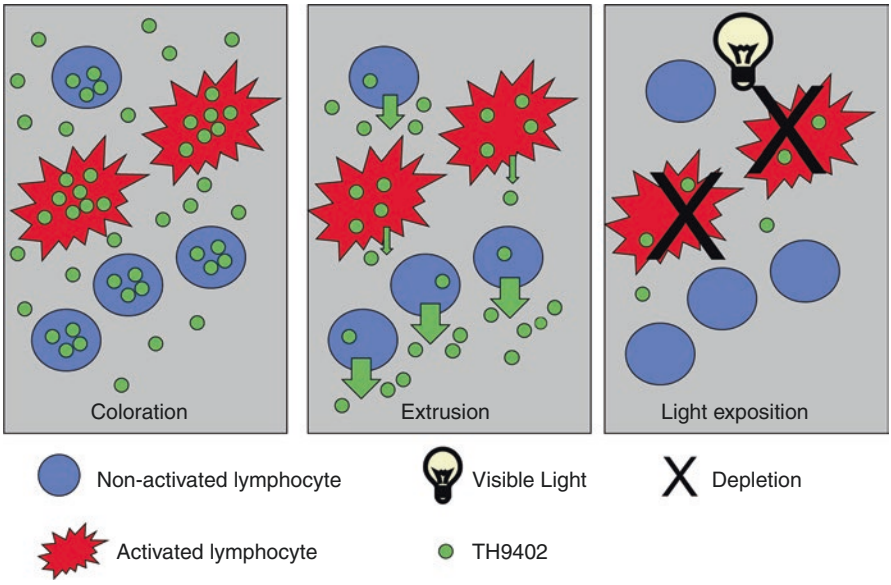

Fig. 6.1 Principle of TH9402-based photodepletion: During coloration the phototoxic drug 4,5-dibromorhodamine 123 (TH9402) is taken up in all lymphocytes. However, activated T-lymphocytes are not able to extrude the dye to the same extent as resting T-cells due to alterations in their MDR pump, resulting in differential dye retention in activated *versus* resting T-cells. When placed in medium without TH9402, cells continue to extrude the dye (extrusion phase). Dye accumulation in activated T-cells is such that light exposure causes elimination of these cells. In contrast, photosensitizer levels in resting T-cells are below toxic levels, allowing these cells to survive light illumination and to remain functional

6.5 Clinical Results in HLA-Mismatched Transplantation

6.5.1 Phase I Clinical Trial

Haploidentical transplants with complete T-cell depletion (TCD) were long known for both their significant TRM, reaching up to 50% after 1 year, and high relapse rates [15]. These high TRM rates observed after TCD haplo-HCT were mainly attributable to infectious (in particular viral) complications as the process required significant *in vivo* and *ex vivo* TCD leaving behind a highly immunocompromised patient prone to severe opportunistic infections. Consequently, photodepletion was first introduced to promote T-cell reconstitution in patients who received a TCD haplo-HCT. The addition of host-nonreactive T-cell post-transplant obviated the need for G*v*HD prophylaxis and offered significant room for improvement in transplant outcomes. In a phase I clinical trial, 19 patients with high-risk hematological malignancies received photodepleted T-lymphocytes at doses between 1×10^4 and 5×10^6/kg of recipient weight as a donor lymphocyte infusion (DLI) approximately 30 days after TBI-based myeloablative conditioning (MAC) and CD34-selected haplo-HCT [16]. This photodepleted DLI did not cause clinically significant G*v*HD (AIR-001 trial). Indeed, acute G*v*HD was limited to four patients developing grade II G*v*HD of the skin, with only one patient also demonstrating hepatic and gastrointestinal involvement. In all cases, G*v*HD was rapidly responsive to treatment with steroids. Of note, patients having received more than 1.3×10^5 allo-depleted T-lymphocytes/kg of recipient body weight showed a decrease in infection rates, a high survival rate (58% at 3 years in a high-risk patient population), and a remarkably low TRM rate of below 20% compared to almost 60% in historical controls receiving similar transplants but without ATIR infusion [17, 18].

In this first clinical trial, the cell treatment process was aimed at small T-cell dose and required several manipulation steps. Several strategies were developed to accommodate larger cell treatment volumes and to comply with good manufacturing practices (GMP). A first GMP-grade procedure was developed at the NIH allowing broader applicability and significant allo-depletion in both HLA-mismatched and HLA-matched transplantations [19]. This approach included the utilization of donor T-cells as antigen-presenting cells, broadening the potential of photodynamic cell elimination [20].

6.5.2 Phase II Clinical Trial

A first international multicenter phase II trial was commenced for patients with hematological malignancies and no HLA-matched transplant donor available (AIR-004 trial). Products were generated using a central production facility in the Netherlands and shipped to the treating centers in Canada and Europe. Patients received a defined dose of 2×10^6 allo-depleted T-lymphocytes/kg of recipient weight as a DLI post $CD34^+$ cell selected transplantation. However, centralized production at this site was challenging, and the trial was terminated early.

After revising the cell manufacturing process, a phase II clinical trial could be reopened with production sites in both North America (Montreal) and Europe (Frankfurt) serving an even larger new multicenter trial (AIR-007 trial). Results of this trial are promising to date, offering a 61% 1-year overall survival (OS) in 23 patients treated with acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) in the first and second complete remission (CR). Again these results, at a median follow-up of 484 days, are most favorable when compared to historical data of patients receiving MAC haplo-HCT of $CD34^+$ cell selected grafts but no ATIR post-transplant [21]. Interestingly, no patient died during the first 3-months post-transplant. Acute G*v*HD grade III–IV was not observed in these Phase I and Phase II trials despite the fact that patients did not receive any G*v*HD immune prophylaxis.

In order to better assess the clinical impact of ATIR-based haplo-HCT *versus* other transplant strategies, clinical results from ATIR patients at 12 months were compared to those from contemporaneous patients with AML, ALL, and myelodysplastic syndrome (MDS) treated according to four other transplant approaches: (1) same type of haplo-HCT using CD34 selected grafts but without the use of ATIR, (2) allo-HCT with HLA-matched unrelated donors (MUD), (3) allo-HCT with 1-locus HLA-mismatched unrelated donors (MMUD), and (4) allo-HCT receiving a double umbilical cord blood transplantation (UCB) [22]. Patients undergoing haplo-HCT with ATIR had a significantly lower TRM and higher OS at 1 year than historical control patients who received a similar haplo-HCT but without the addition of ATIR ($p < 0.005$). In addition, ATIR offered the best G*v*HD-free relapse-free survival (GRFS) compared with all four above transplant groups. However, GRFS for the ATIR haplo-HCT group was not significantly different from that for HLA-MUD transplant patients (p = NS). Interestingly, GRFS for the ATIR group was superior to that of HLA-MMUD and double UCB groups ($p < 0.01$ and $p < 0.005$, respectively) [22]. One has to keep in mind that these results come from a retrospective study rather than a prospective randomized study. With the limitations associated with such study design, results from the ATIR haplo-HCT approach are most interesting and clearly warrant further evaluation as it represents a most appealing treatment strategy to enable G*v*HD-free survival with low relapse rates. The AIR-008 trial is now exploring the feasibility of multiple infusions of ATIR™ to overcome residual infectious complications.

6.6 The Future

The successful transition of this sophisticated process into the clinic clearly underlines its feasibility and most promising clinical results in high-risk patients. Whether photodepletion will become a standard of care procedure remains an open question. This technique of personalized medicine will likely be associated with significant cost and require off-site cell manufacturing. However, when compared to other approaches aiming to improve the outcome of haploidentical stem cell transplantation, photodepletion offers low rates of relapse and, so far, particularly promising

results in terms of GRFS. Importantly, it would be possible to incorporate this strategy to promote immune reconstitution in the various clinical settings of haplo-HCT, whatever the TCD process ($CD34^+$ cell selection, $CD19^+CD3^+$ depletion, αβ-T-cell depletion, etc.) (see Chaps. 2–4). It could also be used to overcome the need for immune suppression and improve patient outcome after HLA-matched transplantation. Moreover, offering haplo-HCT with a low risk of G*v*HD and without post-transplant immunosuppressive agents is most interesting for patients with nonmalignant hematologic disorders who lack a suitable donor. Furthermore, one-time costs can become acceptable when associated with both cure and QoL.

One must also consider that this photodepletion approach represents an allo-immunotherapeutic platform for the treatment of high-risk hematological diseases [23]. In addition, it allows for combination with novel strategies such as CAR T-cells (see volume 3 of this series), BITEs, immunomodulatory drugs, and vaccination strategies that can be applied in the absence of immunosuppression [24, 25]. The TH9402 photodepletion has also been found to eliminate T-cells activated *in vivo* in patients with chronic G*v*HD [17, 26]. The process can also be modulated to spare increased numbers of regulatory T-cells (see Chap. 4), thus offering both elimination of G*v*HD-causing cells and addition of immunosuppressive cells to chronic G*v*HD patients undergoing such a photopheresis approach. These positive developments prompted Kiadis Pharma to now plan and initiate a prospective multicenter international randomized clinical trial where the ATIR™ photodepletion approach will be compared to the PTCy (see Chap. 7) approach in patients receiving haplo-HCT, looking at GRFS as the primary endpoint (AIR-009).

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Post-transplant Cyclophosphamide in Haploidentical Transplantation

7

Shannon R. McCurdy and Ephraim J. Fuchs

7.1 Introduction

HLA-matched sibling donors are identified for only a minority of patients. Utilizing partially HLA-mismatched related donor (HLA-haploidentical or haplo) blood or marrow transplantation (BMT) allows a donor to be identified for the vast majority of patients and represents the least expensive alternative graft source. Haplo BMT has historically been limited by excessive graft-*versus*-host disease (G*v*HD), nonrelapse mortality (NRM), and poor overall survival (OS). Efforts to improve outcomes have centered on T-cell depletion either globally or selectively. Nonselective T-cell depletion of the graft, while achieving acceptable rates of G*v*HD, has been complicated by high incidences of infectious morbidity and mortality. Post-transplantation cyclophosphamide (PTCy) was developed to allow selective depletion of alloreactive T-cells. When given on days 3 and 4 after HLA-haploidentical T-cell replete BMT, high-dose cyclophosphamide (Cy) targets dividing alloreactive T-cells, resulting in low rates of acute graft-*versus*-host disease (aG*v*HD), comparable to HLA-matched transplantation, and chronic graft-*versus*-host disease (cG*v*HD) incidence below that seen with T-cell replete HLA-matched transplantation. Importantly, Cy spares non-alloreactive T-cells, preserving immunity and leading to a low incidence of nonrelapse mortality (NRM) after HLA-haploidentical transplantation. As such, PTCy has improved the safety associated with HLA-haploidentical transplantation, achieving comparable survival to HLA-matched transplantation and thereby widening the pool of eligible allogeneic donors.

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7.2 The Difficulty in Crossing the HLA Barrier in Allogeneic Blood or Marrow Transplantation

In HLA-matched allogeneic hematopoietic cell transplantation (allo-HCT), minor histocompatibility antigen mismatches (mHAgs) may mediate graft rejection or G*v*HD. With haplo BMT, in addition to mHAgs mismatches, the disparate HLA antigens themselves may promote graft-*versus*-host (G*v*H) and host-*versus*-graft (H*v*G) reactions. Specifically, mismatched allogeneic HLA molecules may elicit intense T-cell responses, which leads to high incidences of graft failure (from host T-cell response to donor HLA) and G*v*HD (from donor T-cell response to host HLA) after HLA-mismatched transplants. In fact, 34% of patients transplanted with T-cell replete HLA-haploidentical (haplo) bone marrows in 1983 [1] died from strong alloreactive reactions including engraftment syndrome or hyperacute G*v*HD [2], the latter consisting of a combination of symptoms including pulmonary edema, fever, kidney failure, and volume overload, a syndrome which has now been characterized as cytokine release syndrome [3]. For these reasons, in many studies, HLA mismatching with greater than a single HLA antigen mismatch has been associated with increased risk of severe G*v*HD and treatment-related mortality (TRM), as well as poor event-free survival (EFS) [4, 5] and OS [6].

Naïve T-cells and T-cells primed against recipient alloantigens are associated with aG*v*HD development, whereas memory T-cells that have not been primed against recipient alloantigens have been associated with graft-*versus*-tumor (G*v*T) effects in the absence of G*v*HD [7]. Depletion of naïve $CD4^+$ and $CD8^+$ T-cells leads to reduced alloreactive $CD8^+$ T-cell numbers [8], but tolerization strategies that are effective against naïve T-cell responses are largely ineffective for sensitized T-cells [9]. Inducing tolerance to transplanted tissue in pathogen-free mice proves easier than doing so in primates, including humans. This phenomenon has been attributed to cross-reactive alloreactive T-cell memory, termed *heterologous immunity*. For instance, mice immunized with the Sendai virus develop Sendai virus-specific $CD8^+$ T-cells that are cytotoxic to uninfected allogeneic donor targets due to immunologic cross-reactivity [10]. Unlike naïve T-cells, memory T-cells activate quickly with little need for costimulatory signals. Then, with re-exposure to antigen, they recover effector function [11]. From 1–10% of the naive T-cell repertoire is capable of recognizing foreign major histocompatibility complex (MHC) molecules. T-cell recognition is degenerate [12] such that alloreactive T-cells can be activated after viral infections in experimental models [13, 14]. In fact, 80% of virus-induced T-cell lines exert cross-reactivity against allogeneic HLA [15, 16]. In mice, infusion of $CD8^+$ memory T-cells leads to graft rejection, whereas $CD4^+$ memory T-cell infusion is associated with prolonged graft survival [14]. Henotypes of memory cells include 1) central memory T-cells ($CD62L^{hi}$ $CCR7^+$) that rapidly produce IL-2 and possess proliferative capacity but cannot immediately mediate cytotoxicity, and 2) effector memory T-cells ($CD62L^{lo}$ $CCR7^-$) that produce interferon-gamma, perforin, and are cytotoxic [17]. In a mouse model, infusion of central memory T-cells mediates graft rejection earlier than infusion of effector memory T-cells (18 days *versus* 70 days, $p < 0.01$) [14]. However, both are theorized to contribute to G*v*HD

and graft failure as a consequence of prior viral exposures through T-cell receptor (TCR) degeneracy.

Calcineurin inhibitors (CNIs) were among the first agents utilized to reduce the incidence of aG*v*HD and TRM after HLA-matched BMT, doing so by preventing intracellular signals that lead to activation of alloreactive T-cells [18]. CNIs mediate immunosuppression through prevention of T-cell activation and reduced ability to suppress the activation of memory T-cells. However, CNIs may block tolerance induction by preventing T-cell apoptosis and clonal destruction [19]. Thus, CNI withdrawal may be associated with G*v*HD development due to the residual presence of alloreactive T-cells [20, 21]. Conventional immunosuppression utilized for HLA-matched transplantation, which combines methotrexate with a CNI, has been inadequate to control G*v*HD, graft failure, and TRM after T-cell replete haplo BMT. Methods to reduce G*v*HD and facilitate crossing the HLA barrier have centered on T-cell depletion either globally or selectively. However, this has proven difficult because of the need to remove multiple subsets of cells that mediate G*v*HD: naïve T-cells and primed anti-HLA memory T-cells. Furthermore, nonspecific T-cell depletion has been associated with increased graft rejection, relapse, and infection [22–24].

7.3 Selective Allodepletion with Post-transplantation Cyclophosphamide

Cyclophosphamide (Cy) was initially identified as an ideal agent for drug-induced tolerance because it possessed the highest therapeutic index for suppression of antibody responses in rats [25]. When utilizing Cy to promote skin grafting in mice, it was found that graft survival was longest when Cy was administered between days 0 and 4 after, rather than prior to, skin grafting. Subsequent studies recognized that Cy, when administered with sheep red blood cell (RBC) transfusion, prevented the production of mouse antibodies to current and future sheep RBC transfusion. However, when ox or rabbit RBCs were administered with Cy, mice were still capable of producing antibodies to sheep RBCs. This led to the conclusion that antigen stimulation led to proliferation of a specific lymphocyte clone and that Cy had selective toxicity for rapidly dividing cells, such as those undergoing antigen-stimulated proliferation [26].

Decades later, inspired by these initial studies, Cy was applied in murine models to induce tolerance to allo-BMT [27]. It was found that PTCy, given on day 2 after nonmyeloablative (NMA) conditioning with Cy, fludarabine, and total body irradiation followed by BMT on day 0, is associated with sustained engraftment in 100% of mice. The delay in Cy administration until day 2 was designed to allow host and donor T-cells to react to foreign HLA and mHAgs on days 0–2 after transplant. Then, host and donor proliferating naïve and memory T-cells are destroyed by high-dose Cy given on day 2, while nonproliferating T-cells are spared (Fig. 7.1) [28]. Analyses of V_β subunits of the TCR show that clonal destruction of alloreactive T-cells occurs within days after Cy administration [29]. While proliferating

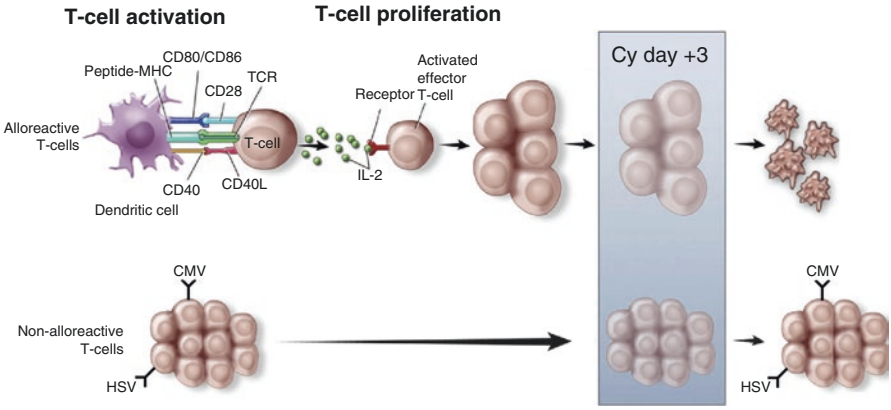

Fig. 7.1 Proposed mechanism for high-dose cyclophosphamide-induced tolerance after allogeneic transplantation. Immediately after bone marrow transplantation, alloreactive T-cells of the host and donor are activated by allogeneic HLA molecules on the surface of donor and recipient antigen-presenting cells, respectively. T-cell recognition of alloantigen, combined with the help of costimulatory signals, leads to T-cell activation, proliferation, and cytokine, including interleukin-2 (IL-2), secretion. During proliferation, replicative DNA synthesis occurs, which leads to sensitivity to cyclophosphamide-induced cytotoxicity and apoptosis induction. Non-alloreactive T-cells are relatively quiescent with a slower growth pattern promoted by IL-15 and IL-7 in response to the lymphopenic environment post-transplant. Given their slower growth, they are relatively resistant to cyclophosphamide and survive to establish the peripheral T-cell pool

T-cells are highly susceptible to Cy, a percentage of resting T-cells also undergoes Cy-mediated apoptosis induction [30]. However, non-alloreactive T-cells, which divide slowly in response to IL-7 and IL-15 in the lymphopenic environment, were highly immune to PTCy cytotoxicity [31]. Given that Cy mediates DNA damage during G1 and S phases of cell division, it was hypothesized that the cells that divide multiple times a day have less time for DNA repair than the more slowly dividing cells. It was also found that dividing naïve cells were the most susceptible to PTCy cytotoxicity. Further, effector memory T-cells have been shown to predominate after PTCy (Kanakry et al. accepted for publication), and, in mouse models, pre-stimulation with alloantigens abrogates PTCy's protective effects from G*v*HD [32]. Murine models of allo-BMT with PTCy have also shown that donor dendritic cells repopulate the thymus and present host antigens to donor T-cells that express TCRs specific for host antigen, which then undergo intrathymic clonal deletion [33].

The protective effects of PTCy with regard to reduction of G*v*HD have also recently been attributed to persistence of FoxP3$^+$ regulatory T-cells or T_{regs}. Murine studies have shown that T_{reg} depletion abrogates the G*v*HD protection of PTCy and Treg adoptive transfer rescues mice from G*v*HD [34, 35]. Donor T_{regs} were found to express high levels of aldehyde dehydrogenase, which detoxifies Cy. High aldehyde dehydrogenase expression has also been proposed to protect hematopoietic stem cells from Cy-mediated cytotoxicity.

7.4 The Graft-*Versus*-Tumor Effect of Haplo-BMT with PTCy: T-Cells, NK Cells, or Both?

Despite the importance of T-cell modulation in the mediation of G*v*HD, significant evidence exists that T-cells play a role in relapse prevention in allogeneic transplant. In T-cell-depleted regimens, relapse rates tend to be higher (range 30–63%) [36–39], and peripheral blood stem cell grafts, which contain a higher proportion of T-cells, are associated with less relapse than bone marrow grafts [40–42]. T-cells that mediate G*v*HD are those with TCR alloreactive to HLA mismatches and mHAgs. However, T-cells that lead to G*v*HD may also prevent relapse, with multiple studies showing that G*v*HD is associated with less relapse risk in HLA-matched transplantation [43, 44]. In addition, work by McCurdy et al. also demonstrates that clinical G*v*HD is associated with less relapse after haplo BMT with PTCy [45]. This supports the role of donor T-cells reactive to HLA antigens or mHAgs in tumor control after PTCy. However, antitumor effects may also be carried out by donor T-cells specific for tumor antigens (such as PRAME, WT1, survivin). It is possible that the level of tumor present immediately after conditioning is minimal and does not lead to clonal T-cell activation and proliferation prior to PTCy. If the tumor recurs with time, new antigens may be displayed that lead to clonal donor T-cell proliferation. If this reaction occurs beyond day 4 post-transplant, then these clones would not have been destroyed by PTCy, and may result in tumor control. In keeping, poor outcomes after nonmyeloablative (NMA) haplo BMT with PTCy are seen in patients transplanted with active disease. With active or perhaps minimal residual disease prior to allo-BMT, NMA conditioning is unlikely to eradicate the tumor, and PTCy may eliminate the donor G*v*T response.

It is also possible that host T-cells play a role in G*v*T effects immediately after transplant. In this scenario, conditioning is cytotoxic to host T_{regs}, but spares tumor-specific host $CD8^+$ T-cells, which lead to G*v*T in the absence of G*v*HD. This mechanism would ultimately end with donor T-cell killing of any remaining host $CD8^+$ T-cells; however, their survival after conditioning could contribute to an initial G*v*T effect. There is some data of the contribution of host $CD8^+$ T-cells to antitumor immunity with post-transplant vaccines studies [46], recipient lymphocyte infusions into mixed chimeric states [47], tumor responses despite graft rejection [48], and host T-cell involvement in antitumor effects after non-engrafting donor lymphocyte infusions [49]. However, as discussed previously, T_{regs} persist after PTCy, and thus this mechanism may play less of a role with PTCy-based immunosuppression.

Highly T-cell-depleted haplo BMT, which is associated with a high infection-related NRM, has demonstrated that natural killer (NK) cells contribute to relapse prevention [24, 50]. NK cells rapidly recover within 2–4 weeks post-transplant after T-cell-depleted haplo BMT. Furthermore, T-cell-depleted haplo BMT from killer immunoglobulin receptor (KIR) alloreactive donors has been associated with less relapse [36]. NK cells have also been shown to recover quickly after haplo BMT with PTCy [51]. In haplo BMT with PTCy, Symons and coworkers also found that alloreactive NK cells contributed to reduction in relapse, utilizing the KIR gene-gene model, which characterizes the KIR genotype by A or B haplotypes [52]. Unlike A

haplotypes, B haplotypes possesses unique KIR genes and greater KIR diversity. Symons and coworkers found that NRM (hazard ratio [HR] = 0.13; confidence interval [CI] = 0.017–0.968; $p = 0.046$), EFS (HR = 0.47; CI = 0.22–1.00; $p = 0.05$), and OS (HR = 0.30; CI = 0.13–10.69; $p = 0.004$) were improved for recipients homozygous for A if they were transplanted from donors with at least one B haplotype. They also found that any inhibitory KIR gene mismatch was associated with reduced relapse (HR = 0.53; CI = 0.31–0.93; $p = 0.025$) and improved OS (HR = 0.37; CI = 0.21–0.63; $p = 0.0003$), and EFS (HR = 0.51; CI = 0.31–0.84; $p = 0.01$), when compared with identical KIR gene content between the recipient and donor.

It is likely that both T-cells and NK cells contribute to relapse prevention after haplo BMT with PTCy's. Importantly, NK cells, while playing a role in relapse prevention, have not been associated with G*v*HD and NK alloreactivity represents a potential unique benefit to haplo BMT. This suggests that it may be possible to separate the G*v*T effects of haplo BMT from G*v*HD by harnessing the alloreactivity of donor NK cells.

7.5 PTCy: Expanding Allogeneic Transplantation to Ethnic Minorities and Developing Countries

One of the biggest barriers to transplantation is identification of a suitable donor. Only 13–51% of patients have an HLA-matched sibling donor (MSD) [53], and an HLA-matched unrelated donor can be identified for anywhere between only 20% of African Americans and up to 80% of Caucasian people of Northern European descent [54]. When combining related and unrelated donor options, approximately 50% of all patients have an identifiable HLA-matched donor, with markedly fewer HLA-matched donors identified for certain ethnicities. In contrast, haplo donors can be identified for the vast majority of patients. Biological parents and children are guaranteed to be HLA-haploidentical to a recipient, and each full or half sibling has a 50% chance of being HLA-haploidentical. Furthermore second-degree relatives like grandchildren, cousins, aunts, uncles, nieces, and nephews are all potential haplo donors. Only recipients who were orphaned, adopted, or possess an inherited disease that prohibits familial donors are routinely unable to find a suitable related haplo donor. This has particular importance for developing countries where an unrelated donor registry may not exist or the health system is unable to afford the costs of acquisition of unrelated donor cells. PTCy may also be particularly well suited for developing countries because it does not require graft manipulation, which requires infrastructure and skilled training. PTCy only requires chemotherapy administration, which would be feasible for any location that is capable of giving induction chemotherapy and transfusion support. Furthermore, haplo BMT with PTCy is the cheapest alternative graft source, less expensive than securing a donor from an unrelated donor or an umbilical cord registry. Thus it is an easily adaptable transplant platform that provides wide donor availability. This is reflected in the fact that now more than 25% of transplants performed worldwide utilize haplo donors, with PTCy being the most commonly adopted haplo platform.

7.6 Haplo-BMT with PTCy: No Longer an Alternative Donor Source

Until recently, haplo BMT was delegated as an acceptable approach only when an HLA-matched related or HLA-matched unrelated donor was unavailable. The initial clinical study of 68 patients undergoing haplo BMT utilizing NMA conditioning and PTCy demonstrated a 13%, 34%, 6%, and 15% incidence of graft failure, grade II–IV aG*v*HD, grade III–IV aG*v*HD, and chronic G*v*HD, respectively [55]. That study was followed closely thereafter with a two-arm parallel multicenter trial comparing haplo BMT utilizing PTCy with double umbilical cord blood transplant (dUCBT). One year cumulative incidence of NRM was 7% and 24%, and 1-year OS was 62% and 54%, after haplo BMT with PTCy and dUCBT, respectively [56]. Since that phase II analysis, many retrospective studies comparing HLA-matched and haplo BMT PTCy have been published, all of which support that there are similar outcomes after HLA-matched and haplo BMT with PTCy (Table 7.1).

In the past, high G*v*HD rates were considered a prohibitive factor associated with haplo BMT. However, with PTCy, the incidence of aG*v*HD after haplo BMT compared with HLA-matched was either similar [57–59] or significantly lower (*p values* <0.001) [60, 61]. In these retrospective studies, the grade II–IV aG*v*HD cumulative incidence ranged from 24% to 50% after HLA-matched related donor (MRD), 19–50% after HLA-matched unrelated donor (MUD), and 14–43% after haplo-BMT [57–62]. The incidence of grade III–IV aG*v*HD after MRD, MUD, and haplo BMT was similar and ranged from 4% to 8%, 4–13%, and 0–11%, respectively [57, 59, 61]. Importantly, there was either a significant reduction [59, 62] or a tendency toward a reduction [57, 58, 60, 61] in the incidence of cG*v*HD after haplo when compared with HLA-matched donor BMT. However, the majority of studies compared haplo BMT with PTCy to HLA-matched donor BMT utilizing methotrexate (MTX) and a CNI. It is important to emphasize that the reduction in cG*v*HD is mostly attributable to PTCy as similarly low rates of cG*v*HD have been demonstrated after myeloablative conditioning (MAC) and HLA-matched BMT utilizing PTCy as the sole G*v*HD prophylaxis [63]. Furthermore, the initial phase II study of PTCy showed that 50 mg/kg of PTCy on both days +3 and +4 compared to 50 mg/kg on day +3 was associated with significantly lower cG*v*HD rates at 5% *versus* 25% ($p = 0.05$), supporting the role for PTCy in cG*v*HD reduction [56].

A major limitation to T-cell-depleted haplo BMT and to historical T-cell replete haplo BMT platforms was a prohibitively high risk of NRM. However, the most promising aspect of haplo BMT with PTCy is the low associated NRM, which was either not significantly different [57, 59, 60, 62, 64] or was significantly lower ($p = 0.02$) [58] after haplo when compared with MRD BMT, ranging at 1 year from 6–24% for MRD, 10–35% for MUD, and 4–24% for haplo BMT with PTCy (Table 7.2) [57, 59, 64, 65]. Furthermore, when conditioning intensity was either similar [59–61] or more intense [57] after haplo BMT with PTCy, NRM was comparable. In contrast, both dUCBT and HLA-mismatched unrelated donor BMT were associated with higher NRM than either haplo, MRD, or MUD allografting in one study [60].

Table 7.1 Summary of comparative studies of HLA-matched and HLA-haploidentical blood or marrow transplantation (adopted from McCurdy et al., Advances in Hematology 2015) [57]

Study	Disease	HLA type and patient #	Conditioning regimens	GvHD prophylaxis	Graft source
Bashey et al. BBMT 2016 [58]	Hematologic malignancies	MRD $n = 181$ MUD $n = 178$ Haplo $n = 116$	MRD or MUD: Bu/Cy Flu/Bu Flu/Bu/Cy Flu/Mel Etop/TBI Flu/Cy Flu/Cy/TBI Cy/TBI Mel/TBI Flu/TBI Bu/Cy/Etop NMA Haplo: Flu/Cy/TBI MAC Haplo: Flu/Bu/Cy Flu/TBI	MRD or MUD: Tac + MTX =/− ATG =/− Alemtuzumab Haplo: PTCy 50 mg/kg D3 and D4, MMF, CNI	PBSC ≫ BM NMA Haplo: BM ≫ PBSC MAC Haplo: PBSC only
McCurdy et al. Blood 2015 [59]	Hematologic malignancies	Haplo $n = 372$ Historical MRD or MUD cohort $n = 614$[a]	Haplo: Flu 30 mg/m^2 D -6 through -2 Cy 14.5 mg/kg D -6 and -5 TBI 200 cGY D -1 MRD/MUD: Flu 120 mg/m^2 Bu 3.2–6.4 IV mg/kg ±ATG	Haplo: PTCy 50 mg/kg D3 and D4, MMF D5–35, Tac D5–90 or 180 MRD/MUD: CNI + MTX ± Sirolimus or CSP with MMF	BM BM or PBSC

Ciurea et al. Blood 2015 [60]	AML	MUD n = 1982 Haplo n = 192	MAC: TBI/Cy TBI/Flu Bu/Cy Mel/Thiotepa/Flu Bu/Flu Bu/Flu/ATG Bu/Thiotepa/Flu NMA: Flu/Cy/TBI	MUD: CNI + MTX or MMF Haplo: PTCy 50 mg/kg D3 and D4, MMF, CNI	PBSC >BM BM > PBSC
Garciaz et al. BMT 2015 [61]	Advanced NHL	MRD = 25 MUD = 28 Haplo = 26	Flu/Bu/ATG NMA Haplo: Flu/Cy/TBI	CsA Haplo: PTCy 50 mg/kg D3 and D4 + CSP + MMF	
Raiola et al. BBMT 2014 [62]	Hematologic malignancies	MRD n = 176 MUD n = 43 mmUD n = 43 UCB n = 105 Haplo n = 92	MAC: Thio/Bu/Flu Bu/Cy Flu/TBI Cy/TBI RIC: Flu/Cy/TBI Thio/Cy ± Mel	MRD: CSP + mini-MTX MUD: CsA + mini-MTX + ATG UCB: CsA + MMF + ATG Haplo: PTCy 50 mg/kg D3 and D4 + CsA + MMF	BM PBSC UCB

(continued)

Table 7.1 (continued)

Study	Disease	HLA type and patient #	Conditioning regimens	GvHD prophylaxis	Graft source
Bashey et al. JCO 2013 [42]	Hematologic malignancies	MRD $n = 117$ MUD $n = 101$ Haplo $n = 53$	RIC or MAC NMA Haplo: Flu 30 mg/m^2 D -6 through -2 Cy 14.5 mg/kg D -6 and -5 TBI 200 cGy D -1 or MAC Haplo: Flu 25 mg/m^2 D -6 through -2, Bu 110–130 mg/m^2 IV D -7 through -4, Cy 14.5 mg/kg D -3 and -2	Standard regimens Haplo: PTCy 50 mg/kg D3 and D4, MMF D5–35, Tac D5–180	PBSC or BM BM for NMA PBSC for MAC
Di Stasi et al. BBMT 2014 [63]	AML or MDS	MRD $n = 87$ MUD $n = 108$ Haplo $n = 32$	MRD/MUD: Flu 120–160 mg/m^2 in four daily doses Mel 140 mg/m^2 or 100 mg/m^2 Haplo: Above + Thiotepa 5–10 mg/kg	MRD: Tac + mini-MTX MUD: Tac + mini-MTX + ATG Haplo: PTCy 50 mg/kg D3 and D4, MMF D5–100, Tac D5–180	BM or PBSC BM or PBSC
Kanakry et al. BBMT 2013 [64]	Peripheral T-cell lymphoma	MRD $n = 22$ Haplo $n = 22$	Bu/Cy Cy/TBI Flu/TBI Bu/Flu Flu/Cy/TBI Fly/Cy/TBI Bu/Cy Cy/TBI	MRD: CSP or PTCy 50 mg/kg D3 and D4 ±MMF ± Tac Haplo: PTCy 50 mg/kg D3 and D4, MMF, Tac or CsA	BM (1 PBSC)

Burroughs et al. BBMT 2008 [65]	Hodgkin lymphoma	MRD *n* = 38 MUD *n* = 24 Haplo *n* = 28	MRD/MUD: TBI 2 Gy TBI 2 Gy + Flu 30 mg/m^2 D -4 through -2 Haplo: Flu 30 mg/m^2 D -6 through -2 Cy 14.5 mg/kg D -6 and -5 TBI 200 cGY D -1	MRD/MUD: MMF or CNI Haplo: PTCy 50 mg/kg D3 (±D4), MMF D4 or 5–35, Tac D4 or 5–180	PBSC BM

HLA human leukocyte antigen, *GvHD* graft-*versus*-host disease, *haplo* HLA-haploidentical, *n* number, *MRD* HLA-matched related donor, *MUD* HLA-matched unrelated donor, *Flu* fludarabine. *D* day, *Cy* cyclophosphamide, *TBI* total body irradiation, *Bu* busulfan, *ATG* anti-thymocyte globulin, *PTCy* post-transplantation cyclophosphamide, *MMF* mycophenolate mofetil, *Tac* tacrolimus, *CNI* calcineurin inhibitor, *MTX* methotrexate, *CsA* cyclosporine, *BM* bone marrow, *PBSC* peripheral blood stem cells, *AML* acute myelogenous leukemia, *MAC* myeloablative conditioning, *Mel* melphalan, *BBMT* biology of blood and marrow transplantation, *Etop* etoposide, *NMA* nonmyeloablative, *mmUD* HLA-mismatched unrelated donor, *UCB* umbilical cord blood, *JCO* Journal of Clinical Oncology, *MDS* myelodysplastic syndrome

[a]Armand et al., A disease risk index for patients undergoing allogeneic stem cell transplantation. July 2012; Blood:120(4) [70]

Table 7.2 Survival outcomes after HLA-matched and HLA-haploidentical blood or marrow transplantation (adopted from McCurdy et al., Advances in Hematology 2015) [57]

	NRM		Relapse		PFS or DFS		OS	
Study	Haplo	Matched (MRD/MUD)	Haplo	Matched (MRD/MUD)	Haplo	Matched (MRD/MUD)	Haplo	Matched (MRD/MUD)
Bashey et al. BBMT 2016 [68	2 yr: 17%	(14%/16%)	2 yr: 29% (30%/34%)		2 yr: 54% (56%/50%)		2 yr: 57% (72%/59%)	
McCurdy et al. Blood 2015 [59]	1 yr: 11%		3 yr: 46%		3 yr: 40%		3 yr: 50%	
			Low: 20%		Low: 65%	66%[a]	Low: 73%	70%
			Int: 48%		Int: 39%	31%	Int: 49%	47%
			High: 67%		High: 25%	15%	High: 37%	25%
Ciurea et al. Blood 2015 [60]	MAC:		MAC:				MAC:	
	1 yr: 12%	14%	3 yr: 44%	39%			3 yr: 45%	50%
	RIC:		RIC:				RIC:	
	1 yr: 6%	16%	3 yr: 58%	42%			3 yr: 46%	44%
Garciaz et al. BMT 2015 [61]	15%	(0%/27%)	19%	(7%/20%)	2 yr: 65%	(80%/68%)	2 yr: 77%	(83%/71%)
Raiola et al. BBMT 2014 [62]	D1000: 18%	(24%/33%)	35%	(40%/23%)	4 yr: 43%	(32%/36%)	4 yr: 52%	(45%/43%)
Bashey et al. JCO 2013 [42]	1 yr: 4%	(10%/10%)	2 yr: 33%	(34%/34%)	2 yr: 60%	(53%/52%)	2 yr 64%	(76%/67%)
Di Stasi et al. BBMT 2014 [63]	1 yr: 24%	(20%/35%)	1 yr: 33%	(28%/23%)	3 yr 30%	(36%/27%)		
Kanakry et al. BBMT 2013 [64]	1 yr: 8%	MRD 6%	1 yr: 34%	MRD 38%				
Burroughs et al. BBMT 2008 [65]	2 yr: 9%	(21%/8%)	2 yr: 40%	(56%/63%)	2 yr: 51%	(23%/29%)	2 yr: 58%	(53%/58%)

NRM nonrelapse mortality, *haplo* human leukocyte antigen (HLA)-haploidentical, *matched* HLA matched, *MRD* HLA-matched related donor, *MUD* HLA-matched unrelated donor, *PFS* progression-free survival, *DFS* disease-free survival, *OS* overall survival, *yr* year, *low* low-risk by disease risk index, *Int* inter-

mediate risk by disease risk index, *high* high or very high risk by disease risk index, *BBMT* biology of blood and marrow transplantation, *D* day, *JCO* Journal of Clinical Oncology

[a]Data based on 614 patients from the original disease risk index study cohort whose outcomes were tabulated and received from P. Armand Dana-Farber Cancer Institute, e-mail, July 24, 2014, personal communication

Given significant improvements in NRM after allo-BMT over the last several decades, relapse now represents the most common cause of treatment failure with modern platforms. In patients with acute myelogenous leukemia (AML) or myelodysplastic syndromes (MDS) treated with similar conditioning platforms followed by either MRD, MUD, or haplo BMT, there was no significant difference in relapse at 28%, 23%, and 33% ($p = 0.75$), respectively [63]. In another study of AML alone, MAC MUD, and MAC haplo BMT were found to be associated with similar relapse risk [60]. However, relapse was lower after NMA MUD when compared with NMA haplo BMT. The authors speculated that the difference in the NMA cohorts may, in part, be explained by the longer duration between diagnosis and BMT, worse performance status, and higher percentage of patients in later disease stages in the haplo BMT cohort.

Haplo BMT is particularly promising with regard to relapse reduction in lymphoma. For instance, in peripheral T-cell lymphoma (PTCL), despite decreased conditioning intensity, there was a similar 1-year cumulative incidence of relapse after MAC MRD at 38% compared with 34% after NMA haplo BMT [64]. Relapse incidence for non-Hodgkin lymphoma was similar at 19% after NMA haplo BMT compared with 20% after NMA MUD transplant in another small study [61]. Finally, in Hodgkin lymphoma, the occurrence of relapse or progression was significantly lower after haplo BMT with PTCy at 40%, when compared with 56% after MRD BMT ($p = 0.01$) and 63% after MUD BMT ($p = 0.03$) [65].

Most importantly, survival has also been demonstrated to be simlar after haplo BMT with PTCy when compared with HLA-matched transplant. At 2 years, OS ranged from 53–76% after MRD, 58–67% after MUD, and 58–64% after haplo BMT with PTCy (Table 7.2) [42, 65]. In a comprehensive comparison of all potential graft sources, there was no difference in 4-year OS after haplo, MRD, MUD, mismatched unrelated, and dUBC transplantation at 53%, 45%, 43%, 40%, and 34%, respectively ($p = 0.10$). In multivariable analysis, survival was inferior after dUBCT ($p = 0.03$), but similar after haplo BMT and MRD BMT ($p = 0.80$) [62].

7.7 Expert Point of View

Utilizing haplo BMT allows identification of a donor for the vast majority of patients and represents the least expensive alternative graft source. With PTCy, haplo BMT leads to highly reproducible, safe outcomes without the requirement for specialized graft modification. By selectively depleting alloreactive T-cells and maintaining T_{regs}, PTCy has enabled haplo BMT with a similar incidence of aG*v*HD, NRM, and lower cG*v*HD than that seen with HLA-matched BMT utilizing a combination of MTX and CNI immunosuppression. PTCy after haplo-BMT results in survival comparable to that after HLA-matched BMT, thereby transporting us across the HLA barrier.

7.8 Future Directions

Certain nonmalignant diseases including autoimmune diseases, immunodeficiency disorders, and hemoglobinopathies have the potential for therapeutic benefit from a transplanted hematopoietic system and Cy-induced tolerance. However, use of

HLA-matched BMT has always been limited by a low tolerance for G*v*HD in these diseases given the absence of a potential benefit from G*v*T effects. With the improved safety associated with PTCy, we may be able to expand further the availability of BMT to nonmalignant diseases with accepatable risks of cG*v*HD and NRM. For nonmalignant transplant indications, the goal of hematopoeitic stem cell transplantation is stable engraftment. Yet, graft failure risk remains high given the lack of prior exposure to marrow-suppressing chemotherapy leading to persistent functionality of host T-cells that can attack the graft. While PTCy may play a role in graft failure reduction in these patients, further alterations to the standard approach, such as inclusion of anti-thymocyte globulin or increasing doses of total body irradiation (TBI), will be necessary to increase engraftment rates in this patient population.

PTCy could also be integrated into platforms for combined solid organ and hematopoietic stem cell transplantation. One of the earliest observations in transplantation immunology was that stable hematopoietic chimerism from a genotypically nonidentical donor was a sufficient condition for the permanent acceptance of solid organ grafts from the same donor [69, 70]. The observation of immunologic tolerance of solid organ allografts via naturally acquired hematopoietic chimerism has led to efforts to induce stable hematopoietic chimerism as a method of achieving transplantation tolerance in the clinic. Post-transplantation lymphoproliferative disease (PTLD), kidney disease, cardiovascular complications, and chronic graft rejection, many of which are attributed to post-transplant immunosuppression, can plague organ transplant recipients. However, transplanting a new immune system that is tolerant to the graft would allow discontinuation of immunosuppression after achievement of stable chimerism. If chronic rejection and organ toxicity from long-term immunosuppression is ameliorated by combined organ-marrow transplantation, then the life of the grafted organ may be prolonged.

Finally, PTCy allows early immunosuppression cessation, with little risk of cG*v*HD, and creates long-term immune tolerance by destruction of alloreactive T-cells. Thus, it may be the ideal platform for early implementation of additional targeted therapies to prevent relapse. Moreover, immune therapies such as immunologic checkpoint blockade, which possess a high risk of post-transplant toxicity by triggering G*v*HD or other immune-related adverse events, may be more safely employed in a transplant setting in which alloreactive T-cells have already been destroyed by PTCy. In that setting, checkpoint blockade may awaken T-cells specific for tumor antigens, without the risk of stimulating HLA-specific T-cells.

7.9 Summary

- Post-transplant cyclophosphamide allows bone marrow transplantation across major HLA barriers.
- Improved safety and a lower nonrelapse mortality have been observed after HLA-haploidentical transplantation using post-transplant cyclophosphamide-based G*v*HD prophylaxis.
- Outcomes of patients treated with HLA-haploidentical transplants are now similar to those with HLA-matched donor transplantation.

- Future directions will include the use of post-transplant cyclophosphamide-based G*v*HD prophylaxis to facilitate hematopoietic stem cell transplantation for nonmalignant diseases, to combine solid organ and hematopoietic transplants, and to enable the early addition of post-transplant maintenance strategies to reduce relapse for patients with hematologic malignancies.

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Part II

Ideal Graft and Donor

8 Graft Source: Marrow or Peripheral Blood with Posttransplant Cyclophosphamide—What Matters?

Paul V. O'Donnell

8.1 Introduction

The choice of bone marrow (BM) or G-CSF-mobilized peripheral blood (PB) as the source of $CD34^+$ cells for transplantation from HLA-haploidentical, related donors is controversial. Short of a randomized clinical trial comparing these two graft sources (which is highly unlikely), it has been necessary to compare multicenter or single-center phase II data retrospectively using either of these two graft sources in the setting of ablative or non-myeloablative conditioning or by comparing registry data. Different approaches to haploidentical transplantation have been taken to circumvent the alloreactivity of donor T-cells which likely led to the high incidence of fatal G*v*HD when haploidentical transplants were first attempted in the 1980s. This chapter will review the data and will discuss whether BM or PB as the source of graft for haploidentical transplantation using PTCy makes any difference in the transplant outcomes of engraftment/hematopoietic recovery, acute and chronic G*v*HD, NRM, relapse, or survival.

8.2 Marrow *Versus* Peripheral Blood in the HLA-Matched Donor Setting

For transplantation from HLA-matched related or unrelated donors, the question regarding utility of donor source has been settled more definitively by randomized clinical trials. But even in those settings, at least with conventional pharmacologic prophylaxis of graft-*versus*-host disease (G*v*HD) using a calcineurin inhibitor (CNI) such as cyclosporine A or tacrolimus combined with short-course methotrexate, there is current controversy over whether BM should be favored over PB for

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transplantation from unrelated donors based on results from the BMT CTN 0201 trial which showed a decreased incidence of chronic G*v*HD in the BM arm without a difference in survival [1]. There is no question that reducing the serious complication of chronic G*v*HD which can markedly diminish a patient's quality of life [2, 3] should be paramount as the field of blood and marrow transplantation evolves.

8.2.1 BM *vs.* PB in Myeloablative Setting

Comparison of BM *vs.* PB was first studied in HLA-matched, related donors in several randomized trials in the 1990s using myeloablative conditioning, which are summarized in a meta-analysis of nine trials conducted by the Stem Cell Trialists' Collaborative Group [4]. An absolute increase of 16% in the incidence of chronic G*v*HD was observed for patients with hematologic malignancies who received PB (overall incidence at 3 years of 67%; 47% incidence of extensive chronic G*v*HD). However, a survival advantage was observed for patients with unfavorable prognostic features, which, probably, influenced the use of PB as a graft source for transplants from HLA-matched related donors. A similar increase in chronic G*v*HD also was seen with PB as the graft source in the BMT CTN 0201 trial of transplantation from HLA-matched unrelated donors following myeloablative conditioning, but in that setting there was no survival advantage for PB, indicating that BM was the winner in terms of reduced complications of G*v*HD with similar survival outcomes [1]. However, it is important to note that in this trial there was still a high incidence of extensive chronic G*v*HD in both arms as scored by Seattle criteria [5]: 32% (95% CI, 26–38%) in the BM arm and 48% (95% CI, 42–54%) in the PB arm. Despite these data, PB still seems to be the favored graft source for unrelated donor transplantation accounting for about 70% of donations in 2015 [6].

8.2.2 BM *vs.* PB in Non-ablative Setting

In the setting of reduced-intensity transplantation, a recent retrospective registry study from the Center for International Blood and Marrow Transplant Research (CIBMTR) showed that the rates of acute G*v*HD, chronic G*v*HD, and overall survival were similar after transplantation of PB compared to BM for patients with acute myeloid leukemia, myelodysplastic syndrome, or non-Hodgkin lymphoma, although the rates of chronic G*v*HD were high with either graft source [7].

8.2.3 BM *vs.* PB: Logistics and Practical Aspects

There are a number of reasons why PB may be a more attractive graft source than BM for hematopoietic cell transplantation, including clinical considerations such as increased rates of engraftment/hematopoietic recovery which may be more important in the non-ablative setting or nonclinical considerations such as (1) ease of

collection of mobilized PB by apheresis without depending on operating room facilities for BM harvesting which may be especially relevant at smaller centers, (2) possible decline in the number of physicians with clinical competence in BM harvesting (although most US centers have a policy for credentialing physicians performing BM harvests [8]), and (3) cost considerations. From a donor perspective, peripheral blood also may be a safer alternative to BM for allograft donors. Recent studies of volunteer, unrelated donors indicated that the potential risk of adverse events was greater for BM donors than for PB donors [9, 10] although both approaches were relatively safe with a risk of life-threatening or permanent side effects of <0.3%. A large safety study for related donors (RDSafe; NCT00948636) is completed, and results should be published in the near future.

8.3 Approaches to Performing HLA-Haploidentical Transplantation

Different approaches to haploidentical transplantation have been taken to circumvent the alloreactivity of donor T-cells which can directly target mismatched HLA, particularly HLA class I antigens [11], ubiquitously present on tissues and which likely led to the high incidence of fatal G*v*HD when haploidentical transplants were first attempted in the 1980s [12, 13].

8.3.1 T-Cell Depleted Allografts

The Perugia Group pioneered the approach utilizing T-cell depletion of PB allografts by positive CD34 selection for transplantation of patients with hematologic malignancies after myeloablative conditioning but without posttransplant pharmacologic prophylaxis of G*v*HD ([14]; see Chap. 2). This approach reduced the incidence of acute and chronic G*v*HD to <10%, but outcomes were limited by high rates of nonrelapse mortality (NRM) primarily due to opportunistic infections in immunodeficient recipients. This approach has been improved upon by either selective depletion of T-cells expressing TCRαβ [15, 16] or by add-back of specific T-cell subsets, e.g., regulatory T-cells (T_{regs}) and NK cells [17] (see Chap. 4).

8.3.2 T-Replete Allografts with Conventional G*v*HD Prophylaxis

All other approaches have used T-replete haploidentical donor allografts. The Beijing Group pioneered an approach using G-CSF-mobilized BM plus PB with aggressive myeloablative conditioning including high-dose cytarabine, busulfan, cyclophosphamide, methyl-CCNU, and ATG combined with G*v*HD prophylaxis including cyclosporine, methotrexate, and mycophenolate mofetil ([18]; see Chap. 5). Hundreds of younger patients (median age 25 years) with acute leukemias have been transplanted in China using this approach with relatively low rates of severe acute and chronic

G*v*HD and excellent survival outcomes [19]. A similar approach has been taken by the group in Italy using exclusively G-CSF-mobilized BM but also using aggressive multidrug myeloablative conditioning and multidrug G*v*HD prophylaxis in younger patients (median age 37 years) with hematologic malignancies resulting in relatively low rates of severe acute and chronic G*v*HD ([20]; see Chap. 5).

8.3.3 T-Replete Allografts with Posttransplant Cyclophosphamide (PTCy)

A different approach, which is being used increasingly worldwide, was pioneered by the group at Johns Hopkins, who introduced the use of high-dose cyclophosphamide given on days 3 and 4 posttransplant (PTCy) to selectively eliminate the highly alloreactive T-cells which could potentially cause fatal G*v*HD ([21, 22]; see Chap. 7). This low-tech and low-cost Hopkins approach to haploidentical transplantation was initially developed to treat hematologic malignancies using non-myeloablative conditioning with BM as the hematopoietic graft source. The largest number of haplo-BM transplants to date performed at a single center was reported in a recent update by McCurdy and coworkers [23]. More recently, the use of PTCy as an effective approach for reducing the complication of G*v*HD after haploidentical transplantation has been extended to myeloablative BM transplantation [24, 25]. Concerns for significant G*v*HD with PB have delayed the use of this graft source in haploidentical transplantation performed with PTCy. However, several groups have started to use PB as the graft source after either non-myeloablative conditioning [26–30] or myeloablative conditioning [31–34].

8.4 Addressing the Question of BM or PB as the Preferred Hematopoietic Graft Source with PTCy-Based Prophylaxis of G*v*HD

The purpose of this chapter is to discuss whether BM or PB as the source of graft for haploidentical transplantation using PTCy makes any difference in the transplant outcomes of engraftment/hematopoietic recovery, acute and chronic G*v*HD, NRM, relapse, or survival.

8.5 Comparison of Outcomes After Haplo-BM *Versus* Haplo-PB Transplantation Following Non-myeloablative Conditioning and PTCy-Based G*v*HD Prophylaxis

Available data to address our central question after non-myeloablative conditioning comes primarily from three studies (Table 8.1). Two of the studies were single-center retrospective studies [26, 29]. The study by myself and an international group

Table 8.1 Haploidentical transplantation using reduced-intensity conditioning and PTCy

	BM			PB		
	Castagna et al. 2014 [26]	Bradstock et al. 2015 [29]	O'Donnell et al. 2016 [35]	Castagna et al. 2014 [26]	Bradstock et al. 2015 [29]	O'Donnell et al. 2016 [35]
N	46	13	43	23	23	43
Median age (yr)	44	53	49	54	44	49
Diagnosis						
% leukemia	8	85	63	19	65	37
% lymphoma	92	15	37	81	22	63
DRI						
% intermediate		46	74		65	74
% high		31	14		17	14
Hematopoietic recovery						
Median *d* to ANC >500	21	15	17	20 (0.18)*	16	18 (NS)
Median *d* to PLT >20K	29	18	25	27 (0.13)	24	24 (NS)
% graft failure	13	0	14	5 (0.18)	13	7 (NS)
% acute G*v*HD						
Grade III/IV	3		0	14 (0.10)	9 (0.65)	5 (95% CI: 1–14)
% chronic G*v*HD	13	29	23	13 (0.21)	32 (0.69)	19 (0.63)
Moderate			16			5 (NS)
Severe			5			2 (NS)
% NRM (2 yr)	22		7	12 (0.96)		12 (0.50)
% relapse	25	44	58	18 (NS)**	24 (0.29)	24 (0.006)
Survival						
Median follow-up (mo)	24	57	60	11	20	39
% DFS (2 yr)	62	38	42	62 (NS)	64 (0.40)	65 (0.03)
% OS (2 yr)	68	53	58	68 (NS)	83 (0.03)	66 (0.47)

**p* value for differences between BM and PB within a given study
***NS, no significant difference stated in publication without *p* value

of collaborators [35] was a comparison of outcomes from two phase II studies, a 16-center clinical trial (BMT CTN 0603) of haplo-BM previously reported by BMT CTN investigators [36, 37], and a collaborative, international study of haplo-PB from centers in the USA, the UK, France, and Australia. In the comparative study, haplo-BM or haplo-PB study subjects were matched for age and disease risk index (DRI). DRI is a composite of disease, disease status, and cytogenetic risk in the case of acute leukemias, which has been shown to independently risk stratify heterogeneous adult patient cohorts with hematologic malignancy regardless of conditioning intensity or graft source [38].

Although the numbers of patients across the three studies in Table 8.1 are relatively small (about 100 patients for each graft source), the studies are quite similar in many aspects. Although there are differences in the percent of patients with

acute leukemias or lymphomas in the studies, the percent of patients with an intermediate or high disease risk index (DRI) is at least 80% of study participants in all of these studies. All patients were transplanted with the same non-myeloablative conditioning consisting of 150 mg/m^2 fludarabine, 29 mg/kg cyclophosphamide, and 200 cGy TBI (Hopkins regimen), and all patients received the same G*v*HD prophylaxis of PTCy (50 mg/kg cyclophosphamide on days 3 and 4) plus a CNI (tacrolimus [target level 5–10 ng/mL] or cyclosporine A [target level 100–200 ng/mL]) plus mycophenolate (1 g three times daily). CNI and mycophenolate were started on day 5 and continued to day 35 (mycophenolate) or day 180 (CNI). In all of the studies, the targeted BM dose was 4×10^8 TNC/kg of recipient weight, and the targeted PB dose was 5×10^6 CD34$^+$ cells/kg of recipient weight, the latter intended to restrict the dose of donor T-cells which was shown in the different studies to be 5–10 times higher in PB grafts compared to BM grafts. Within each study, no significant differences in hematopoietic recovery, T-cell engraftment determined by donor CD3-positive cell chimerism at day 28 post-transplant (graft failure rate was <5–10% donor), grade III–IV acute G*v*HD, or chronic G*v*HD were seen between transplants using either of the graft sources. Across the studies, median time to recovery of neutrophils (ANC >500) ranged from 15 to 21 days for BM and 16–20 days for PB. Median time to hematopoietic recovery of platelets (>20,000, untransfused) ranged from 18 to 29 days for BM and 24–27 days for PB. Primary graft failure ranged from 0% to 14% for BM and 0–13% for PB. The incidence of severe acute G*v*HD (grade III, no grade IV was observed) was low ranging from 0% to 14% for BM and 5–18% for PB. The incidence of chronic G*v*HD scored using NIH criteria [41] between the graft sources ranged from 13% to 23% overall for BM and 13–32% overall for PB. Of importance, the incidence of severe chronic G*v*HD measured by the NIH global severity score was very low even in the PB cohort (2%) which was not expected based on studies of matched donor transplants using conventional G*v*HD prophylaxis [1, 4]. Similarly, NRM was similar for both graft sources ranging from 7% to 22%. Only in the matched pair analysis [35] was there a significant difference in relapse rates between the two cohorts which translated into a significant difference in disease-free survival (DFS) with the PB cohort having a higher rate of DFS at 2 years (65% *vs.* 35%). This difference may be explained by different ratios of acute leukemia/lymphoma between the cohorts (2:1 for BM, 1:2 for PB) although the DRIs were balanced between the two groups. However, in all three studies, there were no significant differences in overall survival which ranged from 53% to 68% for BM and 61–83% for PB. Overall survival was the same in the study, which had the longest median follow-up of >3 years for both BM and PB cohorts [35].

8.6 Comparison of Outcomes After Haplo-BM *Versus* Haplo-PB Transplantation Following Myeloablative Conditioning and PTCy-Based G*v*HD Prophylaxis

There are fewer studies of haplo-BM or haplo-PB transplantation following myeloablative conditioning using PTCy/CNI/MMF as G*v*HD prophylaxis as shown in Table 8.2. Retrospective comparisons of haplo-BM to haplo-PB have not been performed at any center because the centers represented in Table 8.2 have used

Table 8.2 Haploidentical transplantation using ablative conditioning and PTCy

	BM	PB		
	Raiola et al. 2013 [24]	Solomon et al. 2012 [33]	Solomon et al. 2014 [34]	Gaballa et al. 2016 [32]
N	50	20	30	50
Median age (yr)	42	44	46	49
Conditioning	TBF or FluTBI	BuFluCy	FluTBI (12 Gy)	2-Step
Diagnosis				
% leukemia	90	85	93	88
% lymphoma	10	15	7	12
Conventional risk status				
% standard	46	45		
% high	54	55		
DRI				
% intermediate			40	64
% high			47	30
Hematopoietic recovery				
Median *d* to ANC >500	18	16	16	11
Median *d* to PLT >20K	23	27	25	17
% graft failure	4	0	0	4
% acute G*v*HD				
Grade III/IV	6	10	23	6
% chronic G*v*HD	26	35	56	19
Moderate	10		22	
Severe		5	10	4
% NRM (2 yr)	18	10	3	10
% relapse	26	40	24	21
Survival				
Median follow-up (mo)	>8	20	24	38
% DFS (2 yr)	51	50	73	68
% OS (2 yr)	62	69	78	70

only one or the other of the two graft sources. These studies are limited by sample sizes and length of follow-up. However, there is less disease heterogeneity than seen with RIC transplants with almost all of the patients in the four studies having acute leukemia, half of whom were high risk by standard criteria or DRI. Conditioning varied among the haplo-PB studies with the earlier study by Solomon and coworkers [33] using high-dose chemotherapy and the later study by Solomon and coworkers [34] and Gaballa and coworkers [32] using high-dose TBI. The study of Gaballa and coworkers is a unique application of PTCy given prior to stem cell infusion developed by Grosso and coworkers [31]. The haplo-BM study of Raiola and coworkers [24] used chemotherapy-based or TBI-based conditioning in a roughly equal percentage of patients. Time to engraftment appeared similar to what has been reported for haploidentical transplantation using non-myeloablative conditioning, and almost no primary graft failure was observed. Incidences of severe acute and chronic G*v*HD were variable after both haplo-BM and haplo-PB transplantation but certainly within the range seen for conventional G*v*HD prophylaxis. In Table 8.2, there appears to be an increased incidence of severe acute and chronic G*v*HD after TBI conditioning [34] compared to chemotherapy-based conditioning [33] in studies from the Northside Group, which may be significant since G*v*HD grading is conducted by a single investigator in their transplant center. On the other hand, it appeared that survival may be improved with TBI-based conditioning. These differences aside, it remains to be seen whether outcomes after haplo-BM or haplo-PB following myeloablative conditioning will prove to be comparable. Two large, recently reported registry studies from the CIBMTR [39] or EBMT [40] seem to present conflicting data on this point (Table 8.3). In both studies the majority of patients had the diagnosis of acute leukemia although there was heterogeneity in the percent of patients conditioned with myeloablative or non-myeloablative conditioning between the comparator arms. Both studies showed no significant differences in the incidence of grades III and IV acute G*v*HD or survival outcomes between haplo-BM and haplo-PB cohorts. The CIBMTR study showed a significantly higher rate of chronic G*v*HD (scored by Seattle criteria, 5) in haplo-PB transplants which differed from the results of the smaller studies shown in Table 8.1 and the EBMT study although the percent of patients with moderate or severe chronic G*v*HD were the same for both cohorts in the CIBMTR study. In multivariate analyses, the CIBMTR study showed no effect of conditioning intensity on survival outcomes in contrast to the EBMT study. The EBMT study also showed a center effect unlike the CIBMTR study. Both studies had relatively short median follow-up especially in the haplo-PB cohorts. Increased numbers of patients in the different subgroups, greater experience with ablative haploidentical transplantation, and longer follow-up may be necessary to resolve these discrepancies in order to confirm that haplo-BM and haplo-PB are comparable graft sources in the ablative setting.

Table 8.3 Registry studies of haploidentical transplantation

	BM		PB	
	Bashey et al. 2017 [39]	Ruggeri et al. 2016 [40]	Bashey et al. 2017 [39]	Ruggeri et al. 2016 [40]
N	496	260	191	191
Median age (yr)	58		47	
Diagnosis				
% Leukemia	61	73	63	73
% Lymphoma	39	27	37	29
DRI				
% Intermediate	66		48	
% High	22		42	
Conditioning				
Myeloablative	18	61	54	49 (.008)
Non-myeloablative	82	39	46	51
Hematopoietic recovery				
Median *d* to ANC >500	17	18	16 (<.001)*	17 (.001)
Medain *d* to PLT >20K	26		25 (.03)	
% Graft Failure	9		12 (NS)**	
% Acute GvHD				
Grade III/IV	7	3	10 (NS)	8 (NS)
% Chronic GvHD	20	36	41 (.0001)	32 (NS)
Moderate	28		30 (NS)	
Severe	10		12 (NS)	
% NRM (2 yr)	17	23	16 (NS)	23 (NS)
% Relapse	45	26	28 (.009)	22 (NS)
Survival				
Median follow-up (mo)	35	22	20	18
% DFS (2 yr)	41	49	54 (NS)	54 (NS)
% OS (2 yr)	54	55	57 (NS)	55 (NS)

*p value for differences between BM and PB within a given study
**NS, no significant difference stated in publication without p value

8.7 Comparison of Outcomes After BM *Versus* PB Transplantation from HLA-Matched Donors Following Myeloablative Conditioning and PTCy-Based G*v*HD Prophylaxis

Compared to the incidences of acute and chronic G*v*HD routinely seen after HLA-matched donor transplantation using conventional prophylaxis with a CNI and short-course methotrexate (~15% acute grade III/IV G*v*HD [42] and 34% NIH chronic G*v*HD requiring immunosuppressive therapy [43]), the reduced

incidences seen after haploidentical donor transplantation using PTCy-based prophylaxis (5–10% acute grade III G*v*HD and <10% severe NIH chronic G*v*HD) are quite remarkable. Stimulated by those findings, more recent studies have extended use of single-agent PTCy prophylaxis to BM transplantation from HLA-matched related and unrelated donors after myeloablative conditioning with either busulfan/cyclophosphamide or busulfan/fludarabine [44, 45]. The same reduction in incidence of severe acute and chronic G*v*HD was seen as for haploidentical transplants, without increasing the risk of relapse. Therefore, given these findings, a similar question could be asked in the matched donor setting with regard to whether BM or PB graft sources are comparable. Since the BMT CTN 0201 study showed a disadvantage to using PB grafts from HLA-matched unrelated donors due to the increase of chronic G*v*HD [1], it was reasonable to ask whether this disadvantage could be abrogated by use of PTCy after PB transplantation from matched donors. Unlike BM transplantation, single-agent PTCy did not appear to be feasible after PB transplantation. Omission of a CNI in the setting of matched donor PB transplantation was shown in a small study by Bradstock and coworkers [46] to result in an unacceptably high incidence of severe acute G*v*HD which was fatal in three of five cases presumably mediated by the five to tenfold higher number of T-cells in the allografts. Consequently, PTCy was combined with a CNI in two recent prospective phase II studies of matched related and unrelated donor PB transplants after myeloablative conditioning. Results of these two trials are compared to the earlier trials of BM transplantation and single-agent PTCy prophylaxis in Table 8.4. As for haploidentical transplantation using either BM or PB allografts with PTCy-based prophylaxis, it appears that the outcomes after either matched donor BM or PB transplantation using PTCy-based prophylaxis are similar. In each study, the overall incidence of chronic G*v*HD was approximately 16% compared to historical controls in which the incidence of NIH chronic G*v*HD was in the order of 34% [43]. It is important to distinguish the scoring used to assess chronic G*v*HD when comparing across studies. When chronic G*v*HD was scored using the older Seattle criteria [5] in the study by Mielcarek and coworkers [47], the incidence of severe (extensive) chronic G*v*HD was 30% compared to the 16% incidence of chronic G*v*HD requiring immunosuppressive therapy as determined by NIH criteria. Thus, there is an absolute difference in incidence of chronic G*v*HD of about 15% depending on the criteria used ([47] and M. Flowers, personal communication). All studies in Table 8.4 used NIH scoring criteria. In the study by Mielcarek and coworkers [47], cyclosporine A was combined with PTCy, while in the study by Moiseev and coworkers [48], tacrolimus plus mycophenolate was combined with PTCy, the same prophylaxis used in haploidentical transplants. Not only was a reduction in chronic G*v*HD observed in these studies compared to that seen with conventional G*v*HD prophylaxis but no grade III–IV acute G*v*HD was observed in

Table 8.4 HLA-matched donor transplantation using PTCy

	BM		PB	
	Luznik et al. 2010 [44]	Kanakry et al. 2014 [45]	Mielcarek et al. 2016 [47]	Moiseev et al. 2016 [48]
N	117	92	43	86
Median age (yr)	50	49	38	34
% donor				
Related	67	49	28	
Unrelated	34	51	72	100
Conditioning	MAC: BuCy	MAC: BuFlu	MAC: BuFlu (58%); 12 Gy TBI (42%)	MAC: BuCy (24%); RIC: BuFlu (65%)
Diagnosis				
% leukemia	77	96	98	100
% lymphoma	33	4	2	
Conventional risk status				
% standard	42	73	51	72
% high	58	27	49	28
Hematopoietic recovery				
Median *d* to ANC >500	23	21	19	19
Median *d* to PLT >20K	25	24	14	
% graft failure	3	5	2	1
% acute G*v*HD				
Grade III/IV	10	15	0	4
% chronic G*v*HD	10	14	16	16
Moderate		8		
Severe	3	6		
% NRM (2 yr)	15	16	14	16
% relapse				
Overall	44	22	17	19
Standard risk	26	10		
High risk		38		
Survival				
Median follow-up (mo)	26	26	23	12
% DFS (2 yr)				
Overall	39	62	69	65
Standard risk	54	80		
High risk	29	33		
% OS (2 yr)				
Overall	55	67	70	69
Standard risk		80		
High risk		54		

the Mielcarek study and only 4% in the Moiseev study. NRM, relapse, DFS, and OS were also comparable to what has been observed with BM allografts.

8.8 Expert Point of View and Future Directions

So, does it matter if BM or PB is used as the graft source for haploidentical transplants using PTCy-based prophylaxis of G*v*HD? Although much of the data described in this chapter is limited by relatively small numbers of studies, small numbers of subjects, disease heterogeneity, and lack of randomization, it appears likely that BM or PB can be used interchangeably as graft sources after nonmyeloablative conditioning and possibly after myeloablative conditioning. Interestingly, the recent studies of HLA-matched related and HLA-matched unrelated donor transplantation using PTCy were generated on the basis of the excellent G*v*HD prophylaxis obtained in the haploidentical setting. Although not discussed in the chapter, the fact that most transplant patients receiving PTCy-based prophylaxis can be weaned off immunosuppressive therapy early posttransplant (see Chap. 18) provides a platform for adoptive cell therapy to further reduce the likelihood of relapse in high-risk patients who require transplant. Furthermore, data described in this chapter for HLA-matched donor transplants suggests that BM and PB graft sources may also prove to be interchangeable in that setting as well.

Beyond the central question of graft source comparability raised in this chapter, it seems that the field of hematopoietic cell transplantation is primed for a paradigm shift from conventional G*v*HD prophylaxis with a CNI and short-course methotrexate to a different type of prophylaxis, which can more effectively decrease the incidence of debilitating chronic G*v*HD. Although additional data is required, the phase II studies and registry studies presented in this chapter suggest that PTCy-based prophylaxis for transplants from all donor types with either BM or PB as the source of the allograft could potentially meet this need by decreasing the incidence of chronic G*v*HD at least 50%. But a change in standard practice will depend on favorable results from randomized studies. In this regard, two randomized trials of new approaches to prophylaxis of G*v*HD (including PTCy) are currently ongoing in the BMT CTN (Progress I [CTN 1203] of reduced-intensity conditioning with HLA-matched donors and Progress II [CTN 1301] of myeloablative conditioning with HLA-matched donors). Progress I trial has completed accrual so results from this study should be forthcoming. These trials will use the important new endpoint of G*v*HD/relapse-free survival (GRFS) to compare outcomes with contemporaneous controls in which G*v*HD prophylaxis consists of the conventional CNI (tacrolimus) and short-course methotrexate. When these trials are completed, we will hopefully move into a new era of hematopoietic cell transplantation, in which better prevention of acute and chronic G*v*HD will improve the outcomes and quality of life for our patients.

8.9 Key Points

- PTCy appears to reduce the incidence of chronic G*v*HD regardless of graft source.
- Outcomes after non-ablative transplantation with haplo-BM or haplo-PB transplant appear comparable and less clear in the setting of ablative conditioning.
- PTCy may represent a new paradigm for G*v*HD prophylaxis after transplantation from both HLA-haploidentical and HLA-matched donors.

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Anti-HLA Antibodies: Assessment and Mitigating Strategies

9

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9.1 Introduction

Transplantation from one human leukocyte antigen (HLA) haplotype-matched first-degree relatives (haplo-HCT) is the most accessible graft source and has been increasing used as an alternative option for patients without a suitable HLA-matched donor. However, the intense bidirectional alloreactive reactions related to the major HLA mismatch between the recipient and haploidentical donor can result in higher incidence of graft-*versus*-host disease (G*v*HD) and graft failure (GF). While several graft manipulation methods have been developed which are aimed to partially deplete T-cells and reduce graft-*versus*-host (G*v*H) reactions, GF remains a major problem, which has been reported in approximately 10–20% of patients receiving a haplo-HCT [1–5]. The increased risk of GF following haplo-HCT is due, in part, to an enhanced susceptibility of the graft to chemoresistant host natural killer (NK) cell and T-lymphocyte-mediated rejection against mismatched donor cells (cellular rejection) [6, 7]. In addition, antibody-mediated rejection

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(humoral rejection) occurring either by antibody-dependent cell-mediated cytotoxicity or complement-mediated cytotoxicity also has been described [8, 9]. Recipient preformed anti-HLA antibodies against donor HLA antigens are well recognized as a major cause of rapid graft rejection in solid organ transplant [10–13]. Therefore, lymphocyte crossmatch tests have been initially developed for prediction of graft rejection [14, 15], and testing became mandatory in solid organ transplants according to the American Society for Histocompatibility and Immunogenetics (ASHI). In allogeneic hematopoietic cell transplantation (allo-HCT) with HLA-mismatched donors, a positive crossmatch for anti-donor lymphocytotoxic antibodies is associated strongly with GF, mainly in mismatched related donor transplants [16, 17]. Although a lymphocyte crossmatch is an effective tool to evaluate alloimmunization and potential donor-recipient incompatibility, the procedure is labor intensive and may detect non-HLA antibodies, which may not be associated with transplant outcome. Over the recent years, several methods have been developed to more precisely detect and characterize serum anti-HLA antibodies in allo-HCT recipients [18, 19], and also the clear association between the presence of these donor-specific anti-HLA antibodies (DSAs) and the development of primary GF has been established, not only in haplo-HCT but also in all types of transplants with HLA-mismatched donors [11, 20, 21].

In this chapter, we will focus on the role of DSA in the development of primary GF in haplo-HCT as well as the progress made in the treatment of patients with DSA in order to improve engraftment rate and transplant outcomes.

9.2 Human Leukocyte Antigen Sensitization

The major histocompatibility complex (MHC) located on chromosome 6 consists of a linked set of genetic loci containing many genes involved in the immune response, including the HLA genes. The products of these genes are expressed on the cell surface as glycoproteins, of which there are three classes within the MHC region:

- Class I region, which includes the HLA genes HLA-A, HLA-B, and HLA-C, expressed on nearly all nucleated cells
- Class II region, which includes HLA genes HLA-DR, HLA-DQ, and, HLA-DP, only expressed on B-cells and antigen-presenting cells
- Class III region, which includes the genes for components of the complement cascade and cytokines

The MHC class I and II molecules are the most immunogenic antigens, are recognized by preformed anti-HLA antibodies in recipients' serum, and can mediate rejection of transplanted cells. Among these, most immunogenic molecules are HLA-A, HLA-B, and HLA-DRB1 [22].

9.2.1 Risk Factors for the Development of Anti-HLA Antibodies in Allo-HCT Recipients

Anti-HLA antibodies can develop after exposure to non-self HLA antigens. They may be unique to a specific allele or limited group or recognize an epitope that is shared by more than one HLA molecules resulting in cross-reactivity. Pregnancy and transfusion of allogeneic blood products have been identified as common risk factors for development of anti-HLA antibodies [19, 23–25].

Transfusion of allogeneic blood products can introduce foreign antigens into the recipient that persist for a variable amount of time. Blood transfusion recipients who are immunocompetent often develop an immune response to the donor antigens, with several clinical implications. Most commonly involved HLA antigens developed in this fashion are class I shared by platelets and leukocytes and class II presented on some leukocytes, granulocyte-specific antigens, platelet-specific antigens (human platelet antigen), and red blood cell-specific antigens. The consequences of alloimmunization to blood products depend on involved antigens. For instance, alloimmunization against platelet-specific or HLA class I antigens can cause platelet refractoriness, while alloimmunization against HLA antigens in the graft can cause immune-mediated graft rejection in allo-HCT. It is well recognized in solid organ transplantation that repeated transfusion is a major risk factor of developing anti-HLA antibodies and engraftment failure [25–27]. Anti-HLA antibodies developed after multiple transfusion is also an important barrier of successful engraftment in patients with severe aplastic anemia [8] and hemoglobinopathies, like beta-thalassemia, receiving a allo-HCT [28]. It has been reported that preformed anti-HLA antibodies were presented in approximately 55% in pediatric patients with aplastic anemia undergoing allo-HCT. In this report, patients who had anti-HLA antibodies tended to have received more units of blood products before transplantation [29].

Besides transfusion of allogeneic blood products, there is strong evidence to suggest that female sex and pregnancy confer a significant risk for allosensitization, and this risk is further increased with a higher number of pregnancies. The frequency of lymphocytotoxic anti-HLA antibodies has been reported up to 50% in the multigravidous women [30]. We have found a striking association between the sex of patients who experience primary GF and the development of DSA both in haploidentical and unrelated donor transplants mismatched at HLA-DP locus [19, 23]. In HLA-matched unrelated donor (MUD) transplants, these patients were multiparous middle-aged women with a median of three pregnancies; 30% of women *versus* 12% of men had DSAs ($p < 0.0001$) and seven of eight patients with DSAs were women, all of whom except one had at least two prior pregnancies. When the presence of DSAs was evaluated in women with no pregnancies compared with the male recipients, no significant difference was identified. Although the majority of allosensitized individuals in this study were women, 12% of patients with DSAs were men, suggesting that other factors, like transfusion of blood products, are associated

with the development of anti-HLA antibodies in these patients [23]. In haplo-HCT, all patients with DSA were also middle-aged females [19]. According to these two studies, the impact of transfusions appeared to be much lower than the impact of multiple pregnancies in the development of DSA.

9.2.2 Prevalence of Anti-HLA in Haplo-HCT Setting

As mentioned above, healthy individuals can develop anti-HLA antibodies as a consequence of allosensitization during pregnancy or multiple blood transfusions. In patients with hematologic malignancies referred for allo-HCT, the reported prevalence of anti-HLA antibodies ranges between 16% and 40% in adults [19, 20, 31, 32] and is of approximately 20–25% in patients undergoing haplo-HCT [23, 33, 34].

Despite a high prevalence of anti-HLA antibodies reported in allo-HCT patients, these anti-HLA antibodies might not be specific to donor HLA antigens. The increasing use of mismatched donors (haploidentical, cord blood, and HLA-mismatched unrelated donors), in addition to improvements in detection techniques, has facilitated recognizing anti-HLA antibodies that react against donor's antigens as a major cause of graft rejection. With the use of highly sensitive solid-phase immunoassays, DSAs were identified in up to 24% of allo-HCT recipients [3, 20, 21, 32, 33, 35, 36]. While overall, in haplo-HCT, the prevalence of DSAs may range between approximately 10% and 21% [19, 33, 34, 37], this proportion is highly dependent on the recipient's gender with very low prevalence in male recipients (5%) as compared with female recipients (86%) [37]. In addition to a much higher prevalence of DSA in female patients, much higher DSA levels were identified compared with DSA levels in allosensitized male patients [37]. Anti-HLA antibodies detected in female patients are much more often DSAs in the settings of "child-to-mother" haplo-HCT than in the settings of cord blood transplant (CBT) [19, 38]. It is because those anti-HLA antibodies are the results of sensitization during pregnancies by offspring's HLA itself, and it makes it often difficult to locate a donor who is not a target of anti-HLA antibodies. Thus it is particularly important to establish an effective desensitization protocol especially in the setting of haplo-HCT.

9.3 Mechanisms of Antibody-Mediated Graft Rejection in Haploidentical Transplants

Immune-mediated graft rejection is the most common cause of engraftment failure after allo-HCT, reported in approximately 5% using HLA-MUD and in up to 20% or more in CBT or T-cell-depleted haplo-HCT [39, 40]. The immune response to transplanted cells consists of both cellular (lymphocyte-mediated) and humoral (antibody-mediated) mechanisms. Recipient T-lymphocytes and NK cells may cause cellular-mediated graft rejection which depends on the genetic disparity between the donor and recipient and the status of host anti-donor reactivity [41]. This makes mismatched and haplo-HCT recipients likely more susceptible to

develop graft rejection compared with HLA-matched transplants due to stronger alloreactive reactions in this setting. However, in haplo-HCT, the use of myeloablative conditioning (MAC) and high-dose posttransplant cyclophosphamide (PTCy) may facilitate engraftment by eliminating alloreactive recipient's T-cells and NK cells, which are highly sensitive to cyclophosphamide [42].

Humoral or antibody-mediated graft rejection is a form of allograft injury primarily mediated by HLA antibodies against the donor HLA antigens and is likely complement-mediated. Antibody-mediated graft rejection can occur immediately posttransplantation (hyperacute) due to preformed antibodies in recipient's sera. In animal models of allo-HCT, preformed antibodies present at the time of marrow infusion in multi-transfused mice, rather than primed T-cells, have been shown to be a major barrier against marrow engraftment resulting in rapid graft rejection within a few hours in allosensitized recipients of MHC-mismatched bone marrow transplantation, while T-cell-mediated graft rejection takes much longer [9, 43]. The risk of antibody-mediated graft rejection in humans depends on antigen density on the target and capacity of binding to the antibody Fc domain. While many types of preformed antibodies can be detected in alloimmunized stem cell transplant recipients, only antibodies against donor HLA antigens have been shown to have clinical significance [23, 31, 32].

9.3.1 Role of Complement System in Antibody-Mediated Graft Rejection

Antibody-mediated graft rejection after allo-HCT can occur either by antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-mediated cytotoxicity [44]. Evidence from studies in cardiac and renal transplant patients has shown that complement system is activated in the transplanted organ during rejection and can be detected by measuring the products of complement activation in the patients' blood, urine, as well as in the transplanted organ itself [45–48]. In haplo-HCT setting, we recently found that DSAs that bind complement, detected by the C1q assay—the first component of the classical complement pathway—play a more important role in the development of graft rejection in haplo-HSCT recipients. In our study, the presence of C1q-fixing DSAs was found in 41% of patients with DSAs and was associated with a significant higher rate of GF compared with patients who had DSAs but negative C1q. The presence of complement also correlated with significantly higher DSA levels. Moreover, patients who became C1q negative after treatment and before transplant engrafted the donor cells successfully, while five patients who remained C1q positive at transplant experienced engraftment failure [37]. Whereas previous studies by Chen showed that there is no predictability by IgG mean fluorescence intensity (MFI) as to which of the antibodies will bind C1q because fixation is independent of MFI values [49], most patients who had positive C1q in our study had higher median MFI of DSAs (all more than 5000 MFI) compared with those who had negative C1q [37]. These results suggested that the complement fixation might play an important role in antibody-mediated rejection in allosensitized patients. Further studies are needed to elucidate the role of complement in this setting.

9.4 Testing for Donor-Specific Anti-HLA Antibodies

9.4.1 DSA Testing

Pretransplant sera of patient are tested for anti-HLA class I and class II antibodies using multianalyte bead assays performed on the Luminex platform including LABScreen® PRA, LABScreen® Mixed methods for screening; the binding level of DSA is determined by the LABScreen® Single Antigen bead assay [One Lambda, part of Thermo Fisher Scientific (Canoga Park, California, USA)] per manufacturer's instructions, and results are expressed as mean fluorescence intensity (MFI). The samples are read on Luminex-based LABScan™ 100 flow analyzer. Antibody specificity and binding level are analyzed and determined through HLA Visual or HLA Fusion software from the manufacturer. In order to evaluate if a prozone effect exists, the Luminex single antigen bead assay is performed using diluted serum at 1:8 for patients with DSA to the selected donor.

9.4.2 C1q Testing

Complement-binding antibodies are detected for patients with DSA using the C1q assay. The complement component (C1q) bound by the antigen-antibody complex is detected with an R-PE labeled anti-C1q antibody. Fluorescence intensity is measured using Luminex-based LABScan™ 100 flow analyzer. DSA specificity and binding level are determined by the C1qScreen™ assay per manufacturer's instructions [One Lambda, part of Thermo Fisher Scientific (Canoga Park, California, USA)]. The samples are read and C1q-specific antibody specificity and binding levels are analyzed and determined.

9.5 Impact of Anti-HLA Antibodies on Transplant Outcomes

It is now well established that DSAs are associated with primary GF in either HLA-mismatched related (haploidentical) and HLA-matched and HLA-mismatched unrelated donor or CBT transplants (Table 9.1). This association appears more important in haplo-HCT presumably due to the close relationship and higher likelihood of sharing the mismatched HLA antigens with DSAs against within the immediate family.

Convincing evidence of the association between DSAs and primary GF in allo-HCT with mismatched donors was initially provided by the MD Anderson group [19]. We initially tested 24 consecutive patients who received a total of 28 T-cell-depleted haplo-HCTs for the presence of DSAs determined by a highly sensitive and specific solid-phase/single antigen assay. DSAs were detected in 21% of the patients, 75% of which failed to engraft, compared with only 5% without DSAs ($p = 0.008$). All four patients who experienced primary GF had second haplo-HCT, and one patient who had persistent high titer of DSAs developed a second GF, while

Table 9.1 Donor-specific anti-HLA antibodies (DSAs) and transplant outcomes

Reference	Donor	Test	*N*	% anti-HLA^{+}	% DSA^{pos}	Graft outcome (DSA^{pos}/DSA^{neg})
Ciurea 2009 [19]	TCD Haplo	Luminex SA	24	NA	21	GF was 75% *vs.* 5% ($p = 0.008$)
Spellman 2010 [20]	MMUD	FlowPRA, Luminex SA	115	37	8.7	24% of GF group has DSAs *vs.* 1% of control group had DSAs
Ciurea 2011 [3]	MUD, 1Ag MMUD	Luminex SA	592	21	1.4	GF was 37.5% *vs.* 2.7% ($p = 0.0014$)
Yoshihara 2012 [33]	Haplo	Luminex SA	79	20	14	GF was 27% *vs.* 4% CI of neutrophil engraftment was 61.9% *vs.* 94.4% ($p = 0.026$)
Ciurea 2015 [37]	Haplo	Luminex SA	122	NA	18	GF was 32% *vs.* 4% ($p < 0.001$)
Chang 2015 [34]	Haplo	NA	345	25.2	11.3	Primary graft rejection was 20% *vs.* 0.3% ($p = 0.002$) Primary poor graft function was 27.3% *vs.* 1.9% ($p = 0.003$)
Takanashi 2010 [38]	Single UCB	FlowPRA, Luminex SA	386	23.1	5	CI of neutrophil engraftment was 32% *vs.* 83% ($p < 0.0001$) Patients with DSA had significant lower EFS and OS compared with no DSA
Brunstein 2011 [35]	Double UCB	Luminex SA	126	41	24% had DSAs target to 1 UCB, 12% had DSA target to both UCB	GF was 17% *vs.* 22%
Cutler 2011 [21]	Double UCB	Luminex SA	73	NA	24.6	GF was 18.2% and 57% in patients who had DSAs against 1 and 2 UCB, respectively, *vs.* 5.5% in patients without DSAs ($p = 0.01$) The rates of death or relapse within 100 days for the group of patients without DSAs, with DSAs against a single UCB unit, or DSAs against both UCB units were 23.6%, 36.4%, and 71.4%, respectively ($p = 0.01$)

(continued)

Table 9.1 (continued)

Reference	Donor	Test	*N*	% anti-HLA^{+}	% DSA^{pos}	Graft outcome (DSA^{pos}/DSA^{neg})
Ruggeri 2013 [32]	Single UCB, double UCB	Luminex SA	294	21	4.7	GF was 56% *vs.* 23% The presence of DSA was associated with lower survival (42% *vs.* 29%; $p = 0.07$)

MMUD HLA-mismatched unrelated donor, *MUD* HLA-matched unrelated donor, *GF* graft failure, *DSA* donor-specific anti-HLA antibody, *TCD Haplo* T-cell-depleted haploidentical hematopoietic cell transplantation, *UCB* umbilical cord blood, *EFS* event-free survival, *OS* overall survival, *NA* not available (Adapted from [59] with permission)

two out of three engrafted patients had the absence of DSAs. Patients in this study had DSAs directed against high-expression HLA loci, including class I HLA antigens (HLA-A and HLA-B) and class II (HLA-DRB1) antigens [19]. In a subsequent study, we found that anti-HLA antibodies directed against low-expression loci (HLA-DPB1 and HLA-DQB1) in patients with mismatched donors can also cause primary GF, however, to a lower extent. In our large prospectively tested patients for anti-HLA antibodies of 592 HLA-MUD transplant recipients, anti-HLA antibodies that were not reactive with donor loci were identified in 116 patients (19.6%), whereas DSAs were found only in 1.4% in this population, all directed against the HLA-DPB1 molecule. While overall, GF occurred in only 3.4% of the patients, 37.5% of patients with DSAs rejected the graft compared with only 2.7% patients without DSAs ($p = 0.0014$). Anti-HLA antibodies not directed against donor HLA antigens did not predict for graft failure. In multivariate analysis, DSA was the only factor that predicted GF in these patients [23]. More recently, we reported outcomes of 122 patients receiving haplo-HCT treated with PTCy including 22 patients with DSAs. Results from this study were consistent with the previous reports: a significantly higher proportion of DSA-positive patients experienced GF (32%) compared with DSA-negative patients (4%; $p < 0.001$) [37].

In another study in haplo-HCT by Yoshihara and colleagues, the authors tested anti-HLA antibodies in patients receiving T-cell replete haplo-HCT. Among 79 screened patients, 16 (20.2%) were allosensitized, 11 with DSAs. The cumulative incidence of neutrophil engraftment was significantly lower in DSA-positive *versus* DSA-negative patients (61.9% *versus* 94.4%, $p = 0.026$) (33). Furthermore, the Chinese group led by Prof. Huang confirmed these findings in a large cohort of 345 haplo-HCT patients treated with a T-cell replete G-CSF-mobilized bone marrow graft, and different G*v*HD prophylaxes confirmed a significantly higher rate of primary graft rejection (20% *versus* 0.3%) and poor graft function (27.3% *versus* 1.9%) in haplo-HCT patients who developed DSAs pretransplant compared with recipients without DSAs [34].

The clinical importance of DSAs has also been confirmed in other donor types of grafts for allo-HCT such as HLA-MUD [20] and cord blood [21, 32, 35, 38], as

summarized in Table 9.1. Besides impact on engraftment, several studies have shown that patients with DSAs had also a significantly lower survival compared with those without DSAs [21, 32, 38].

Although these studies have clearly confirmed that the presence of DSAs influences graft outcomes and survival in haplo-HCT, DSA levels which increase the risk of graft rejection remain unclear due to the fact that too few patients have been studied so far. It is generally accepted that positive DSA levels are 500–1000 MFI. In a case-control study conducted by us, a MFI 500 or more was considered positive [23]. Different levels of MFI may be significant for different donor sources, or, for the same type of donor, different factors like conditioning intensity, source of graft, or graft manipulation (like T-cell depletion) may be important. In haplo-HCT, MFI values of more than 1500 or 5000 were appreciated as significant by our group [19] and by Yoshihara and coworkers [33], respectively. An important difference between these two studies is that our study was done in patients treated with a T-cell-depleted graft, while the second one in patients treated with a T-cell replete graft and PTCy-based G*v*HD prophylaxis. It is possible that stem cells without T-cells are more exposed to the HLA antigens as the only targets available for the DSAs, in addition to the lack of contribution of donor T-cells to engraftment and eradication of recipient's alloreactive T-cells. Recently, Chang and colleagues also showed that positive DSAs at MFI of 10,000 or more were correlated to primary graft rejection, while MFI of 2000 or more was strongly associated with primary poor graft function [34].

As mentioned above about the possible role of complement fixation in development antibody-mediated GF, we recently have found a strong correlation between DSA levels and complement system activation determined by C1q assay. In our study, haplo-HCT recipients who had C1q positivity had DSA levels >5000 MFI pretransplant (median 15,279) and more than half of them suffered from GF, while patients with negative C1q had significant lower MFI (median 2471) [37]. This result suggests that the ability of DSA to fix complement is higher with a higher DSA level. However, the minimum level of DSA and other factors that are associated with the possibility of complement activation and can influence transplant outcomes still need to be investigated.

So far, the conclusion from these published studies is that a higher DSA level (>5000), which may be revealed by serum dilution or titration for those false-low or false-negative antibodies defined by the MFI in the solid-phase immunoassays, poses an absolute contraindication to transplantation (in the absence of treatment), whereas very weak antibodies (2000 MFI or less) may be considered as a relative contraindication for transplantation. Although the standard cutoff level of DSAs that is considered safe for transplant still needs to be determined, it is likely that other transplant factors need to be taken into consideration. It is possible that complete T-cell depletion and lower intensity conditioning (non-myeloablative) may be predisposing patients with low-level DSAs to experience engraftment failure, while the use of a peripheral blood graft (rather than bone marrow) may be less risky for patients with low DSA levels.

9.6 Desensitization Therapy

Preformed antibodies present at the time of hematopoietic graft infusion are generally unaffected by standard transplantation conditioning regimens or T-/B-cell immunosuppressive strategies given in the peri-transplantation period. To reduce the risk of GF, a number of studies have reported beneficial effects of a variety of interventions used to reduce total anti-HLA antibody load, predominantly by using a combined approach [50]. Reversal of DSA-mediated graft rejection and reduction in antibody load by using plasmapheresis, intravenous immunoglobulin (IVIg), cyclophosphamide, polyclonal anti-lymphocyte antibodies, monoclonal antibodies to $CD20^+$ B lymphocytes (rituximab), and proteasome inhibitor against alloantibody-producing plasma cells (bortezomib) have been described in solid organ transplantation [47]. However, their effectiveness is, in general, modest and unreliable [51–55]. These treatment modalities also have been used to desensitize DSAs in haplo-HCT and HLA-mismatched transplant recipients with a variety of graft outcome as summarized in Table 9.2. The first case was reported by Barge and colleagues in 1989; a patient with positive crossmatch test with donor lymphocytes was treated with plasmapheresis before haplo-HCT but did not result in a negative crossmatch before transplant and subsequently developed GF [44]. Maruta and coworkers confirmed that repeated high-volume plasmapheresis does not effectively eliminate preformed anti-HLA antibodies and applied adsorption of HLA antibodies to irradiated donor lymphocytes before marrow transplantation for a successful engraftment [56]. We have used for the first time a combined approach with plasmapheresis, IVIg, and rituximab with mixed results: out of the first four patients treated with this approach, two achieved a significant reduction in antibody levels and engrafted the donor cells, whereas the other two patients maintained high levels of DSAs and experienced primary GF [22]. Yoshihara and coworkers have tried three desensitization approaches for five patients who were to receive either bone marrow or peripheral blood stem cell grafts from haploidentical donors. Treatment regimen in this study was a combination of plasmapheresis, rituximab, antibody adsorption with platelets, and administration of the proteasome inhibitor, bortezomib. One of the two patients treated with plasmapheresis and rituximab received plasmapheresis on day −11 and the other received plasmapheresis on days −17, −15, and −13. Both were given a single dose of rituximab at 375 mg/m^2. DSA reduction was achieved in only one of two patients. However, both engrafted. Some of the most impressive reductions of DSAs were achieved by using 40 units of platelet transfusion from healthy donors selected to have the HLA antigens corresponding to the DSAs [33]. In a more recent study, in addition to three doses of alternating plasmapheresis every other day followed by one dose of IVIg and rituximab, we added an irradiated buffy coat infusion on day −1 prepared from 1 unit of blood on day −2 to block remaining circulating antibodies after the initial treatment. The rationale was to infuse donor HLA antigens, which will presumably bind the remaining of DSAs and spare the stem cells (Fig. 9.1) [37]. Moreover, in this study we have also found that more important appears to be the conversion of C1q positivity to negativity posttreatment and pre-stem cell infusion not merely the reduction

Table 9.2 Desensitization strategies in haploidentical and HLA-mismatched related allo-HCT

Reference	Donor type (N)	Anti-HLA abs test	Desensitization method	DSAs posttreatment	Graft outcome
Barge 1989 [44]	Haplo ($N = 1$)	CDC	2 cycles of plasmapheresis	NA	Graft failure
Maruta 1991 [56]	Mismatched related ($N = 1$)	AHG-CDC	CyA 2 weeks, methylprednisolone 1 week, plasmapheresis, DLI	Negative XM	Engrafted
Braun 2000 [60]	Haplo ($N = 1$)	FCXM	Staphylococcal protein A immunoadsorption	Negative XM	Engrafted on day +69 posttransplant
Ottinger 2002 [17]	Mismatched related ($N = 2$)	DTT-CDC	Plasmapheresis, mismatched platelet transfusion	1 patient with negative XM, 1 patient with positive XM	Patient with negative XM posttreatment engrafted, while patients with positive XM had GF
Pollack 2004 [61]	Mismatched HLA-A68 related ($N = 1$)	FCXM	Donor's platelet transfusion, plasmapheresis (2 attempts), IVIg	No antibodies detected at day 86 posttransplant	Engrafted at 1 week after second transplant
Narimatsu 2005 [62]	Mismatched related ($N = 1$)	AHG-LCT	4-weekly administrations of 600 mg rituximab, 40 units of donor's platelet transfusion	Negative AHG-LCT	Engrafted on day +14 posttransplant
Ciurea 2009 [19]	Haplo ($N = 4$)	Luminex MFI >500	2 weekly doses of 375 mg/m^2 of rituximab, 2 cycles plasmapheresis	1 negative, 1 low titer, 2 high titer	Patients with DSAs negative and low titer posttreatment engrafted, 2 patients with high titer had GF

(continued)

Table 9.2 (continued)

Reference	Donor type (N)	Anti-HLA abs test	Desensitization method	DSAs posttreatment	Graft outcome
Yoshihara 2012 [33]	Haplo ($N = 5$)	Luminex MFI >500	Plasmapheresis + rituximab ($N = 2$), platelet transfusion ($N = 2$), bortezomib + dexamethasone ($N = 1$)	1 patient had temporary DSA reduction and 1 patient had significant reduction post-plasmapheresis, 2 patients had a significant reduction post-platelet transfusion, 1 patient had moderate DSA reduction after bortezomib and dexa	All patients engrafted
Ciurea 2015 [37]	Haplo ($N = 12$)	Luminex MFI >500	3 cycles of plasmapheresis + 1 dose of 375 mg/m^2 of rituximab + IVIg 1 g/kg ($N = 5$), Plasmapheresis + rituximab + IVIg+ donor's buffy coat infusion ($N = 7$)	No significant change of MFI before transplant All patients cleared DSA after transplant	5 patients with C1q positive posttreatment had GF while patients who became C1q negative engrafted
Leffell 2015 [58]	Haplo ($N = 13$) MMUD ($N = 2$)	Luminex MFI >1000	Single-volume plasmapheresis + IVIg 100 mg/kg	Mean reduction of DSAs posttreatment was 64.4%. 1 patient failed to reduce DSAs to the level that was thought to be safe for transplant	All 14 of 14 transplanted patients engrafted

MFI mean fluorescence intensity, *CDC* complement-mediated cytotoxic, *XM* crossmatch, *FCXM* flow cytometric crossmatch, *GF* graft failure, *AHG-LCT* anti-human immunoglobulin lymphocytotoxicity test, *NA* not available, MMUD HLA-mismatched unrelated donor (Adapted from [59] with permission)

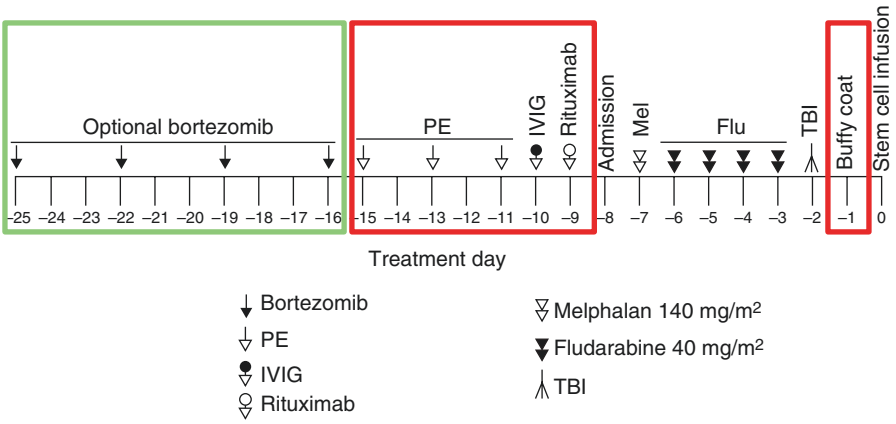

Fig. 9.1 Desensitization approach for patients with DSAs undergoing HLA-haploidentical transplantation at MD Anderson Cancer Center. Reproduced from [59] with permission

of antibody levels. Although a small number of patients have been treated with this approach so far, all five patients who remained C1q positive after treatment with plasmapheresis, IVIg, and rituximab with or without buffy coat experienced engraftment failure, whereas all four patients who became C1q negative after treatment/before transplant engrafted the donor cells [34]. Although antibody level did not significantly change early on, all patients eventually clear the antibodies completely in the first few weeks posttransplant [37]. These results suggested to us that a reduction to non-complement-binding level of DSAs should be the goal of treatment rather than clearing of the non-complement-binding DSA, which appear to clear more slowly in the immediate posttransplant period and became undetectable in all patients within the first few weeks after transplant, similar with prior experience [57]. Although our experience is limited, this approach has been very successful as none of the patients treated as such experienced primary GF. A different approach was developed by the Johns Hopkins group from treatment of allosensitized recipients of solid organ transplantation using a combination of repeated plasmapheresis, IVIg, and immunosuppressive medications. This group treated 15 patients with HLA-mismatched grafts including 13 haplo-HCT with alternate day of single-volume plasmapheresis followed by 100 mg/kg of IVIg, tacrolimus (1 mg/day, intravenous), and mycophenolate mofetil (1 g twice daily) starting 1–2 weeks before the beginning of transplant conditioning, depending on patient's starting DSA levels. Reduction of DSA to the level that thought was safe for transplant was seen in 14 of 15 patients, all of these 14 patients engrafted with donor cells [58]. Using this approach, the treatment continues until DSA levels decrease significantly exposing the patients to a higher risk of disease relapse due to the continuation of therapy for an undetermined amount of time. Even though the majority of these studies have been anecdotal and included only a small number of patients, these results indicate that reduction of DSA levels and clearance of complement-binding DSA are possible and can permit successful engraftment even in highly allosensitized recipients.

9.7 Expert Point of View

Evidence has grown in the past 5 years that the presence of anti-HLA antibodies directed against the donor cells can affect transplant outcomes. It is now well accepted that the presence of DSA pretransplant is associated with allograft rejection in patients with HLA-mismatched donors, a problem which may be more important in haplo-HCT recipients. Testing for DSAs should be mandatory in all patients receiving $CD34^+$ graft from HLA-mismatched or haplo-HCT to prevent immune-mediated graft rejection by choosing a different donor or treat patients with DSA levels with no better donor options. The introduction of the more sensitive methods to detect both donor and non-donor-specific anti-HLA antibodies has led to an increase in the number of highly sensitized patients but also to the knowledge that the presence of DSA is not always a contraindication but rather a risk factor based on DSA levels and presence of complement-binding antibodies. To date, there is no standard recommendation on anti-HLA desensitizing methods. Nevertheless, combined approaches using plasmapheresis, IVIg, cyclophosphamide, polyclonal anti-lymphocyte antibodies, monoclonal antibodies to $CD20^+$ B lymphocytes (rituximab), and proteasome inhibitor against alloantibody-producing plasma cells (bortezomib) seem to be the most effective methods resulting to a significant reduction of DSAs. In addition, the addition of an irradiated buffy coat infusion prepared from the donor cells may prove to be the most effective way to mitigate the impact of DSA on graft outcome and survival.

9.8 Future Directions

Future studies will explore the pathogenesis of antibody-mediated rejection and develop effective therapies for allosensitized recipients. Complement activation has been recently identified as an important mechanism of antibody-mediated graft rejection. In addition to removing preformed antibodies by using combined modality, complement-modulating strategies are the possible therapy for antibody-mediated graft rejection that need to be investigated in the future.

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Haloidentical Transplants and NK Cell Alloreactivity

10

Andrea Velardi

10.1 The Inherent Contradiction of Allogeneic Hematopoietic Transplantation

Allogeneic hematopoietic cell transplantation (Allo-HCT) is the most powerful immunotherapy for high-risk acute leukemia. Donor T-cells recognize host alloantigens on leukemic cells and eradicate leukemia (graft-*versus*-leukemia, G*v*L, effect). They also attack non-hematopoietic tissues such as skin, gut, and liver and mediate graft-*versus*-host disease (G*v*HD), a major cause of morbidity and mortality. Posttransplant pharmacological immune suppression is necessary to antagonize excessive alloreactivity and help prevent G*v*HD. However, by the same token it may compromise the G*v*L effect. Indeed, whoever the donor and whatever the allo-HCT strategy, posttransplant relapse rates are approximately 30% for patients transplanted in remission and much higher for patients transplanted in relapse [1] (see Chap. 19).

Matching donor and recipient at HLA level is crucial for optimal transplant outcomes with acceptable non-relapse mortality (NRM). However, only 25% of individuals have an HLA-identical sibling who could serve as donor. Alternative hematopoietic graft sources are HLA-matched unrelated volunteers, unrelated umbilical cord blood units and full HLA haplotype-mismatched ("haploidentical") family members which are, however, associated with up to 40% NRM due to diverse combinations of graft failure, G*v*HD, hepatic sinusoidal obstruction syndrome, and infections.

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10.2 Haploidentical Transplantation: The Contradiction at Its Extreme

Bone marrow transplant centers are becoming more and more interested in haploidentical transplantation as it offers the advantages of immediate donor availability for almost all patients and access to donor-derived immune cell therapies after transplantation. Recent popular approaches have used non-T-cell-depleted ("unmanipulated") grafts combined with enhanced strategies to attenuate/modulate donor T-cell alloreactivity and help prevent G*v*HD. For example, Huang and coworkers (see Chap. 5) first applied G-CSF priming of unmanipulated haploidentical blood and marrow grafts and intensive posttransplant immune suppression to modulate/downregulate donor T-cell alloreactivity [2, 3]. Using G-CSF-primed bone marrow, Di Bartolomeo and coworkers achieved very encouraging 3-year probabilities of overall and disease-free survival for standard-risk, and even high-risk, patients [4]. In a different approach, low risk of acute and chronic G*v*HD and encouraging rates of transplant-related mortality (TRM) were observed after unmanipulated haploidentical bone marrow transplantation and posttransplantation high-dose cyclophosphamide [5, 6] (see Chaps. 7 and 8).

10.3 How T-Cell-Depleted Haploidentical Transplants Provided the Opportunity to Discover the Benefits of NK Cell Alloreactivity

Historically, the 1990s saw what had been major drawbacks of haploidentical transplantation, i.e., very strong host-*versus*-graft (H*v*G) and graft-*versus*-host (G*v*H) allo-responses which led respectively to rejection and G*v*HD (i.e., "the contradiction"), being overcome through use of high-intensity conditioning regimens and transplantation of a megadose of extensively T-cell-depleted peripheral blood (PB) hematopoietic progenitor cells [7, 8] (see Chap. 2). T-cell depletion (TCD), however, delayed immune reconstitution and was associated with high transplant-related/infectious mortality rate. On the other hand, posttransplant immune recovery in the absence of any immune suppression created an opportunity for discovering innovative forms of immunotherapy. It favored natural killer (NK) cell development and revealed donor-*versus*-recipient NK cell alloreactions which eradicated acute myeloid leukemia (AML) and improved survival [9]. We discovered that, because of specific HLA class I ("KIR ligand") mismatches recognized by NK cell inhibitory receptors ("KIRs"), haploidentical donors were able to mount donor-*versus*-recipient NK cell alloreactions. In 2002, a description of the benefits of donor-*versus*-recipient NK cell alloreactivity in haploidentical TCD hematopoietic cell transplantation for acute leukemia generated international enthusiasm for, and interest in, NK cell alloreactivity as a form of leukemia immunotherapy. It eradicated AML, favored engraftment, protected from G*v*HD, and greatly improved survival, as demonstrated by integrating clinical and preclinical data [10–12]. Unlike AML, acute lymphoblastic leukemia (ALL) in adults was not susceptible to NK cell alloreactivity [9, 12].

10.4 How HLA Educates NK Cells to Tolerate Self and React to Missing Self

Human NK cell function is regulated by a balance between activating and inhibitory receptors [13]. Clonally distributed inhibitory receptors termed killer cell immunoglobulin-like receptors (KIRs) recognize HLA class I allele groups (KIR ligands): HLA-C alleles with a Lys^{80} residue ("group 2" alleles), HLA-C with an Asn^{80} residue ("group 1" alleles), and HLA-B alleles sharing the Bw4 specificity [14]. All KIR genes are randomly expressed, and KIR distribution varies on NK cells. Only NK cells which express inhibitory KIRs for self-HLA ligands become "licensed/educated" [15–17]. When confronted with an allogeneic target, *educated NK cells* with a KIR that does not recognize the allogeneic HLA as their only inhibitory receptor for self-HLA sense the missing expression of their inhibitory ligand and mediate alloreactions ("missing self-recognition") [9–12]. NK cells that do not express inhibitory receptors for self (and are, thus, potentially autoreactive) are retained in the repertoire in an anergic (or "hypofunctional") state, perhaps reflecting lack of education and/or appropriate signaling during NK cell development [18, 19]. Combined evidence from *in vitro* studies, murine models, and clinical trials indicated the ability of NK cells to mediate donor-*versus*-recipient alloreactivity rested on "missing self-recognition" (Fig. 10.1). In haploidentical

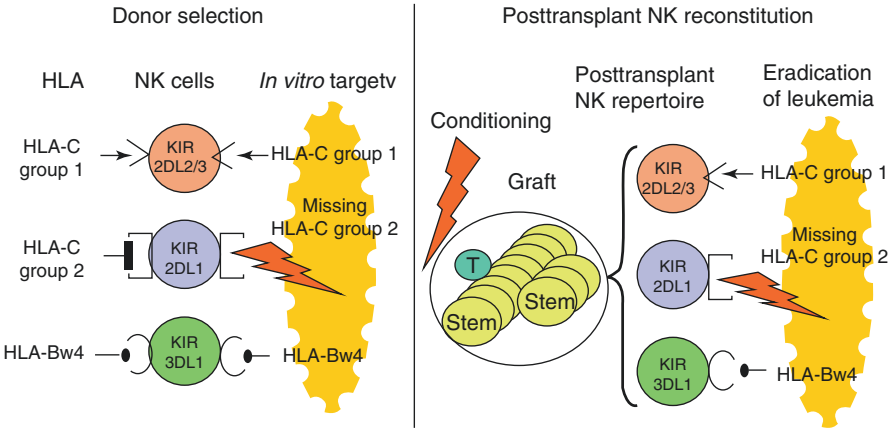


Fig. 10.1 NK cell alloreactivity and its regeneration after allogeneic hematopoietic cell transplantation. Left panel: Example of donor-*versus*-recipient NK cell alloreactivity. The donor possesses HLA-C1, HLA-C2, and HLA-Bw4 KIR ligands and consequently possesses a repertoire of HLA-"educated" NK cells composed of cells expressing inhibitory receptors specific for HLA-C1, HLA-C2, and HLA-Bw4 alleles (i.e., KIR2DL2/3, KIR2DL1, and KIR3DL1). When confronted with targets from a haploidentical recipient that is homozygous for HLA-C1 group alleles and therefore lacks HLA-C2 alleles, donor NK cells expressing the HLA-C2-specific inhibitory receptor, KIR2DL1, will not find their inhibitory HLA ligand on target cells and will be activated to kill (by "missing self-recognition"). Right panel: Following transplant, the exact same donor NK cell repertoire is regenerated in the recipient. It is "reeducated" by donor HLA (expressed on transplanted hematopoietic cells) to become tolerant of self (the donor) and recipient alloreactive and, consequently, capable to eradicate leukemia

hematopoietic cell transplantation, engrafted progenitor cells gave rise to an NK cell repertoire of donor origin, which included alloreactive clones that killed recipient cryopreserved leukemic cells [9–12]. Thus, donor-derived mature NK cells which developed from engrafted stem cells matured in a bone marrow microenvironment in which they were predominantly exposed to, and "licensed/educated" by, donor HLA. This process shaped their repertoire to be both donor-tolerant and recipient-alloreactive. They are, therefore, enabled to recognize, and react to, missing self on recipient targets.

The same educational process was also observed in HLA-mismatched unrelated donor transplants [20]. The responsiveness of different NK cell subsets was assessed as a function of their expression or lack of expression of self-HLA-specific inhibitory receptors. The data showed that the donor's HLA KIR ligands determined a fully effective NK cell education process, thus demonstrating that in this setting also, the NK cell educator was of donor hematopoietic cell origin. This donor-ligand-driven NK cell education generated an NK cell repertoire that was able to mediate donor-*versus*-recipient NK cell alloreactions [9–12]. Similarly, data from an NK cell differentiation assay supported a ligand-instructed model of NK cell education; recognition of HLA class I by an inhibitory receptor suppressed subsequent expression of a second receptor of related specificity [18]. Moreover, in investigating cytokine production and killing potential of reconstituting NK cells after unrelated donor or umbilical cord transplant, one study provided further evidence that donor HLA drove NK cell education and led not only to cytotoxicity but also to cytokine production [19].

10.5 Effectiveness of Donor-*Versus*-Recipient NK Cell Alloreactivity in Haploidentical Transplantation

In TCD haploidentical transplantation, NK cells exerted donor-*versus*-recipient alloreactions by virtue of KIR ligand mismatches in the G*v*H direction: HLA-C1 present in donor/missing in recipient, HLA-C2 present in donor/missing in recipient, and HLA-Bw4 present in donor/missing in recipient. Such mismatches can be found in about 50% of haploidentical transplant pairs. In an updated analysis by Mancusi and coworkers [21] of 161 patients with AML, 69 patients received transplants from NK alloreactive donors and 92 patients from non-NK alloreactive donors. Donors were considered able to exert donor-*versus*-recipient NK cell alloreactivity when (1) *HLA-C* and *HLA-B* typing showed KIR ligand mismatches in the G*v*H direction, that is, the donor possessed an HLA-class I KIR ligand (either HLA-C groups 1 or 2 or HLA-B group Bw4) that was absent in the recipient); (2) donors possessed the relevant inhibitory *KIR* gene(s) for missing self-recognition of recipient targets (*KIR2DL2* and *KIR2DL3* were present in everyone, *KIR2DL1* was absent in about 3% of individuals, and *KIR3DL1* was absent in about 10%) [21]; and (3) donors possessed alloreactive NK cell clones against recipient targets. As our previous analyses in large numbers of individuals had shown they possessed alloreactive NK clones against HLA-C group mismatched targets [11–13], NK cell

cloning and allo-cytotoxicity assays were not considered necessary and were therefore not routinely performed in HLA-C group mismatched transplant donors. Thus, HLA typing and donor *KIR* genotyping were considered sufficient to define HLA-C group mismatched transplant donors as NK alloreactive. As previous analyses had shown alloreactive NK clones against HLA-Bw4 mismatched targets are undetectable in about 1/3 of *KIR3DL1*-positive individuals [13], NK cell cloning and allo-cytotoxicity assays were considered necessary and were therefore routinely performed. In agreement with previous studies [11–13], multivariate analyses showed donor-*versus*-recipient NK cell alloreactivity was associated with reduced relapse (transplantation from NK alloreactive donors *versus* non-NK alloreactive donors: HR 0.42; 95% CI 0.18–0.96; $P = 0.04$) and improved EFS in patients with AML (but not ALL) (transplantation from NK alloreactive donors *versus* non-NK alloreactive donors: HR 0.60; 95% CI 0.37–0.96; $P = 0.034$ in TCD haploidentical transplants) [21].

As mentioned above, interaction of inhibitory KIRs with self-HLA ligands renders NK cells "licensed/educated" to become fully functional and, therefore, alloreactive against targets that do not express self-HLA KIR ligands. NK cells that do not express inhibitory receptors for self (and are, thus, potentially autoreactive) are anergic (or "hypofunctional") [18, 19]. These cells are found in individuals who possess inhibitory KIRs for the three major HLA ligands but carry only one or two KIR ligands. Upon transfer into the recipient, such anergic (or "hypofunctional") NK cells were hypothesized to become activated and exert a G*v*L effect with no need for KIR ligand mismatching (the so-called "missing ligand" model) [22–29]. When we analyzed outcomes of HLA-haploidentical and HLA-matched sibling transplants from such donor-recipient combinations, prognosis was disappointingly worse than that in KIR ligand-mismatched transplants [13]. These data indicated that donor NK cell recognition of "missing self" on recipient targets was crucial for triggering effective NK cell-mediated alloreactions. Concurring with these observations, a recent study demonstrated that NK cells that did not express receptors for self-HLA remained hypofunctional throughout NK cell reconstitution after HLA-matched transplantation, thus precluding any clinical benefits through breaking of NK cell tolerance [30]. Results from pediatric clinical trials showed that NK cell alloreactivity was an effective form of immunotherapy in also for pediatric ALL. A lower incidence of ALL relapse was observed after transplantation of TCD hematopoietic grafts from NK alloreactive donors [22, 23, 31].

10.6 Optimal Transplantation Protocols for Development of NK Cell Alloreactivity

NK cell alloreactivity has been investigated in unrelated donor transplants [31–42]. Most studies showed no advantage in transplantation from KIR ligand-mismatched donors [31–37], whereas a few observed an increased G*v*L effect [38–42]. Interestingly, the study that reported the most marked survival advantage was performed in KIR ligand-mismatched transplant recipients who received pre-transplant

antithymocyte globulins (ATGs) that exerted *in vivo* TCD [38]. In cord blood transplants, Willemze and coworkers [43] demonstrated KIR ligand mismatching in the G*v*H direction significantly reduced the incidence of relapse and improved leukemia-free survival, particularly in patients with AML. However, in an analysis of the combined results of single unit and the dominant engrafting unit of double cord blood transplants, Brunstein and coworkers [44] did not demonstrate an advantage of selecting cord blood units based on KIR ligand mismatching. These studies differ because of the complexities of three-party interactions in double-unit transplants, diagnoses (AML *versus* a variety of hematological malignancies), conditioning regimens (myeloablative *versus* reduced intensity regimens), proportion of patients receiving ATG and sources of ATG (rabbit *versus* horse source), as well as posttransplant G*v*HD prophylaxis (without *versus* with mycophenolate mofetil). Likewise, haploidentical transplants, when performed with T-cells, appear to antagonize/obscure NK cell alloreactivity-mediated G*v*L effects [45, 46]. Intriguingly, however, an innovative approach to TCD haploidentical hematopoietic transplantation combining T-regulatory and T-effector cell add-backs showed NK cell recovery/maturation was, if anything, enhanced [47] and the clinical benefits of NK cell alloreactivity were maintained [21] (see Chap. 4).

What emerges from all this evidence is that TCD and no posttransplant immune suppression are crucial for the development of NK cell alloreactivity.

10.7 NK Cell Alloreactive Donors with Concomitant Activating KIRs Provide Additional Clinical Benefits

In the setting of TCD haploidentical transplantation without posttransplant immunosuppression, it was recently demonstrated that alloreactive NK cells also beneficially impact NRM when they concomitantly express activating KIRs [21]. Molecular homologues of the inhibitory KIRs, with shorter cytoplasmic tails, activating KIRs transduce activating signals that regulate NK cell functions. Approximately 25% of whites are homozygous for group A KIR haplotypes that contain the main inhibitory KIR genes. The others are either heterozygous or homozygous for group B KIR haplotypes that also carry various combinations of activating KIR genes (KIR2DS1, 2, 3, 5, and 3DS1). It is well established that KIR2DS1 binds HLA-C group 2 molecules (HLA-C2) with less affinity compared to KIR2DL1. Little is known about the ligands of other activating KIRs. As activating KIRs are heterogeneously expressed in the population, we recently investigated the role of donor activating KIRs in haploidentical TCD hematopoietic transplants for acute leukemia transplants was preliminarily grouped according to presence *versus* absence of KIR ligand mismatches in the GvH direction (i.e., of donor-*versus*-recipient NK cell alloreactivity). In the absence of donor-*versus*-recipient NK cell alloreactivity, presence of activating KIRs in the donor had no effects on outcomes. In the 69 transplant pairs with donor-*versus*-recipient NK cell alloreactivity and concomitant presence of activating KIR genes in the donors, multivariate analyses showed transplantation from donors with *KIR2DS1* and/or *KIR3DS1* was associated

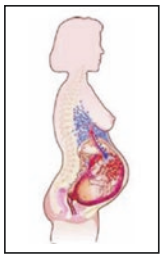
with reduced risk of NRM , largely infection-related (*KIR2DS1* present *versus* absent HR 0.25, $P = 0.01$; *KIR3DS1* present *versus* absent HR 0.18, $P = 0.006$), and better relapse-free survival (RFS) (*KIR2DS1* present *versus* absent HR 0.31, $P = 0.011$; *KIR3DS1* present *versus* absent HR 0.30, $P = 0.008$). Transplantation from donors with *KIR2DS1* and/or *KIR3DS1* was also associated with a 50% reduction in infection rate ($P = 0.003$). The study included patients transplanted under two protocols, that is, our standard TCD $CD34^+$ hematopoietic progenitor cell graft protocol [7, 8] and the more recent protocol with T_{reg}/T_{con} add-backs [47, 48] (see Chaps 3 and 4). It might be questionable to include in the analysis patients who received T_{reg}/T_{con} cell add-backs as T-cells in the graft were shown to antagonize NK cell recovery and prevent clinically relevant NK cell effects in unrelated and unmanipulated haploidentical transplants [49]. However, in patients receiving T_{reg}/T_{con} add-backs, we previously demonstrated that reconstitution of donor-*versus*-recipient alloreactive NK cells was as efficient as in patients under the TCD transplant protocol [47]. In fact, the survival advantage of transplantation from NK alloreactive donors with activating *KIR* genes was more significant in the two cohorts combined (HR 0.33; 95% CI 0.14–0.76; $P = 0.009$) than in the TCD cohort (HR 0.37; 95% CI 0.15–0.88; $P = 0.025$). Finally, *in vitro* analyses showed KIR2DS1 binding to its HLA-C2 ligand upregulated inflammatory cytokine production by alloreactive NK cells in response to infectious challenges (*Aspergillus fumigatus*).

In conclusion, these studies identify a further advantage of donor-*versus*-recipient NK cell alloreactivity. It demonstrates that transplantation from NK alloreactive donors who possessed *KIR2DS1* and/or *KIR3DS1* activating genes was associated with markedly reduced infection rate and infectious mortality and significantly better event-free survival. Apparently, KIR ligand mismatches and the consequent NK cell release from recipient HLA blockade allowed activating KIRs to enhance NK cell functions upon binding to their ligands on recipient cells. As 40% of donors able to exert donor-*versus*-recipient NK cell alloreactivity carry *KIR2DS1* and/or *KIR3DS1*, searching for them may become a feasible, additional criterion in donor selection in TCD haploidentical transplants.

10.8 Pregnancy: The Ultimate *Ex Vivo* Graft Manipulation to Enhance Outcomes of T-Cell-Depleted Haploidentical Transplantation

Transplacental trafficking of maternal and fetal cells during pregnancy establishes long-term, reciprocal microchimerism in both mother and child. As a consequence, the immune system of the mother may become sensitized to paternal histocompatibility antigens. In fact, antibodies directed against paternal HLA antigens [50] and T-lymphocytes directed against paternal major and minor histocompatibility antigens were detected in multiparous women [51, 52] (Fig. 10.2).

More recently, it was hypothesized that mother's "exposure" to paternal HLA haplotype antigens during pregnancy may affect transplantation outcomes when the mother is the donor for the child. Indeed, survival after TCD haploidentical hematopoietic



Microchimerism of maternal origin persists in adult life. Maloney et al. J Clin Invest 1999

Leucocyte antibodies in sera of pregnant women. Van Rood JJ et al. Nature 1958

Pregnancy can induce long-persisting primed CTLs specific for inherited paternal HLA antigens. Van Kampen et al. Human Immunol, 2001

Pregnancy induces minor istocompatibility antigen-specific cytotoxic T cells: implications for stem cell transplantation and immunotherapy. Verdijik et al. Blood, 2004

Fig. 10.2 Mother's "exposure" to paternal HLA haplotype antigens during pregnancy may affect transplantation outcomes when the mother is the donor for the child

transplantation was improved using the mother as donor (*vs.* all other family members) [53, 54].

However, maternal donors were associated with increased incidence of G*v*HD and decreased survival after HLA haploidentical unmanipulated (i.e., T-cell-replete) blood and marrow transplants [55].

A retrospective EBMT registry-based study was performed in a combined series of adult ($n = 333$) and pediatric ($n = 105$) patients with acute leukemia (AML = 268, ALL = 160, mixed phenotype = 10). Seventy-eight percent of patients received *ex vivo* TCD transplants, and 22% received *in vivo* TCD transplants. As preliminary analyses showed transplantation outcomes from family members other than mothers did not differ from one another, such transplants were combined for analyses. When compared with transplantation from all other family members ($n = 338$), transplantation from mother donors ($n = 100$) was associated with better RFS (43% *vs.* 21%, $P < 0.001$) and trends toward lower relapse incidence (RI) (28% *vs.* 39%, $P = 0.07$) and NRM (29% *vs.* 39%, $P = 0.08$). Multivariate analyses showed transplantation from mother donors was an independent factor predicting better RFS (other donors *vs.* mothers: HR 1.42; CI 1.01–2.00; $P = 0.043$) and lower RI (other donors *vs.* mothers: HR 1.85; CI 1.12–3.06; $P = 0.016$). In addition, transplantation in relapse (*vs.* remission) predicted worse RFS (HR 2.36, CI 1.83–3.04, $P < 0.001$), higher RI (HR 3.29, CI 2.29–4.73, $P < 0.001$), and higher NRM (HR 1.70, CI 1.19–2.43, $P = 0.003$). Age ≥18 (*vs.* <18) adversely impacted RFS (HR 1.40, CI 1.00–1.95, $P = 0.049$) and NRM (HR 3.01, CI 1.73–5.23, $P < 0.001$). Thus, our retrospective analyses in 438 HLA haploidentical TCD hematopoietic transplants for acute leukemia patients (pediatric and adult) show that transplantation from mother donors, when compared with transplantation from any other family member, is an independent factor predicting better outcomes, i.e., better RFS and lower relapse incidence [56]. Mothers should therefore be preferred when selecting an HLA haploidentical family donor in TCD transplant setting. Further clinical and preclinical studies are needed to unveil the mysteries underlying mother-to-child immune interaction during pregnancy and its bearing on the reproductive success of the human species.

10.9 Expert Point of View

Donor-*versus*-recipient NK cell alloreactivity is now established as a key therapeutic element in haploidentical hematopoietic transplantation for acute myeloid leukemia. From a practical standpoint, donors may be classified as NK alloreactive

against their recipients when (1) they possess HLA class I KIR ligand(s) which are missing in the recipients (i.e., they are KIR ligand mismatched in the G*v*H direction with their recipients) and (2) they possess inhibitory KIR gene(s) for missing self-recognition on recipient targets. The latter assessment is necessary because in a survey of 198 individuals, we observed that, although the HLA-C group 1 receptor genes (KIR2DL2 and/or KIR2DL3) are present in 100% of individuals, the HLA-C group 2 receptor gene (KIR2DL1) is present in 97% and KIR3DL1, the inhibitory receptor for HLA-Bw4 alleles, was found in about 90% of individuals. Functional in vitro analyses performed in large cohorts of donor recipient pairs demonstrated donors that are KIR ligand mismatched in the GvH direction with their recipients (and carry the relevant inhibitory KIR genes) all possessing alloreactive NK clones against recipient targets [21]. Importantly, such donors are found in approximately 50% of haploidentical transplant pairs. Clinical trials demonstrated that donor-*versus*-recipient NK cell alloreactivity is a key therapeutic element in haploidentical transplants for AML in adults and ALL in children.

After haploidentical hematopoietic transplantation, donor-derived NK cells mature in a bone marrow microenvironment in which they are predominantly exposed to, and "licensed/educated" by, donor HLA, shaping their repertoire to be both tolerant to self (i.e., to donor) and recipient alloreactive. They are, therefore, enabled to recognize, and react to, missing self on recipient targets. Data from clinical trials using diverse protocols show that crucial for the development of NK cell alloreactivity are the transplant of large numbers of hematopoietic progenitor cells, extensive TCD of the graft, and no posttransplant immune suppressive drugs for G*v*HD prophylaxis. Consequently, NK cell alloreactivity is expected to be boosted by, for example, transplanting CD3 or alfa/beta TCD grafts (which in fact contain large numbers of NK cells) [57, 58] (see Chap. 3). In contrast, protocols using T-cell-replete bone marrow or PB $CD34^+$ cells are becoming more and more popular [2–6]. Under these protocols, the benefits of NK cell alloreactivity might be expected to be antagonized/obscured as was reported in unrelated donor and cord blood transplantation. The only exception so far documented is the haploidentical hematopoietic cell transplant trial with T_{reg}/T_{con} add-backs that, however, do not use any posttransplant pharmacologic immunosuppressive G*v*HD prophylaxis [47, 48].

Additional studies identified a further advantage of donor-*versus*-recipient NK cell alloreactivity. Transplantation from NK alloreactive donors who concomitantly possessed *KIR2DS1* and/or *KIR3DS1* activating KIR genes was associated with markedly reduced infection rate and infectious mortality and significantly better event-free survival. As 40% of donors able to exert donor-*versus*-recipient NK cell alloreactivity carry *KIR2DS1* and/or *KIR3DS1*, searching for them is an additional, complementary criterion in NK cell alloreactive donor selection.

Finally, studies in haploidentical TCD hematopoietic transplants for acute leukemia patients (pediatric and adult) show that transplantation from mother donors, when compared with transplantation from any other family member, is an independent factor predicting better outcomes, i.e., better RFS and lower relapse incidence. In contrast, maternal donors were associated with increased incidence of G*v*HD and decreased survival after HLA haploidentical unmanipulated (i.e., T-cell-replete) blood and marrow transplants [55]. No data are available from other protocols using

unmanipulated bone marrow grafts, such as transplantation of G-CSF-primed bone marrow followed by intensified posttransplant immune suppression or transplantation of unmanipulated bone marrow followed by post-transplant cyclophosphamide (see Chaps. 2–7). Consequently, on the basis of currently available data, mothers should be preferred when selecting a haploidentical family donor for TCD hematopoietic transplants. As expected, NK cell alloreactive maternal donors provided better outcomes than non-NK alloreactive mothers and NK alloreactive fathers (non-NK alloreactive fathers were in fact the worst donors) [53].

10.10 Future Directions

Studies are needed (1) to establish a hierarchy between the two types of "good prognosis" donors described in this review in TCD transplantation and (2) to document whether they play a role in non-T-cell-depleted (unmanipulated) bone marrow transplants.

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11 Myeloablative *Versus* Nonmyeloablative Conditioning Regimen in Haploidentical Transplantation: Does It Matter and How Best to Select Between the Two?

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11.1 Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) from an HLA-haploidentical family donor is the fastest growing donor type in Europe [1].

Several haploidentical hematopoietic cell transplantation (haplo-HCT) platforms have been successfully established, with promising results, due to improved ways to overcome the HLA barrier. *Ex vivo* T-cell depletion (TCD) dominated the scene in the 1980s, including the $CD34^+$ "megadose" approach of Perugia [2] and the more recent selective TCRαβ depletion (see Chaps. 1, 2, and 3) [3]. Myeloablative conditioning (MAC) regimens are used to ensure engraftment with *ex vivo* TCD platforms. In the last decade, several programs of unmanipulated or T-cell-replete haplo-HCT have been described, including protocols based on anti-thymocyte globulin (ATG) [4] or on posttransplantation cyclophosphamide (PTCy) [5] or both. These platforms have used both nonmyeloablative (NMA) and MAC regimens and will be discussed in this chapter.

11.2 Myeloablative (MA) Regimens

11.2.1 *Ex Vivo* T-Cell depletion

Programs using *ex vivo* TCD have historically used MAC regimens, as a means to ensure engraftment of HLA-mismatched grafts but also to treat acute leukemias, often in advanced stages [2]. High-dose total body irradiation (TBI), combined with

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thiotepa and busulfan (Bu), a regimen designed by the Perugia group in the 1990s (Table 11.1), has been the standard for HLA-haploidentical grafts using $CD34^+$-selected peripheral blood (PB) grafts [2]. This program has been adopted by other centers, especially in the pediatric setting, less frequently in adults, due to a high rate of infectious complications: results in Europe have been reviewed [6]. In a 2005 paper, Aversa and coworkers reported the outcome of 67 patients with acute myeloid leukemia (AML) and 37 with acute lymphoblastic leukemia (ALL), grafted with $CD34^+$-selected haploidentical stem cells, after a myeloablative regimen combining total body radiation (TBI), thiotepa, and fludarabine (Flu) [7]: engraftment was achieved in 94 patients, G*v*HD was seen in 8 patients, non-relapse mortality (NRM) was reported to be 36%, and actuarial survival was 47% for remission patients and less than 10% for relapsed patients [7]. More recently selective alfa-beta T-cell and $CD19^+$ depletion has been successfully implemented, instead of $CD34^+$ selection [3, 8, 9]: the conditioning regimen has remained very similar, but engraftment, and especially immune recovery, has been greatly improved [8].

A variation of selective *ex vivo* TCD has been reported by the Perugia group [10]: this program involves a MAC regimen, followed by the infusion of expanded regulatory T cells, followed by combination of $CD34^+$-selected cells and a fixed number of mature T cells, and has been associated with an extremely low incidence of leukemia relapse (see Chap. 4).

11.2.2 Unmanipulated Haploidentical Transplants

Possibly the first published series of patients transplanted with unmanipulated haploidentical grafts, following a myeloablative regimen, is the paper by Dao Pei and coworkers [4], reporting comparable survival of leukemias receiving transplants from HLA-identical siblings or family haploidentical donors. In 2006, when this paper was published, it seemed hard to believe that the outcome of sibling and haploidentical-mismatched donors could be comparable. Nevertheless, time showed that the Peking group was right and unmanipulated haploidentical grafts, following MA regimens, especially but not only for acute leukemia, have been growing very rapidly, especially in recent years [1], and have been shown to produce outcome equivalent to HLA-matched siblings or HLA-matched-unrelated donors [11, 12].

11.2.3 Myeloablative Conditioning Regimens in Combination with ATG

The Peking group reported an intensive conditioning regimen with a combination of semustine, high-dose cytarabine, cyclophosphamide (Cy), and Bu [4] (Table 11.1); prophylaxis of G*v*HD included rabbit ATG, cyclosporine (CsA), mycophenolate mofetil (MMF), and methotrexate (MTX). An update of that study 9 years later, with over 700 patients receiving unmanipulated haploidentical grafts, and the intensive chemotherapy-based regimen, confirmed excellent 3-year disease-free survival (DFS) of 68% and 49%, respectively, for standard- and high-risk patients [13].

Table 11.1 Conditioning regimens for haploidentical transplantation

Reference number	Conditioning regimens	$CD34^+$ cell source	G*v*HD prophylaxis
Myeloablative			
[2]	Thiotepa 10 mg/kg, Flu 200 mg/m^2, sTBI 8 Gy	G-PB	$CD34^+$ selection ATG
[4]	Bu 9.6 mg/kg; cytarabine 8 g/m^2; Cy 120 mg/kg; semustine 250 mg/m^2	G-BM+ G-PB	ATG + MTX + MMF + CsA
[12]	cytarabine 6 g/m^2; Cy 90 mg/kg ; fTBI 10 Gy	G-BM	ATG + MTX + MMF + CsA + Basiliximab
[15]	Thiotepa 10 mg/kg; Bu 9.6 mg/kg; Flu 150 mg/m^2	G-BM	ATG + MTX + MMF + CsA + Basiliximab
[17]	Thiotepa 10 mg/kg; Bu 9.6 mg/kg; Flu 150 mg/m^2	BM	PTCy + MMF + CsA
[21]	Treosulfan 42 g/m^2, Flu 150 mg/m^2; Mel 140 mg/m^2	G-BM	PTCy + MMF + Sirolimus
[22]	fTBI 12 Gy; Flu 75 mg/m^2	G-PB	PTCy + MMF + TK
[23]	fTBI 10.5 Gy; Flu 120 mg/m^2	G-PB	PTCy + MMF + TK
Nonmyeloablative			
[5]	Cy 14.5 mg/kg; Flu150 mg/m^2; TBI 2 Gy	BM	PTCy + MMF + TK
[30]	Cy 14.5 mg/kg; Flu 150 mg/m^2; TBI 2 Gy	G-PB	PTCy + MMF + TK
Reduced intensity			
[26]	Flu 150 mg/m^2; Cy 14.5 mg/kg; TBI 2 Gy; Bu 6.4 mg/kg	G-PB	PTCy + MMF + TK
[29]	Flu 150 mg/m^2; Cy 14.5 mg/kg; Bu 6.4 mg/kg	G-PB	PTCy + MMF + TK
[28]	Flu 160 mg/m^2; Mel 70 mg/m^2; Bu 9.6 mg/kg	G-PB	PTCy + MMF + TK
[19]	Flu 125 mg/m^2 ; Bu 9.6 mg/kg	G-PB	PTCy + MMF + TK
[27]	Flu 160 mg/m^2; Mel 100 mg/m^2; TBI 2 Gy	BM	PTCy + MMF + TK

Flu fludarabine, *Cy* cyclophosphamide, *TBI* total body irradiation, *sTBI* single TBI, *fTBI* fractionated TBI, *Bu* busulfan, *TK* tacrolimus, *PTCy* posttransplant cyclophosphamide, *MMF* mycophenolate mofetil, *CsA* cyclosporine, *MTX* methotrexate, *BM* bone marrow, *G-PB* G-CSF and peripheral blood graft, *G-BM* G-CSF and bone marrow graft

A modification of this protocol was published by another group from China [14]: G*v*HD prophylaxis included ATG, CsA, MMF, and MTX as in the Peking protocol but was supplemented with anti-CD25, basiliximab. Also, the graft source was modified, from a combination of G-CSF-mobilized marrow and blood to G-CSF-mobilized BM alone, and the conditioning was TBI based (10 Gy) (Table 11.1): G*v*HD grades II–IV was seen in 10% of patients, NRM was 31%, and relapse-related death (RRD) was 16%.

The basiliximab-based G*v*HD prophylaxis regimen [14] has been used by Di Bartolomeo and coworkers [15], with a non-TBI-based conditioning consisting of thiotepa, Bu, and Flu (TBF), originally described by Sanz and coworkers [16]: engraftment was not a problem, and severe acute G*v*HD (aG*v*HD) was seen only in 6% of patients; however, NRM was 36%, with a 3-year overall survival (OS) of 54% and 33% for standard- and high-risk patients.

11.2.4 Myeloablative Conditioning Regimens and Posttransplant Cyclophosphamide

We have reported the same MAC regimen (i.e., TBF regimen) followed by unmanipulated haploidentical bone marrow (BM), as compared to G-CSF-mobilized marrow, and PTCy [17]. Compared to the Baltimore PTCy regimen [5], we introduced two modifications: PTCy was given on days +3 and +5 (rather than +3 and +4) and CsA plus MMF was started before high-dose cyclophosphamide [17]. Grade II–III G*v*HD was seen in 12% of patients, NRM in 9% *vs*. 26% for patients in complete remission (CR) and advanced leukemias, and DFS at 18 months 62% *vs.* 51%, respectively [17]. A follow-up of that study confirmed these results [18]. The Atlanta group used a Flu-Bu conditioning, perhaps a little less ablative than TBF, combined with unmanipulated haploidentical G-CSF-mobilized PB graft and PTCy on days 3 and 4, tacrolimus, and MMF [19, 20]: the dose of Flu and Bu was reduced after the first five patients to Flu 25 mg/m^2 × 5 and Bu 110 mg/m^2 × 4 [19] (Table 11.1); aG*v*HD III–IV was 10%, NRM 10%, and 1-year survival 88% for standard-risk patients. The group in Milan developed a treosulfan-based regimen [21] (Table 11.1): they treated 40 patients and reported a very low rate of aG*v*HD III–IV (3 patients), a NRM of 17% at 1 year and DFS of 70% for remission patients and 20% for advanced disease [21]. This study proves that unmanipulated haplo-HCT can be performed with a calcineurin inhibitor (CNI)-free regimen.

TBI is still frequently used for young patients with acute leukemias: haploidentical grafts with myeloablative TBI doses have been reported and results are encouraging. The Atlanta group has reported haploidentical grafts following TBI for acute leukemia patients in remission [22]: in 30 high-risk patients, G*v*HD grades III–IV was 23%, NRM 3%, and OS 78%. All patients were in remission, but the median age was 46 years. We have also used ablative TBI (9.9–12 Gy) with results comparable to chemotherapy-based regimens [17]: however, it should be noted that patients receiving ablative TBI were significantly younger than patients given chemotherapy-based regimens. Di Persio and coworkers reported 52 patients receiving a regimen of

Flu + fractionated TBI (fTBI) 10.5 Gy (1.75 × 6) followed by G-mobilized PB grafts: severe G*v*HD (III–IV) was in the range of 20% and NRM 25% [23]. Thus, severe G*v*HD and NRM seem higher after PB rather than BM grafts for haplo-HCT, following MAC haplo-HCT, but prospective trials have not been performed.

11.3 Nonmyeloablative Regimens

11.3.1 Nonmyeloablative Regimens and Posttransplant Cyclophosphamide

Luznik and coworkers [5] published a milestone paper, showing safety and efficacy of high-dose PTCy, for the prevention of graft rejection and G*v*HD following a NMA regimen and a T-cell-replete, HLA-haploidentical, BM transplant. The protocol consisted of Cy 12.5 mg/kg, on days −6 and −5, Flu 30 mg/m^2 from day −6 to day −2, and 2 Gy of TBI on day −1, followed by BM graft infusion on day 0 (Table 11.1). On day 3, or days 3 and 4, patients received 50 mg/kg of Cy. Additional G*v*HD prophylaxis in the form of tacrolimus and MMF started after PTCy. Patients also received filgrastim support, starting on day 4 and continuing until recovery of neutrophils. The median time to neutrophil and platelet recovery was 15 and 24 days, respectively. [5]. Graft failure occurred in 13% of patients and was ultimately fatal in one patient. The probabilities of grade II–IV and III–IV aG*v*HD by day 100 were 34% and 6%, respectively, without a statistically significant difference for patients receiving one *versus* two doses of PTCy. However, chronic G*v*HD (cG*v*HD) was more frequent when using only 50 mg/kg of PTCy, rather than 100 mg/kg. The cumulative incidence of NRM was low (4% and 15% at 100 days and 1 year posttransplant, respectively), but the probability of relapse at 1 year was 51%. Patients with lymphoid malignancies had a better DFS as compared to those with myeloid malignancies [5]. The investigators explained the high relapse rate as the consequence of a population of high-risk patients, one third of them, having relapsed after prior autologous hematopoietic cell transplantation.

Nevertheless, this study showed that unmanipulated BM transplants from family HLA-haploidentical donors, with PTCy in combination with MMF and tacrolimus, were associated with a low incidence of graft rejection and acute and chronic G*v*HD despite high relapse rates possibly also due to the NMA conditioning regimen.

An update of the Baltimore group, with the same transplant platform, confirmed that relapse rate remains high in patients with high-risk disease [24]**.** They enrolled 372 consecutive adult patients with hematologic malignancies: the probability of NRM at 6 months was 8%, and the probability of severe G*v*HD (grades III–IV) was 4%. Three-year probability of relapse, DFS, and OS were 46%, 40%, and 50%, respectively. Using the refined disease risk index (DRI) [25], patients who had low-risk disease had estimated DFS of 65%, whereas patients with intermediate and high/very high risk had 37% and 22% DFS at 3 years ($P = 0.0001$). The investigators concluded that the outcome of patients grafted on the Baltimore platform was strongly associated with the DRI [24].

11.3.2 Modified Nonmyeloablative Regimens

Several other groups adopted PTCy, with modifications, such as increased intensity of the conditioning regimen or use of PB (instead of BM) as the graft source. The concern was that the original protocol was not able to control advanced malignancies and that PB $CD34^+$ graft would be easier to collect.

A recent study from Sugita and coworkers [26] (Table 11.1) evaluated the addition of busulfan to the NMA regimen from Luznik with the use of PB (instead of BM), to increase disease control. This prospective phase II multicenter trial included 31 patients with high-risk malignancies; 61% of the patients were not in remission at the time of transplant using the Baltimore conditioning (Flu, Cy, and 2 Gy; Table 11.1), with the addition of Bu 3.2 mg/kg/d on days −3 and −2 followed by unmanipulated PB graft infusion and PTCy on days +3 and +4. Additional immunosuppression was achieved with tacrolimus and MMF. Results from this study showed excellent neutrophil and platelet engraftment of 87% and 86% at a median of 19 and 35 days, respectively. The cumulative incidence of grades II–III and III–IV aG*v*HD at day 100 was 23% and 3%, respectively, with cumulative incidence of cG*v*HD at 1 year of 15%. The NRM was 23% at 1 year. The cumulative incidence of relapse was 45% at 1 year. The OS was 45% at 1 year and DFS was 34% at 1 year. Nine patients died of relapse and seven of NRM. These results do not seem very different from the ones reported by Baltimore group. The MD Anderson group reported a combination of Flu, Mel, and TBI 2 Gy, followed by unmanipulated BM [27], whereas Jaiswal and coworkers reported a combination of Flu, Mel, and Bu followed by G-mobilized PB grafts [28]. Finally the group from Nice reported an intensification of the Baltimore regimen, with the addition of Bu 6.4 mg/kg [29].

It is not easy to compare the effect of different conditioning regimens, in these phase II studies with small number of patients and different diagnoses: it is clear that haplo-HCT can be performed after reduced intensity conditioning (RIC) regimens, with addition and adjustments of Bu and/or Mel doses; stable donor engraftment is achieved and the incidence of acute and chronic G*v*HD is low.

11.3.3 Nonmyeloablative Regimens: Peripheral Blood *Versus* Bone Marrow as Graft Source

In a multicenter study, Raj et al. [30] reported the outcome of 55 patients with high-risk hematologic malignancies who received G-mobilized PB, after a NMA regimen, based on the use of Flu and low-dose TBI. Engraftment was rapid, but aG*v*HD grades II–IV was 61%; grade IV aG*v*HD was not described. The cumulative incidence of cG*v*HD was 18% and NRM was 23% at 2 years after transplantation. The investigators used a fixed dose of $CD34^+$ cells in the PB allograft ($5–6 \times 10^6$/kg) for two reasons: firstly, it approximated the median $CD34^+$ cell dose in BM allografts reported previously for this particular protocol; secondly, it standardized the T-cell dose, since an increased number of T cells in PB products, compared with BM, have

been associated with increased rates of acute and chronic G*v*HD, in the setting of HLA-matched-related or HLA-unrelated donors.

In a retrospective study, Castagna and coworkers [31] analyzed the outcome of 69 patients. Forty-six and 23 of patients received BM and PB grafts, respectively, from HLA-haploidentical donors, with PTCy, following a NMA regimen. They reported a higher risk of relapse (33%) for patients transplanted with advanced disease, compared with 14% for those in CR; they failed to show a difference between BM and PB patients, in terms of neutrophils and platelet recovery, or G*v*HD. They concluded that the use of T-cell-replete haploidentical PB instead of BM, after a NMA regimen, did not appear different in terms of either G*v*HD or engraftment rate. [31].

Bradstock and coworkers [32] compared the outcome of two retrospective cohorts of patients undergoing a RIC transplant using haploidentical-related donors and G*v*HD prophylaxis, with PTCy, tacrolimus, and MMF. The graft source was BM and PB in 13 and 23 patients, respectively. The investigators concluded that the OS for all 36 patients differed significantly between the BM and PB graft groups (log rank test $P = 0.028$) and at 2 years posttransplant it was 52.7% and 83.4%, respectively [32]. This study has the limitation of a significantly longer follow-up for survivors in the BM group (57 months) as compared to the PB group (20 months), so these are early results, which need to be confirmed over longer period of time.

11.3.4 Nonmyeloablative Conditioning in Older Adults

One important question is whether the Baltimore platform of haplo-HCT can be successfully used in older patients, considering the high rates of malignancies in older adults (aged over 60 years). In a retrospective analysis, Kasamon and coworkers [33] described the results of haplo-HCT in 271 patients with hematological malignancies who received a NMA haplo-BMT with PTCy. The patients' ages were between 50 and 75 years with a mean age of 61. The NRM at 1 year, for patients in their 50s, 60s, and 70s, was 9%, 14%, and 11%, respectively. Given the limited number of patients aged over 70 years (27 patients), outcomes were evaluated in patients aged 60–75 years ($n = 156$). In patients over 60 years, the NRM was 9% at day 180 and 13% at 1 year ($P = 0.08$ *vs.* ages 50–59 years) [33]. On univariate analysis older age was associated with a higher incidence of grade II–IV aG*v*HD. In this population, higher HCT-CI risk categories were not statistically significantly associated with greater NRM. Progression-free survival (PFS) and OS were 37% and 47%, at 3 years from transplant. These data were recently confirmed by Devillier and coworkers [34], in a cohort of 46 older patients with hematological malignancies undergoing an unmanipulated PB haplo-HCT following the Baltimore NMA regimen. In addition to PTCy, G*v*HD prophylaxis consisted of cyclosporine A (CsA) combined with MMF from day +5. These recent papers confirm that NMA haplo-HCT with PTCy are feasible in older patients, also with some intensification [29], and disease phase remains the major factor influencing the outcome, while age should not be considered a limiting factor.

11.3.5 Nonmyeloablative Regimen in Hodgkin's Disease

One particular disease deserves to be mentioned in the context of haplo-HCT, and this is Hodgkin's disease (HD). The Baltimore and Seattle groups have reported improved results with HLA-haploidentical donors, as compared to conventional transplants from HLA-identical siblings or HLA-unrelated donors [35]: in this report 28 haplo-HCT, using the Baltimore approach, were compared to 34 HLA-identical sibling and 24 unrelated transplants. All 90 patients received an almost identical NMA conditioning regimen, consisting of Flu+ TBI 2 Gy: NRM and relapse were both significantly lower with haploidentical donors, as compared to the other two HLA-matched donor types, producing a superior 2-year PFS of 51%, compared with 23% for HLA-identical siblings and 29% for unrelated donors [36]. These results have led to the preferential selection of a haploidentical donor for all HD patients undergoing an allo-HCT in Seattle and Baltimore. We have confirmed the excellent result of haplo-HCT with the Baltimore regimen in HD [36]: in the first 26 patients, we reported a 3-year OS of 77%, a NRM of 4%, and a relapse risk of 31%. The extremely low NRM should be compared with the average NRM of 21% with RIC regimens [37]. Relapse remains a problem, which can be successfully treated in a proportion of patients with haploidentical donor lymphocyte infusions (DLIs) [38], and is predicted by PET status of patients at the time of transplant. The introduction of checkpoint inhibitors, nivolumab and pembrolizumab, either before or after haplo-HCT, may further improve our ability to treat advanced HD refractory to induction chemotherapy or relapsing after an autotransplant [39].

These results suggest that a haplo-HCT should be seriously considered for all patients with advanced HD: a study comparing outcome of haplo-HCT *vs*. HLA-matched-unrelated donors would answer the question whether these excellent results are due to the Baltimore platform or the donor.

11.4 Expert Perspective

Despite 30 years of solid data on the existence of a graft-*versus*-leukemia (G*v*L) effect derived from the immune system of the donor and directed against the recipient and his hematologic disease, the intensity of the conditioning regimen still plays a role in the eradication of leukemia. It could be a direct role, through killing of leukemic cells; it could be indirect, by promoting G*v*L via inflammatory cytokines. Whatever the mechanism, MAC regimens reduce the risk of relapse, and we know this from a prospective randomized study [40]. This is particularly important in patients with acute leukemia. Therefore, does the use of a NMA or MAC regimen make a difference? The answer is *YES,* and how do we choose: rather easy.

11.4.1 Young Patients with Leukemias

For young patients with acute leukemia, MAC regimens should be preferred, because we know for certain that the tumor burden will be reduced and we also know that minimal residual disease (MRD) predicts posttransplant relapse [41]: it could well be

that G*v*L has a greater chance of being effective with a low tumor burden. Ablative dose of TBI is usually reserved for patients under the age of 50 years and especially for patients with ALL. Young patients undergoing *ex vivo* TCD would also be prepared with a MAC regimen.

11.4.2 Older Adults with Leukemias

For patients with acute leukemia, above 50 years of age, a NMA regimen, such as the Baltimore regimen, is probably not the best choice, as it is associated with a high risk of relapse [5]. Older patients may benefit of RIC regimens, such as a modified Baltimore platform, with the addition of one alkylating agent (Mel or Bu) [26–29]: these regimens are well tolerated and may provide sufficient reduction of the patients' tumor load. The combination of two alkylating agents (such as busulfan and thiotepa) results in a MAC regimen, which can be used up to the age of 70, with excellent tolerability and efficacy [18].

11.4.3 Primary Myelofibrosis

These are fragile patients with a disease, which is not easy to eradicate. The conditioning regimens have been historically based on the combination of one alkylating agent and fludarabine: Flu-Bu [42], Flu-thiotepa [43], or Flu-Mel [44], with encouraging results for HLA-matched sibling donors, but not for alternative donors [44]. We have recently used haploidentical donors following a combination of two alkylating agents (Bu and thiotepa) with Flu (TBF regimen), with significant reduction of both NRM and relapse [45].

11.4.4 Hodgkin's Disease

Patients with HD appear to do so well with the Baltimore NMA platforms [35, 36] that one wonders if this can be further improved: relapse remains a problem for patients who come to transplant with disease, but programs with checkpoint inhibitors may offer an additional opportunity to control the disease [39].

11.4.5 Other Chronic Lymphoproliferative Disorders

Patients with lymphoproliferative diseases can be offered either the Baltimore platform or the RIC regimen developed in Houston [27].

11.5 Future Directions

It seems clear that haploidentical grafts can be performed with a variety of regimens: perhaps the choice should be different for acute leukemias and for chronic diseases. The number of patients undergoing haploidentical grafts has been growing

so rapidly that it will be soon possible to run prospective trials and compare different regimens: all other components of the transplant will need to be the same in the two arms, since modification of the graft or G*v*HD prophylaxis (ATG or PTCy based) may well produce significant changes.

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Part III

Disease-Specific Pediatric Population

Haloidentical Transplants for Nonmalignant Diseases in Children

12

Christian Seitz, Patrick Schlegel, and Rupert Handgretinger

12.1 Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) offers a curative treatment strategy to a variety of nonmalignant hematological and immunological disorders as well as inborn errors of metabolism. Successful allo-HCT is able to restore functional hematopoiesis and immune function and can substitute disabled enzymatic activity. Nevertheless, allo-HCT can also be associated with serious risks for transplantation-related morbidities or even mortalities like graft-*versus*-host disease (G*v*HD) or life-threatening infectious complications. Especially in nonmalignant disorders (NMD), risks and benefits have to be carefully balanced on an individual patient basis. Up to now, human leukocyte antigen (HLA)-matched siblings are the preferred source of hematopoietic graft [1, 2]. However, only about one third of patients have HLA-matched sibling donor (MSD) [3]. This number further decreases in patients with inherited disorders as siblings might be carriers or affected as well. Therefore HLA-matched unrelated donors (MUD) have become an important alternative. Despite the tremendous efforts to establish international donor registries, HLA-MUDs are still unavailable for many patients in need for allo-HCT. Chances of finding a HLA-MUD are particularly dismal for individuals belonging to certain ethnic groups, with often less than 10% compared to approximately 75% in the Caucasian population [4, 5]. Therefore, alternative donor sources have to been taken into account, especially when the clinical condition of the patients does not allow a further delay of the allo-HCT. The advantages and disadvantages of the different alternative donor sources are listed in Table 12.1. This chapter will focus on HLA-haploidentical hematopoietic cell transplant (haplo-HCT) in NMD. It will highlight recent developments in graft manipulation utilizing safe application of haplo-HCT

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Table 12.1 Comparison of alternative donor options

Graft source	Matched unrelated	Umbilical cord blood	HLA-haploidentical
Availability	Depending on country and ethnic group <10% to 70%	>90%	Up to 100%
Time delay to transplantation	Depending on infrastructure and availability	No delay	No delay
Experience	Historical "gold standard," many published studies	Moderate	Less, but many ongoing studies
Graft composition	Reproducible quality and composition, options for graft engineering	Low cell number, single use only	Reproducible quality and composition, options for graft engineering
Engraftment	Good	Slow hematopoietic engraftment due to small amount of stem cells	Historically inferior, but reliable and quick with recent protocols
Immune reconstitution	Good	Delayed	Delayed after T-cell depletion, major improvements with no or partial depletion
Risk for severe infection	Low	Moderate due to delayed immune reconstitution	Historically high, major improvements with recent protocols
Risk for graft-*versus*-host disease	Moderate (10–50%), higher if unmanipulated bone marrow or PBHCs	Moderate (10–50%)	Depending on graft manipulation, historically high (>50%), low if T-cell depleted (<25%)
Posttransplant immunotherapy	Donor lymphocytes Available	None	Donor lymphocytes Available, donor always at hands for virus-specific T-cells, etc.
Transplantation-related mortality	Low, depending on experience and disease (0–30%)	Moderate due to delayed immune reconstitution depending on disease (0% to >50%)	Historically high, major improvements with recent protocols (<10%)

grafts and discuss important advantages which might lift haplo-HCT to a standard therapy in NMDs in the near future.

12.2 A Short History of Haploidentical Transplants in Nonmalignant Disorders

In contrast to HLA-MSD and HLA-MUD, haploidentical donors are available for almost every individual. These include siblings, parents, or even more distant relatives, sharing one haplotype with the patient. Physiologically, haplo-HCT is impaired by bidirectional alloreactivity in which both the donor's immune system and the

host's immune system can cause host-*versus*-graft (H*v*G) reactivity and consequently graft rejection as well as G*v*HD. And indeed, early attempts to transplant unmanipulated haploidentical bone marrow were limited by unacceptable transplantation-related mortality (TRM) due to graft failure, G*v*HD and infections, exceeding 50% in some studies [1, 6–8]. The first successful haplo-HCTs were carried out in the early 1980s in patients with severe combined immunodeficiency (SCID). Because of the lack of H*v*G reactivity due to T-cell deficiency in the host, novel approaches for graft engineering techniques toward G*v*HD prevention could be investigated. Soybean agglutinin (SBA) and erythrocyte-rosetting with sheep erythrocytes were used to deplete T-cells from the bone marrow graft. This method highlighted the efficacy of T-cell depletion (TCD) for G*v*HD prevention, even in the absence of posttransplant pharmacological prophylaxis, and achieved impressive cure rates especially in SCID patients transplanted within the first year after birth [9, 10]. Engraftment in non-SCID patients was improved by host T-cell depletion (TCD) using intensified conditioning regimes including thiotepa, cyclophosphamide (Cy), total body irradiation (TBI), and antithymocyte globulin (ATG), as well as by increasing the $CD34^+$ cell dose infused by seven- to tenfold ("megadose") [11, 12]. Graft engineering was further refined by the introduction of magnetic activated cell sorting (MACS), enabling precise enrichment and depletion of specific cell populations [13]. This technology led to an evolution in graft engineering starting with positive selection of hematopoietic grafts by anti-CD34 beads, followed by negative selection of T- and B-cells using anti-CD3 and anti-CD19 beads or certain subsets of T-cells by anti-TcRαβ or most recent anti-CD45RA beads [14, 15]. Whereas haplo-HCT using $CD34^+$ purified was hampered by delayed immune reconstitution and associated infections, specific depletion of possibly alloreactive T-cells offers excellent G*v*HD prevention while sparing other lymphocyte populations (NK-cells, γδ-T-cells, or memory T-cells) to augment posttransplant immune defense against pathogens.

12.3 T-Cells: To Deplete or Not to Deplete?

In the recent years, four competing but not mutually exclusive strategies for graft engineering and haplo-HCT have emerged. A comparison of these regiments, (1) TCD by positive $CD34^+$ selection with "megadose," (2) TCD by negative selection (CD3, TCRαβ, CD45RA), (3) GIAC (acronym for *G*-CSF-stimulation of donor, *i*ntensified immunosuppression (cyclosporine A (CsA) plus mycophenolate mofetil (MMF) and methotrexate (MTX)), *A*TG, *c*ombination of mobilized peripheral blood (PB) and bone marrow (BM) graft; see Chap. 5), and (4) posttransplantation cyclophosphamide (PTCy; see Chaps. 7, 8, and 18), is provided in Table 12.2. The primary goal in all regiments for haplo-HCT in NMD is the limitation of transplant-related morbidity and mortality due to G*v*HD and impaired immune reconstitution, while securing the sufficient substitution of preexisting deficiencies by successful engraftment. TCD-based regimes can be seen as the technologically most advanced approach in haplo-HCT. Automated positive immunomagnetic selection of $CD34^+$ HCS from

Table 12.2 Comparison of recent protocols for haplo-HCT

Protocol	Conditioning	Immunosuppression	Graft preparation
T-cell depletion by positive $CD34^+$ selection	Myeloablative conditioning based on TBI or busulfan, +fludarabine or cyclophosphamide, ±thiotepa	Pretransplant ATG No posttransplant immunosuppression	Positive immunomagnetic selection of $CD34^+$ HSC from G-CSF-stimulated apheresis, "megadose" grafts $>1 \times 10^7$/kg $CD34^+$
T-cell depletion by negative CD3 or TCRαβ selection	Myeloablative conditioning (see above) or reduced-intensity conditioning using treosulfan and fludarabine	Pretransplant ATG No posttransplant immunosuppression	Negative immunomagnetic selection of $CD3^+$ or $TCR\alpha\beta^+$ T-cells from G-CSF-stimulated apheresis, NK-cell ± γδ-T-cell-containing graft
GIAC protocol	Myeloablative conditioning using cytarabine, busulfan, cyclophosphamide, semustine	Pretransplant ATG mycophenolate mofetil, and cyclosporine A from day −9 pretransplant till day 60 and 180–300 posttransplant, MTX on days 1, 3, 6, and 11 posttransplant	G-CSF-stimulated, T-cell-replete PB and bone-marrow graft
Posttransplantation cyclophosphamide	Reduced-intensity conditioning using fludarabine, low-dose cyclophosphamide, low-dose TBI, and posttransplant (day 3 and 4) high-dose cyclophosphamide	Mycophenolate mofetil, tacrolimus	T-cell-replete bone marrow or G-CSF-stimulated PB grafts

BM or G-CSF-mobilized PB is a highly effective method to achieve a median purity of 97% and extensive T- and B-cell depletion below the threshold of 2.5×10^3 T-cells/kg recipient body weight, which is associated with a very low risk of G*v*HD [16]. Haplo-HCT using $CD34^+$-enriched grafts after myeloablative conditioning (MAC) is feasible in children with NMD, offering minimal incidence of G*v*HD (<10%) even in the absence of posttransplant immunosuppression and with robust engraftment [17, 18]. On the negative side, extensive TCD is associated with a significantly delayed immune reconstitution associated with high rates of TRM due to severe and often lethal infections. In attempts to accelerate immune reconstitution, more recent protocols use negative depletion of T-cells by immunomagnetic depletion of $CD3^+$ or $TCR\alpha\beta^+$ T-cells. In contrast to CD34 positive selection, these grafts contain high numbers of NK- and myeloid cells as well as γδ-T-cells in the latter case. Besides the intended graft-*versus*-leukemia (G*v*L) effect in hematologic malignancies, these

ancillary immune cells function as an immunological supplementation to bridge the time until functional αβ-T-cells recover. γδ-T-cells have been shown to mediate effector function against viral pathogens and might function as precursors for αβ-T reconstitution [19, 20]. Further, NK-cells support engraftment by eliminating host hematopoiesis facilitating reduced-intensity conditioning (RIC) regimes [21]. Both $CD3^+$ and TCRαβ TCD in combination with B-cell depletion via anti-CD19 for prevention of posttransplant lymphoproliferative disease (PTLD) have demonstrated an improved immune reconstitution and a reduction of infectious complications and TRM with a low incidence of G*v*HD [22–28]. Finally, depletion of $CD45RA^+$-positive naïve T-cell associated with pathogenesis of G*v*HD and the infusion of $CD45RO^+$ memory T-cells might offer further benefits for NMD patients, as theoretically the donor's complete cellular immunological memory should be transferred to the recipient [15]. More clinical trials must be performed to verify this hypothesis.

In contrast to TCD grafts, the GIAC protocol and PTCy are regimens using unmanipulated grafts. GIAC is based on the induction of hyporesponsiveness and polarization toward a T_H2 phenotype in T-cells due to G-CSF-stimulation and mixing of PB and BM allografts in combination with intensified posttransplant immune suppression (see Chap. 5) [29]. GIAC was extensively studied in hematopoietic malignancies achieving comparable results to HLA-MSD allo-HCT with engraftment in all patients and moderate incidence of G*v*HD (acute G*v*HD 36–40%, chronic G*v*HD 42–55%) and TRM (13–22%) [30, 31]. The role of GIAC in NMD has to be further evaluated. The biological background of PTCy is the induction of allo-tolerance due to direct elimination of alloreactive host T-cells responding to donor antigens as well as donor T-cells activated by host antigens and the generation of tolerogen-specific suppressive regulatory T-cells [32, 33]. Importantly, non-alloreactive T-cells as well as $CD34^+$ cells are not damaged, providing an extended T-cell repertoire and sufficient amount of $CD34^+$ cells for the recipient. Clinical data in patients with hematopoietic malignancies, using RIC regimes and PTCy on days 3 and 4 posttransplant, demonstrated feasibility with primary engraftment in 87%, acute G*v*HD in 40%, chronic G*v*HD in only 5%, and TRM in 15% of patients [34]. Several groups have extended the indication for this approach to a number of different nonmalignant conditions, which will be discussed below. Since no special laboratory equipment is necessary, haplo-HCT and PTCy can be performed in centers which lack such laboratory infrastructure or in countries where such infrastructures are not available due to financial or regulatory hurdles.

12.4 Haploidentical Transplant in Primary Immunodeficiency

Primary immunodeficiencies (PID) arise from genetic alterations leading to impaired development or effector function of various immune cells. Broadly, PIDs can be divided into severe combined immunodeficiency (SCID) and non-SCID including hemophagocytic syndromes and autoimmune and immunoregulatory disorders. Table 12.3 provides an overview on the most common PIDs and their underlying genetics. Allo-HCT from a healthy donor can restore the majority of these

Table 12.3 Overview on nonmalignant disease in children suitable for allo-HCT

Disease	Phenotype	Genetics
Primary immunodeficiencies		
Severe combined immunodeficiency (SCID)		
	No T-, no B-, no NK-cells	ADA deficiency, reticular dysgenesis
	No T- and no B-cells	Alteration in RAG 1/2, Artemis, Cernunnos, DNA ligase 4, DNA PK
	No T- and no NK-cells	Alteration in common gamma chain, Jak-3
	No T-cells	Alteration in IL-7Rα, CD3, coronin 1A, others
Omenn syndrome	Severe combined immunodeficiency with autoreactive T-cells causing chronic inflammation	Various SCID-associated alterations
Non-SCID variants		
	Variable	Deficiencies in Zap70 kinase, MHC class I or II, CD40, CD40 ligand, PNP, CD25, STAT5b, DOCK8, MALT1, BCL1o, CARD11, others
Wiskott-Aldrich syndrome	Eczema, thrombocytopenia, immune deficiency	Mutation in WASP gene
Hemophagocytic syndromes		
Familial hemophagocytic lymphohistiocytosis	Pancytopenia, hemophagocytosis, fever, hepatosplenomegaly, lymphadenopathy	Mutations in HPLH1, PERF1, UNC13D, STX11, STXBP2
Chédiak-Higashi syndrome	Recurrent pyogenic infection, albinism, peripheral neuropathy	Mutation in CHS1 gene
Phagocytic disorders		
	Variable	Deficiencies in ELA2, GFI1, LAD 1–3, RAC2, beta-actin, IL12p40, IL-12 and IL-23 β-chain, interferon-γ receptor 1 or 2, STAT1, others
Chronic granulomatous disease	Granuloma, recurrent infections	Mutations in p91-PHOX, CYBA, NCF1, NCF2
Kostmann syndrome	Severe neutropenia	Mutations in ELANE, GFI1, HAX1, G6PC3, VPS45, WASP
Shwachman-Diamond syndrome	Neutropenia or pancytopenia, exocrine pancreas dysfunction, growth retardation	Mutation in SBSD gene

Table 12.3 (continued)

Immune dysregulation		
Autoimmune lymphoproliferative syndrome	Lymphadenopathy, hepatosplenomegaly	Mutations in FAS, FAS ligand, caspase 8, caspase 10
IPEX syndrome	Autoimmunity	Dysfunction in FOXP3
Inborn errors of metabolism		
Mucopolysaccharidosis		
Hurler syndrome	Progressive deterioration, mental retardation, hepatosplenomegaly	Mutations in α-L-iduronidase
Scheie syndrome	Like Hurler but less severe	Mutations in α-L-iduronidase
Hunter syndrome	Frequent infection, abdominal hernia, hepatosplenomegaly, joint stiffness, mental retardation	Mutations in iduronate-2-sulfatase
Leukodystrophies		
X-linked adrenoleukodystrophy	Progressive demyelination, vegetative state, sexual dysfunction, adrenal insufficiency	Mutations in ABCD1 gene
Metachromatic leukodystrophy	Progressive demyelination, muscle wasting, convulsions, paralysis, dementia	Mutations in arylsulfatase A
Bone marrow failure syndromes		
Fanconi anemia	Pancytopenia, cancer predisposition most often AML	Mutations in FANCA, FANCB, FANCC, FANCD1 (BRCA2), FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ (BRIP1), FANCL, FANCM, FANCN (PALB2), FANCP (SLX4), FANCS (BRCA1), RAD51C, XPF
Dyskeratosis congenita	Abnormal skin pigmentation, nail dystrophy, leukoplakia, bone marrow failure, cancer predisposition	Mutations in CTC1, DKC1, TERC, TERT, TINF2, NHP2, NOP10, WRAP53
Congenital amegakaryocytic thrombocytopenia	Thrombocytopenia	Mutations in MPL gene
Diamond-Blackfan anemia	Normocytic or macrocytic anemia Variety of congenital abnormalities	Mutations in DBA1-13, TSR2, RPS28, GATA1
Paroxysmal nocturnal hemoglobinuria	Anemia, thrombosis	Spontaneous mutations in PIGA

Table 12.3 (continued)

Disease	Phenotype	Genetics
Acquired severe aplastic anemia	Anemia, leukocytopenia	Various acquired alterations
Hemoglobinopathies		
α-Thalassemia	Depending on genotype: −/α α/α asymptomatic, −/− α/α or −/α −/α mild anemia, −/− −/α anemia and hepatosplenomegaly, −/− −/− hydrops fetalis	Alterations in HBA1 and HBA2 genes (four homologue copies of α-chain)
β-Thalassemia	Depending on genotype: −/β mild anemia, −/− anemia, and hepatosplenomegaly	Alterations in HBB gene or regulatory regions
Sickle cell disease	Sickle cell crisis, pain, vasoocclusion, hemolysis	Mutation in HBB gene

deficiencies by replacing the patient's defect cellular immune system. Currently, the preferred donor source in PIDs is HLA-MSD due to best overall survival (OS). Despite genetic heterogeneity, untreated SCID uniformly leads to death by infection within the first years of life. HLA-MSD allo-HCT can improve survival to >95% [35]. Importantly, time to transplant, younger age, and absence of preexisting infections are crucial prognostic factors [35, 36]. Therefore, decisions for an alternative donor source have to be made in a timely manner if no HLA-MSD is available. Besides cord blood (CB) transplants, reviewed in volume one of this series, haplo-HCT offers an immediate strategy for almost every patient. Importantly, in young patients (<3.5 months) preferentially with a $T^{-}B^{+}$ SCID phenotype, haplo-HCT can be performed without pervious conditioning [36]. A comprehensive study, comparing different donor sources for SCID in 240 patients in the period between 2000 and 2009, demonstrated inferior survival (79% in patients without and 66% undergoing any kind of conditioning) compared to HLA-MSD but similar incidence of G*v*HD (15–20%) in patients receiving haploidentical grafts using TCD (51% SBA and E-rosette, 36% CD34 selection, 9% other TCD). Survival in CB recipients was 58% and 53% in MUDs [35]. Encouragingly, first reports for TCRαβ-depleted haploidentical grafts demonstrate further improvement, and in one study, all eight patients, three with active viral infection, were successfully transplanted and remarkably also achieved donor B-cell engraftment [28]. In another study, five patients were successfully transplanted and alive [27].

PTCy has also been successfully applied in SCID [37]. Despite the usually less severe clinical presentation, allo-HCT in non-SCID remains challenging due to acquired infections [38]. Non-SCID comprises widely different diseases, and due to later and often less severe manifestation, risk and benefit of allo-HCT have to be discussed individually. Besides HLA-MSD, HLA-MUD becomes an additional donor source. Also haplo-HCT is an option worth considering due to the improvements in the recent years. Two studies demonstrated the efficiency of TCRαβ-depleted haploidentical grafts, leading to survival in five out of five patients without incidence of

G*v*HD and five out of five survivors with only one patient experiencing limited G*v*HD [27, 28]. Also PTCy offers a reasonable option for non-SCID PIDs, achieving survival in five out of five patients with an incidence of mild G*v*HD in one patient, and one patient had to be reconditioned and was successfully re-transplanted from the same donor [39]. In addition to excellent engraftment and survival, haplo-HCT offers the opportunity for adoptive transfer of donor-derived immune cells. Virus-specific T-cells against the most common and dangerous viral infections in the allo-HCT setting, such as CMV, EBV, and ADV, can be routinely generated from the donor, conferring protection against these pathogens in 70–90% of cases [40–43]. These technologies offer new options for pre- or posttransplant antiviral therapy. A further strategy for virus control might be the add-back of limited numbers of CD45RA-depleted $CD45RO^+$ memory T-cells after TCD haplo-HCT [44]. Alternatives to allo-HCT might be gene therapeutic approaches. By substituting or in the future replacing the mutated gene in autologous HSC, patients can be causally cured. Early attempts for gene therapy in the 1990s have been hampered by high incidence of leukemia due to oncogenic transformation mediated by the retroviral vectors used for gene transfer [45, 46]. Improvements in the vector design have increased the safety of this approach. Clinical trials for a variety of genetic defects causing PIDs, including SCID, ADA deficiency, and Wiskott-Aldrich syndrome (WAS), have been performed or are under way [47, 48]. However, the risk of oncogenic transformation needs to be reduced to a minimum before gene therapy can become a standard therapy for these patients.

12.5 Haploidentical Transplant for Inborn Errors of Metabolism

Like PIDs, inborn errors of metabolism (IEM) are caused by inherited genetic alterations. IEMs are a heterogenetic group of disorders characterized by mutations in enzymes, coenzymes, or transporter leading to loss or impairment of function, which leads to the accumulation of toxic substrates and intermediate metabolites or the lack of vitally required products. IEMs typically present with onset in infancy or early childhood and rapidly progressing systemic manifestations including growth and development delay, impairment of cardiopulmonary status and visceral organ functions, as well as neurological and cognitive function, resulting in major disabilities and early death in more severe phenotypes. Timely diagnosis and management are essential. There are genotype-phenotype correlations, demonstrating that the degree of functional loss correlates with onset and progression of the disease. Consequently, enzyme substitution should stop or at least delay disease progression. Enzyme-replacement therapy (ERT) by repetitive application of recombinant enzymes is becoming available for a growing number of IEMs. Although ERT can be successful in certain diseases, there are limitations in terms of biodistribution, especially penetrance to the CNS and immunological responses against the therapeutic agent [49, 50]. In contrast, allo-HCT can offer a permanent therapeutic option in a number of IEMs, including deficiencies in lysosomal enzymes (lysosomal storage diseases LSDs) or peroxisomal function. In these cases, the transplanted, healthy

HSC-derived tissue is capable to sufficiently substitute missing enzyme function [51]. Allo-HCT has become standard of care in certain LSDs including mucopolysaccharidosis type-1, Hurler phenotype (MPS-1H), or lysosomal leukodystrophies like metachromatic leukodystrophy (MLD), X-linked adrenoleukodystrophy (X-ALD), or Krabbe disease and is under investigation in various others, listed in Table 12.3. Importantly, allo-HCT might only stop the progression of the disease but not reverse already acquired deficiencies [52]. Therefore, the decision to transplant has to be based on a balance of risk and potential benefit. Especially patients diagnosed very early or presenting with slow progression are eligible candidates. In MPS-1H, the best studied IEM treated with allo-HCT, HLA-MSD grafts after MAC achieve best event-free survival (EFS) rates, with up to 95% in recent reports [53]. However, it has to be recognized that most HLA-MSDs in certain IEMs are carriers, influencing posttransplant enzyme levels, which might impact long-term outcomes [54]. As the period from initiation of the donor search until the date of transplant is crucial, readily available alternative donor sources have been evaluated. A comprehensive study, comparing different donor sources for MPS-1H in 258 patients in the period between 2000 and 2007, demonstrated equal EFS in patients receiving six of six HLA-matched CB grafts compared to MSD, both 81%, *versus* 68% in five of six CB, 57% in four of six matched CB, 66% in HLA-MUD, and only 41% in mismatched unrelated donors using mostly TCD grafts [55]. It is important to mention that clinical data concerning the outcome of modern haplo-HCT approaches are limited but promising, even after primary graft failure [56, 57]. The RIC regimen in combination with negatively selected TCD haploidentical grafts or PTCy might reduce transplant-related toxicity and improve long-term survival. Moreover, as already mentioned, enzyme dose delivered by transplant appears to be important for patient's long-term outcome [54]. Consequently, haploidentical donors could be selected due to enzyme expression. In addition, transfusion of donor-derived mesenchymal stem cells might improve outcome [58]. Finally, gene therapeutic approaches will certainly impact future therapy for IEM. Efficacy of gene therapy for X-ALD and MLD using autologous HSCs cells, which have been gene-modified to express wild-type ABCD1 or arylsulfatase A (ARSA) gene, has been successfully demonstrated [59, 60], and several clinical trials are about to open. Further modifications in terms of enhanced enzyme expression or improved secretion or targeted delivery might additionally improve outcomes. In conclusion, haplo-HCT is currently not state of the art in IEM but might offer certain benefits that have to be evaluated in clinical trials.

12.6 Haploidentical Transplant for Bone Marrow Failure Syndromes

Bone marrow failure syndromes (BMFS) are either inborn or acquired deficiencies of isolated or multiple hematopoietic lineages. An overview on genetics and phenotype is provided in Table 12.3. Importantly, BMFS may progress via clonal evolution into myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML)

depending on the underlying condition in up to 55% [61]. This risk might be averted by successful allo-HCT [62]. However, allo-HCT is not capable to reverse extramedullary manifestations as seen in some inherited BMFS or cancer predispositions like in Fanconi anemia or dyskeratosis congenita. Moreover, conditioning in preparation to allo-HCT might increase the risk for malignant transformation. In severe aplastic anemia (SAA), immunosuppressive therapy (IST) might be effective, but relapses occur in more than 30% and EFS rates are significantly improved after allo-HCT [63, 64]. Therefore, allo-HCT should be considered on a risk to benefit ratio as a curative therapeutic approach. HLA-MSDs are the preferred donor source leading to cure rates of 80–90% and graft failure in 9–11% [65, 66]. Importantly allo-HCT for HLA-MUD achieves similar results using RIC in combination with alemtuzumab or ATG [63, 66]. CB transplantation in SAA was associated with high rates of graft failure (49%) and inferior OS (38%) [67]. Importantly outcomes for haplo-HCT have dramatically improved. Using a GIAC-like regime, engraftment in 17 of 17 pediatric patients was achieved with moderate rates of aG*v*HD (31%) and cG*v*HD (21%) and OS in 72% [68]. Also PTCy was successfully evaluated in 16 patients, leading to primary engraftment in 94%, aG*v*HD in 12.5%, no cG*v*HD, and OS of 67% [69]. Using $CD3^+$-depleted grafts in pediatric patients, primary engraftment was achieved in 11 of 12 cases; 3 patients experienced graft failure and were successfully re-transplanted from a 2nd haploidentical donor. All patients survived and were transfusion independent [70]. In contrast, two of four patients transplanted with TCRαβ TCD grafts experienced graft failure, and one patient died due to infection. This was contributed to insufficient immune-suppressive preparation [28]. Like in SAA, outcomes for inherited BMFS after allo-HCT have significantly improved with OS between 60% and 90% using HLA-MSD, HLA-MUD, or CB [71–73]. As mentioned above, allo-HCT in BMFS with predispositions for cancers like Fanconi anemia (FA) or dyskeratosis congenita is more challenging as genotoxic conditioning might increase the risk for secondary malignancies [74]. Therefore, fludarabine-based RIC protocols with reduced Cy and limited field or no radiation in combination with or without TCD via ATG and/or ex vivo depletion have been introduced increasing 5-year survival rates from 30% to above 90% in the last two decades for HLA-MSDs as well as HLA-MUDs in FA [75–77]. In the absence of a matched donor, haplo-HCT offers an excellent alternative for patients suffering from FA. Studies using CD34-purified grafts resulted in good primary engraftment (75%) with low rates of aG*v*HD (17%) and cG*v*HD (35%) and a 5-year OS of 83% [78]. Also RIC in combination with PTCy has been tested in three patients with mixed results [79]. Negative TCD by CD3/CD19 depletion led to survival in three of three FA patients without any signs of G*v*HD [24] and successful transplantation in four of four FA patients using TCRαβ-depleted grafts [28]. These results are promising and might allow further reduction of conditioning intensity due to the positive effect of NK- and γδ-T-cells on engraftment. Finally, haplo-HCT does allow donor selection beyond HLA match. In several malignancies, donor selection-based certain killer immunoglobulin-like receptor (KIR) ligand incompatibilities or KIR haplotype can translate into significant survival benefit [80, 81]. Further clinical investigation will have to demonstrate whether this is also the case

for BMFS with malignant transformation or might even cause protection against secondary malignancies. In summary, haplo-HCT represents a reasonable alternative donor source in acquired as well as hereditary BMFS.

12.7 Haploidentical Transplant for Hemoglobinopathies

Hemoglobinopathies are among the most common monogenic human diseases with an estimated 7% of the world's population being carriers. They can be caused by defined single nucleotide mutation in the β-globin gene like in sickle cell disease (SCD) or heterogenic mutations or depletions like in β-thalassemia, leading to impaired function of globin genes [82]. A summary on genetics and phenotype is listed in Table 12.3. Clinical presentation varies from hydrops fetalis in α-thalassemia major over transfusion dependency in β-thalassemia to intermittent crisis in SCD. Allo-HCT offers a curative therapeutic option by replacing the patient's dysfunctional hemoglobin synthesis with a healthy erythropoiesis. Early transplantation should be favored to avoid transfusion-dependent late toxicities like gonadal and growth failure and to reduce risk for graft failure due to allosensitization. Best results have been achieved using HLA-MSD transplants with disease-free survival rates of 90% [83, 84]. Besides the fact that HLA-MSDs are only available in 1/3 of cases, siblings are often carriers leading to minor variants after transplant which might be associated with certain disabilities. Another constraint at present times is the insufficient availability of HLA-MUDs due to the underrepresentation of unrelated donors in the registries of similar ethnic background as the patients [5]. Consequently, alternative donor sources are in need to meet increasing demands. In thalassemia, the introduction of treosulfan-based RIC regimens to reduce toxicities and the use of ATG, hydroxyurea, azathioprine, and thiotepa as prophylaxis against graft rejection have significantly improved outcomes for HLA-MUD transplantation with transfusion-free survival (TFS) at 82% [85]. In the absence of HLA-MSD and HLA-MUD, CB might be a valid option, achieving up to 77% EFS after MAC at selected institutions [86]. To overcome donor limitations, haplo-HCT is of major significance. In one study, 23 patients received CD34-positive selected PB grafts and 8 patients CD3/CD19-depleted BM grafts. Overall TFS was 70% with OS of 92% [87]. Recent data using TCRαβ-depleted grafts showed successful transplantation in one patient [28]. Promising results have also been demonstrated using PTCy, resulting in 94% TFS, moderate aG*v*HD in 31%, and transient cG*v*HD in 17% [88]. Interestingly, analysis of transplanted patients has shown that mixed donor chimerism is sufficient to achieve independence from transfusion [89]. Consequently, further reduced-intensity conditioning in combination with PTCy for tolerance induction might offer a sufficient and economical strategy for broad application. First experience has been obtained in 14 SCD patients receiving haploidentical grafts and PTCy. While all patients survived and mild G*v*HD was seen in only one patient, graft failure occurred in 43% [90]. Further studies should identify the most effective approach. Finally, like in most monogenetic diseases, gene therapeutic strategies are on the way to the clinic. Several trials for gene replacement using

lentiviral vectors for globin expression are recruiting. Further strategies for gene correction or gene editing in order to reactivate the expression of fetal hemoglobin are in preclinical evaluation. In conclusion, haplo-HCT is an attractive method to meet the existing needs for alternative donor sources in hemoglobinopathies, being available for almost all patients and achieving comparable results [82].

12.8 Future Directions

Recent improvements in graft engineering and preparative regimes have tremendously increased safety of haplo-HCT, underscoring its value as readily available donor source. Further refinements will additionally increase safety of haplo-HCT. Besides depletion of potentially alloreactive T-cells using immunomagnetic depletion of TCRαβ$^+$ or CD45RA$^+$ T-cells, other methods have been developed to actually deplete T-cells after confirming their alloreactivity. After incubation with patient-derived antigen-presenting cells, alloreactive donor T-cells are depleted either by targeting surface markers of activation and proliferation or by selective accumulation of photoactivatable cytotoxic dyes [91]. Another strategy is based on the concurrent transfusion of donor-derived regulatory T-cells (Tregs) in order to suppress alloreactive T-cells *in vivo*. One study with 43 adult leukemia patients demonstrated feasibility with only 15% experiencing aG*v*HD and 0% cG*v*HD in the absence of posttransplant immunosuppression [92]. To further increase safety, suicide genes might be integrated in the graft. These genes can be pharmacologically activated in case of G*v*HD or other graft-related problems, leading to cell death induction and elimination of donor-derived cells genetically modified to express the suicide gene. Several constructs have been tested in clinical trials. In one study T-cells engineered to express thymidine kinase have been infused after haplo-HCT. After occurrence of aG*v*HD in ten patients, T-cells were successfully abrogated by application of ganciclovir [93]. Similar results have been obtained using inducible caspase 9 as a suicide switch [94].

12.9 Expert Point of View

Whereas haploidentical donors have been considered in the past as a last option for hopeless cases without alternatives, they are now on the brink of becoming frontline choice not only in malignancies but also in several NMDs. Several clinical studies have demonstrated applicability as summarized in Table 12.4. Besides improvements in safety and the broad availability, a major advantage of haplo-HCT for NMDs in children is its availability even after posttransplant period in the absence of HLA-MSD. Therefore, haplo-HCT can serve as a platform for pre-, inter-, and posttransplant cellular therapy. As highlighted above, immune cells like NK- or different subsets of T-cells can be quite easily generated from the donor to support engraftment and immune reconstitution. In case of preexisting or acquired viral infections, virus-specific T-cells derived from the donor can be administered to clear the

Table 12.4 List of select haplo-HCT studies for nonmalignant diseases

Disease	*n*	Protocol	Conditioning	Graft failure	aG*v*HD	cG*v*HD	DFS	OS	Ref.
PID									
SCID	77	TCD SBA/E-r.	None	0%	36%	n.g.	78%[a]	78%	[10]
SCID	138	TCD SBA/E-r. (51%), CD34$^+$ (36%)	63% none, 18% MAC, 12% RIC, 7% IS	24%	21%	16%	79%[a] 66%[a]	79% 66%	[35]
SCID	8	TCD TCRαβ$^-$	Treo, Flu, ATG	0%	n.g.	0%	100%	100%	[28]
SCID	5	TCD TCRαβ$^-$	Treo, Flu, ATG	20%	40%	20%	100%	100%	[27]
SCID Non-SCID	13 27	TCD CD34$^+$	Bu, Cy	8%	24%	3%	47.5%[a]	47.5%	[38]
Non-SCID	5	TCD TCRαβ$^-$	Treo, Flu, ATG	20%	20%	0%	100%	100%	[27]
Non-SCID	5	PTCy	Mel, Flu, ±ldTBI, Alem	0%	20%	0%	100%	100%	[39]
BMFS									
SAA	12	TCD CD3$^-$	Flu, Cy, ±TBI, ATG	25%	33%	22%	100%	100%	[70]
SAA	16	PTCy	Flu, Cy, ldTBI	8%	12,5%	0%	63%	63%	[69]
SAA	17	GIAC	Bu, Flu, Cy, ATG	0%	31%	21%	72%	72%	[68]
FA	12	TCD CD34$^+$	Flu, Cy, ATG	17%	17%	35%	83%	83%	[78]
FA	4	TCD TCRαβ$^-$	Treo, Flu, ATG	0%	n.g.	0%	100%	100%	[28]
HGP									
β-thal	31	TCD CD34$^+$ (23), CD3$^-$ (8)	Bu, Flu, TT, Cy, ATG	23%	0%	0%	70%	94%	[87]
β-thal	31	PTCy	Bu, Flu, ATG	6%	31%	17%	94%	97%	[88]
SCD	14	PTCy	Flu, Cy, ldTBI, ATG	43%	0%	0%	57%	100%	[90]

n number, *g. fail.* graft failure, *aGvHD* acute graft-*versus*-host disease, *cGvHD* chronic graft-*versus*-host disease, *DFS* disease-free survival, *OS* overall survival, *Ref.* reference, *PID* primary immunodeficiencies, *SCID* severe combined immunodeficiency, *BMFS* bone marrow failure syndromes, *SAA* severe aplastic anemia, *FA* Fanconi anemia, *HGP* hemoglobinopathies, *β-thal* β-thalassemia, *SCD* sickle cell disease, *TCD* T-cell depletion, *SBA* soybean agglutination, *E-r.* erythrocyte-rosetting, *CD34$^+$* positive selection, *CD3$^-$ or TCRαβ$^-$* negative selection, *PTCy* post-transplant cyclophosphamide, *MAC* myeloablative conditioning (various), *RIC* reduced-intensity conditioning, *IS* immunosuppression, *Treo* treosulfan, *Bu* busulfan, *Mel* melphalan, *Flu* fludarabine, *Cy* cyclophosphamide, *TT* thiotepa, *ATG* antithymocyte globulin, *Alem* alemtuzumab, *TBI* total body irradiation, *ldTBI* low-dose TBI, *n.g.* not given or not provided

[a]Varying degree of lymphocyte alterations or immunoglobulin substitution

infection or can be stored to be able to immediately react in case of reactivation. Donor-derived mesenchymal stromal cells might be infused to suppress alloreactive T-cell in case of G*v*HD [95]. Finally, stem cell boosts can be prepared from the haploidentical donor in case of delayed or insufficient engraftment [96]. Therefore, haplo-HCT should no longer be regarded as a last-option approach but can be offered frontline to patients in need of a transplant missing a HLA-MSD or HLA-MUD.

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Haloidentical Hematopoietic Cell Transplantation in Children with Neoplastic Disorders

13

Mattia Algeri and Franco Locatelli

13.1 Introduction

For almost 50 years, allogeneic hematopoietic cell transplantation (allo-HCT) from a human leukocyte antigen (HLA)-matched donor, either related or unrelated, has increasingly been used to treat children affected by several neoplastic and nonmalignant disorders. Thanks to this procedure, thousands of patients have been cured of their original disease [1]. Despite this remarkable success, only 25% of patients in need of an allograft have an HLA-identical sibling available, and for less than 70% of the remaining patients, a suitable, HLA-compatible, unrelated volunteer can be found [2]. This proportion can be even lower for patients belonging to ethnic groups poorly represented in the registries. Furthermore, the search for an HLA-matched unrelated donor (HLA-MUD) may result in unacceptable delay for children with aggressive hematological malignancies, in whom the goal is to proceed to transplantation while the patient is in remission or has minimal disease burden after conventional chemotherapy. In the absence of an HLA-matched donor, alternative donors/sources of hematopoietic grafts such as unrelated umbilical cord blood (UCB) and HLA-haploidentical relatives are being increasingly used to offer the chance of an allograft to any patient in need of transplantation [2]. In particular, the majority of patients has a family member, identical for one HLA haplotype (i.e., HLA-haploidentical), who can immediately serve as transplant donor [3, 4]. Besides availability for almost all patients, transplantation from a full haplotype-mismatched family member offers other several other advantages such as no delay in obtaining

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the graft, the possibility to select the best donor from a panel of candidate members, and easy access to donor-derived cellular therapies if required after transplantation. Despite many advantages associated with haplo-HCT, widespread use of this procedure has been slow, for many years, by relevant complications mediated by bidirectional alloreactivity toward incompatible HLA molecules, responsible for high rates of graft rejection and for development of severe graft-*versus*-host disease (G*v*HD) [5–7]. Since donor-derived T lymphocytes contained in the graft are the major mediators of severe alloreactions in haplo-HCT, various attempts have been employed to overcome the risk of G*v*HD by depleting T-cells from the graft prior to infusion (see Chaps. 2–6). The first *ex vivo* T-cell-depleted (TCD) bone marrow (BM) transplants using soybean agglutinin and rosette formation with sheep red blood cells were performed in children with immunodeficiency syndromes [8]. It can now be estimated that hundreds of SCID patients have been transplanted worldwide using an HLA-haploidentical related donor, with a high rate of long-term, either partial or complete, immune reconstitution [9]. Such encouraging results, however, were not initially replicated in leukemia patients, in whom haplo-HCT was associated with an unacceptably high incidence of graft failure [10]. In this setting, extensive TCD of the graft dramatically affected the balance between competing host and donor T-cells, leading to rejection of transplanted hematopoietic progenitor cells by anti-donor cytotoxic T lymphocytes (CTLs) of the patient surviving the preparative regimen [10] (see Chap. 2). Consequently, early attempts to use HLA-haplotype-mismatched donors in leukemia patients were only partially successful.

Over the past two decades, an impressive amount of translational immunologic research has resulted in a variety of promising techniques to control the intense alloreactivity in haplo-HCT and reduce transplantation-related mortality (TRM). These strategies have yielded encouraging results, with high rates of successful engraftment, effective G*v*HD control, and favorable outcomes, at the point that retrospective analyses of adult cohorts published within the past 10 years have demonstrated similar survival after haplo-HCT and HLA-matched-related or HLA-matched-unrelated transplants [11, 12]. Therefore, haplo-HCT is no longer regarded as a last-resort treatment, but it is increasingly offered to patients with an indication for transplantation who do not have a HLA-MSD or a HLA-MUD identified within a reasonable time.

13.2 Analysis of Data in Pediatric Population: What Are the Limitations?

Unfortunately, in the pediatric population, with neoplastic diseases, the number of published studies regarding haplo-HCT remains limited, with most studies reporting only small series of heterogeneous patients [13–20]. In particular, most reports include patients with neoplastic and nonmalignant diseases, adolescents and young adults, and even patients who received a transplant from one HLA-antigen disparate related donor, whose outcome may be similar to HLA-matched donor hematopoietic

cell transplant (HCT). Besides this, several other heterogeneous aspects of these studies are of concern: conditioning regimen (total body irradiation [TBI] or busulfan [Bu]-based regimen with different additional drugs or combinations), cell doses, cell manipulation, and G*v*HD prophylaxis. These limitations hamper the possibility of drawing definitive conclusions on the role of haplo-HCT in specific pediatric hematologic disorders.

Although several transplantation strategies have been developed to successfully overcome historical barriers to haplo-HCT, it must be emphasized that the great majority of studies regarding haplo-HCT in children are largely limited to TCD approaches. Indeed, the use of unmanipulated, T-cell replete graft, despite the large amount of impressive results obtained in the adult setting, has been so far investigated only by a few pediatric studies [21–23]. To date, no prospective and randomized trial comparing different regimens, cell doses. or cell manipulation methods exists, and consequently, common standards for haplo-HCT in pediatric hematologic disorders are lacking.

In this chapter, we will summarize clinical results obtained with haplo-HCT in pediatric hematologic malignant diseases, analyzing major advantages and drawbacks of various approaches and discussing strategies to further improve outcome.

13.3 The CD34⁺ Cell "Megadose" Breakthrough

One of major advances in TCD haplo-HCT came from murine experiments, demonstrating that the use of "megadoses" of granulocyte colony-stimulating factor (G-CSF)-mobilized PB-derived CD34⁺ cells was capable of overcoming the barrier of HLA incompatibility, eluding the residual anti-donor T-lymphocyte reactivity of the recipient [24] (see Chap. 2).

In an effort to reduce the high rate of graft failure of haplo-HCT, this preclinical evidence has translated into clinical reality by the group from Perugia, Italy. In a pilot study performed in adults with acute leukemias, Aversa and colleagues used a combination of donor BM and G-CSF-mobilized peripheral blood (PB) graft, which together allowed the collection of seven- to tenfold higher levels of hematopoietic progenitors than those found in BM allografts alone. After harvesting, allografts were depleted of T-cells using soybean agglutination and erythrocyte rosetting. The conditioning included TBI, thiotepa, cyclophosphamide (Cy), and rabbit antithymocyte globulin (ATG) without immunosuppressive therapy posttransplant. The reported engraftment rate was above 90%, with a cumulative incidence of grade II–IV acute and chronic G*v*HD below 10%. Just two patients in this study relapsed, although non-relapse mortality (NRM) occurred in 9 of 17 patients [25].

A further improvement was represented by the ability to purify CD34⁺ cells via immunomagnetic selection, which drastically reduces the T- and B-cell content of the graft, allowing the infusion of more than 10×10^6/kg of recipient weight CD34⁺ cells of recipient body weight, with a mean CD3⁺ cell inoculum of a 3×10^3/kg BW (see also Fig. 13.1). In this other seminal study, Aversa et al. documented sustained engraftment in 41 of 43 adults and pediatric patients (age range 4–53 years) with

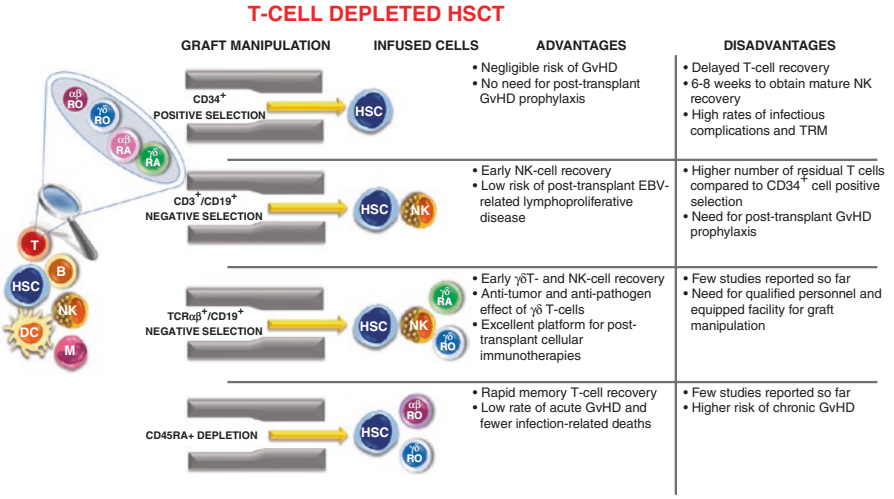

Fig. 13.1 Different types of graft manipulation currently used for haplo-HCT, with the related advantages and disadvantages

advanced leukemias without acute or chronic G*v*HD; long-term disease-free survival (DFS) was 28%. Conditioning regimen consisted of TBI, thiotepa, fludarabine, and ATG, and no pharmacological G*v*HD prophylaxis was employed [13].

Although the administration of $CD34^+$ "megadoses" partially addressed the rejection problem, removal of T-cells from the graft entailed prolonged lymphopenia and delayed immune reconstitution, as $CD4^+$ T-cells remained low for more than a year after transplantation. Consequently, NRM was 40%, with two thirds of these deaths resulting from opportunistic infections [13].

Subsequent publications by the Perugia group reported larger patient series, including both adult and children: transplant-related deaths occurred in 16.6% and 38.8% of patients who were in remission and in relapse at time of transplantation, respectively. The probability of relapse was 12% for patients with acute myeloid leukemia (AML) and 28% for patients with acute lymphoblastic leukemia (ALL) who received transplants in first or second complete remission. At a median follow-up of 6.9 years (minimum follow-up, 4.9 years), event-free survival (EFS) for patients who received transplants while in remission was 74% for AML patients and 59% for ALL patients [26].

A similar $CD34^+$ positive selection approach was employed in a German study on 39 children, of whom 31 with neoplastic hematologic diseases, after a myeloablative conditioning (MAC) [16] (also see Chap. 12). A rapid primary engraftment was seen in 36 patients, and acute G*v*HD occurred in only 6 patients (5 patients with grade I and 1 patient with grade II). The LFS for patients with malignant diseases was 28% (39% for children in complete remission, 14% for children not in remission), and NRM was 34%. Interestingly, a faster immunological recovery was observed when the number of transplanted $CD34^+$ cells was 20.3×10^6/kg of recipient body weight. Consequently, incidence of fatal infections was lower (18%) compared to that reported by the Perugia

group, with the main cause of death being relapse of the neoplastic disease. In a later study from the same group, the DFS of 28 patients with ALL and non-Hodgkin lymphoma (NHL) in remission, who received positively selected haploidentical grafts, was comparable with that of a similar historical control group receiving an unmanipulated HLA-MUD transplant (48% *vs.* 38%; $P = 0.6$). Moreover, no significant difference in the 3-year probability of relapse was observed between the two groups [16].

Two studies from the UK resulted in similar findings. Ortin et al. [20] reported excellent survival in 16 children with neoplastic conditions who received HLA-partially matched related donor allografts. The average $CD34^+$ cell dose was 11.13×10^6/kg recipient weight. Fifteen of 16 patients with neoplastic disorders received TBI plus Cy, and one, with leukemia, received Bu plus Cy. With a follow-up of 1.5 years, 13 had survived free of disease, and the incidence of grade III–IV G*v*HD was low [20]. Marks and colleagues investigated the role of haplo-HCT in 34 patients with acute leukemias (median age 11 years, range 1–16 years). Patients were conditioned with Cy and TBI (14.4 Gy in eight fractions) and received TCD PB grafts with a median $CD34^+$ cell dose of 13.8×10^6/kg of recipient weight (range 4.2–35.1) and 0.7×10^4 $CD3^+$ cells/kg of recipient weight . The actuarial survival at 2 years was 26% (13–41%, 95% CI). Survival at 4 years was 23% (10–38%). Being not in remission at the time of transplant was significantly associated with poorer outcome ($P = 0.033$), and, in particular, none of the nine patients with AML transplanted while not in remission survived. Relapse ($n = 14$) and viral or fungal infections ($n = 10$) were the major causes of mortality [19].

Given the high heterogeneity of previous reports and with the goal to evaluate the role of haplo-HCT in childhood ALL, an EBMT Pediatric Disease Working Party study analyzed a homogenous cohort of 127 children (<16 years) transplanted between 1995 and 2004 using related donors with at least 2 HLA-antigen disparities and after a relatively homogenous procedure of TCD, namely, positive selection of $CD34^+$ cells [27]. The median number of $CD34^+$ and $CD3^+$ cells infused was 12.3×10^6/kg BW (range, 1.36–95 $\times 10^6$/kg) and 5.0×10^4/kg BW (range, 0–12.9×10^4/kg). *In vivo* TCD by ATG or antilymphocyte globulin (ALG) was used in 78% of reported transplantations. All patients received a MAC regimen; TBI was used in 76% of patients. Twenty-two patients underwent haplo-HCT in first, 48 in second, and 32 in third complete remission. Twenty-five patients were in relapse at the time of transplantation. The 5-year LFS was 30%, 34%, 22%, and 0%, respectively. This observation confirmed findings of previous reports, showing better results in patients transplanted in CR than in those children who were not in remission, and the authors suggested that haplo-HCT should not be considered an option in patient active/resistant disease. A risk factor analysis was performed for patients transplanted in remission ($n = 102$). Five-year NRM, relapse incidence, and LFS were 37%, 36%, and 27%, respectively. A trend toward improved LFS and decreased relapse incidence was observed for children given a graft containing higher number of $CD34^+$ cells (adjusted $P = 0.09$ and $P = 0.07$, respectively). In a multivariate analysis, haplo-HCT performed in larger centers (performing >231 allo-HCT in the studied period) was associated with improved LFS rate and decreased relapse incidence (adjusted $P = 0.01$ and $P = 0.04$, respectively) [27].

These findings demonstrated that TCD haplo-HCT was a technically demanding procedure requiring experience, whose results are influenced also by the $CD34^+$ cell content of the graft. Major drawbacks for its universal application were the high TRM associated with delayed immune reconstitution (Fig. 13.1) and substantial inefficacy in patients not in remission at the time of transplantation.

13.4 The Impact of NK Cell Alloreactivity

The efficacy of allogeneic HCT in patients with malignancies is mainly due to the so-called "graft-*versus*-tumor" (G*v*T) effect, which, in unmanipulated transplants, is largely mediated by donor-derived T-cells present in the graft recognizing allogeneic peptide-HLA complexes displayed by malignant cells [28] (see Chaps. 10 and 19). Given the role played by donor T-cells in mediating the "graft-*versus*-leukemia" (G*v*L) effect, it was anticipated that a significant proportion of patients with acute leukemia given TCD haplo-HCT would have experienced disease relapse. However, this expectation was only partly confirmed by clinical results, as it became evident, initially in adult patients affected by AML, that a subgroup of patients was characterized by a particularly low risk of relapse. This subset included patients transplanted from a donor having natural killer (NK) cells that were "alloreactive" toward recipient targets [29, 30]. Since this seminal observation, several groups have investigated the impact of NK cells in shaping the outcome of haplo-HCT [31–34].

NK cell function is finely regulated by an array of receptors transducing either inhibitory or activating signals [35]. Seminal studies have shown that the signals delivered by inhibitory receptors, present on the surface of NK lymphocytes, are even more important than the activating signals [35, 36]. Among receptors that negatively regulate NK cell function, a crucial role is played by those interacting with HLA class I molecules. These receptors include killer immunoglobulin-like receptors (KIRs), specific in humans for determinants shared by groups of HLA-A, HLA-B, and HLA-C allotypes (referred to as KIR ligands), and the CD94/NKG2A heterodimer, specific for the nonclassical, class I molecule HLA-E. They avoid that NK cells attack autologous normal cells and allow that cells, in which HLA class I expression is downregulated (e.g., by tumor transformation or viral infection), be killed [35, 37–40]. In an allo-HCT setting, NK cells can kill nonself cells through the mechanism of "missing self-recognition," provided that the donor (1) expresses a KIR ligand missing in the recipient HLA genotype and (2) expresses the specific KIR, leading to a KIR/KIR ligand mismatch in the graft-*versus*-host direction.

According to the concept of "missing self-recognition," donor NK cell alloreactivity can be predicted to occur in approximately 50% of patients given haplo-HCT [31]. In comparison to alloreactive T lymphocytes, NK cells offer the advantage of inducing a G*v*L effect without G*v*HD development. Indeed, healthy non-hematopoietic tissues of the recipient are protected from donor NK cell-dependent alloreactivity, since they lack activating receptors, which, by contrast,

are expressed by both tumor and hematopoietic cells [31]. Alloreactive NK cells have been demonstrated to positively affect the outcome of TCD HCT from an HLA-haploidentical relative in both adults with AML [29, 30] and children with ALL [33, 34]. Indeed, in patients receiving the graft from a NK alloreactive donor, the probability of leukemia recurrence was particularly low, while the probability of LFS was found to be at least as good as that of patients transplanted in a similar disease phase from an HLA-matched sibling or unrelated volunteer. The donor NK-mediated G*v*L effect was particularly evident when patients with acute leukemias were transplanted in CR and, in children and young adults, when the donor was the mother [41].

In the last decade, several clinical studies have focused on the influence that NK cell-activating receptors exert on the outcome of allogeneic HCT recipients [42–44]. In this regard, it is known that two basic groups of KIR haplotypes can be found in the human genome: *group A haplotype*, which has a fixed number of genes encoding inhibitory receptors (with the exception of the activating receptor KIR2DS4), and *group B haplotype*, which has a variable gene content that includes additional activating KIR genes [45, 46]. Patients affected by AML given an unmanipulated allogeneic HCT from an HLA-matched unrelated volunteer have been reported to have a significantly improved outcome when *B/x haplotype* donors are employed, as compared with *A/A donors* [42]. Venstrom and colleagues, who investigated a large cohort of patients with AML, have recently confirmed the importance of specific *activating* KIR receptors in terms of protection against both leukemia recurrence and infections. In their analysis, patients receiving allogeneic HCT from donors positive for KIR2DS1, 33% of the whole population of donors, had a significantly reduced relapse rate than those transplanted from donors negative for KIR2DS1 [47]. Importantly, this benefit disappeared when donors with HLA-C2/C2 were employed, since high levels of HLA-C2 in HLA-C2/C2 donors reduce NK-cell reactivity. These clinical results are corroborated by experimental data showing that NK clones from KIR2DS1-positive donors with HLA-C1/C1 or C1/C2 genotypes exhibited higher cytotoxic activity against leukemia targets than clones derived from HLA-C2/C2 donors [48, 49].

An algorithm based on donor KIR B gene content has provided a further advancement in understanding the role of activating receptors. More specifically, *group A and B KIR haplotype* have distinctive centromeric (Cen) and telomeric (Tel) gene-content motifs. In patients with AML, compared with A haplotype motifs, centromeric and telomeric B motifs both contributed to protection against recurrence and to improved survival; Cen-B homozygosity had the strongest effect [43].

Similar results have been obtained in children with B-cell precursor (BCP)-ALL after TCD haplo-HCT: in these patients, not only the presence of KIR haplotype B but, even more so, the selection of donors with a high KIR B content score >2 conferred better protection against relapse [32].

In the future, biological and clinical studies will further elucidate the contribution of different activating receptors to NK cell alloreactivity and clarify the hierarchy of the factors related to NK cell-mediated G*v*L effect to be considered in the selection of the best donor for haplo-HCT.

13.5 Refinements to T-Cell Depletion Strategies: From Negative to Positive Selection

The high rate of NRM due to delayed recovery of adaptive immunity and the increased risk of infections represents the major drawbacks of classical haplo-HCT platform based on $CD34^+$ positive selection, especially when compared with HLA-matched unmanipulated allografts. Moreover, in a seminal study performed to investigate the kinetics of NK cell reconstitution, it was shown that after transplantation of $CD34^+$-selected cells, mature, fully functioning NK cells, derived from the differentiation of HSC, emerge in the recipient peripheral blood only several weeks after the allograft, while in the early posttransplant period, immature, poorly functioning NK cells predominate [31, 33]. Therefore, patients transplanted with TCD $CD34^+$ grafts from an NK-alloreactive relative cannot benefit from the NK-mediated G*v*L effect in the early posttransplant period. These observations provided the rationale for investigating alternative approaches of TCD, encouraged by technical improvements in immunomagnetic cell manipulation (Fig. 13.1).

The Tübingen and Memphis groups introduced CD3/CD19 cell depletion rather than $CD34^+$ cell selection in order to produce a graft containing other CD34 negative cells (such as NK cells, monocytes, dendritic cells, and other myeloid cells; Fig. 13.1), which might enable better immune recovery without leading to G*v*HD [50].

Handgretinger et al. [15] provided evidence that a reduced-intensity conditioning (RIC) with fludarabine, thiotepa, melphalan, and serotherapy with OKT-3 followed by infusion of CD3/19-depleted PB grafts was a feasible option for children with high-risk hematologic malignancies. The efficiency of TCD with this method was lower than after $CD34^+$ selection with 3.5–4 log depletion, and although this correlated with increased rates of G*v*HD, the TRM associated with this strategy was low, with only 1 of the 38 patients dying as a result of toxicity [15] (see Chap. 21). Primary engraftment occurred in 83%, and this number rose to 100% after a second procedure. Similar studies in adults and children with hematologic malignancies undergoing TCD haplo-HCT after RIC also demonstrated improved results in terms of low TRM and overall survival (OS) with some survival rates reportedly comparable to HLA-MUD HCT [14, 51–55]. Data also suggest that outcomes have improved over the years to such an extent that HCT could be a suitable option for all children with high-risk malignancy, regardless of matched donor availability [56]. In particular, a study from the St. Jude group reported an impressive 5-year OS of 88% in children with very-high-risk leukemia receiving CD3/19-depleted haplo-HCT and a 25.7% incidence of grade II–IV G*v*HD [56].

Recently, the Tübingen group reported the results of haploidentical transplantation using CD3/CD19 cell selection after myeloablative conditioning in 46 children with ALL ($n = 26$), AML ($n = 17$), or advanced MDS ($n = 3$). Twenty patients had active disease, and 19 received second or third HCT. The patients received a median number of 14.5×10^6 $CD34^+$ progenitor cells/kg of recipient weight. The extent of TCD was 4.16 log (median, range 3.6–5.1), and the median number of residual $CD3^+$ T-cells was 59.6×10^3 cells/kg of recipient weight. Primary engraftment occurred in 88% of patients (100% after re-transplantation). Grade II–IV acute

G*v*HD occurred in 20%, grade III–IV acute G*v*HD in 7%, and chronic G*v*HD in 21% of children. With a median follow-up of 4.3 years (range 1.3–6.2 years), 12 of the 46 patients survived free of disease. Remission status significantly influenced survival: patients in any CR had a 3-year EFS of 31%, whereas patients with active disease had a 3-year EFS of 15% (P<0.02). TRM was 8% at 1 year and 20% at 5 years. Relapse occurred in 63% of patients during 2 years of follow-up. The overall probability of relapse at 2 years was 38% in the patient group with CR receiving first HCT, while patients who were not in remission at time of transplantation or who received a subsequent HCT had a significantly higher risk of relapse (75% and 88%, respectively; $P < 0.05$). Overall, the use of CD3/CD19 cell selection might slightly reduce NRM but seems to carry a substantially greater risk of G*v*HD compared with $CD34^+$ cell selection. Indeed, as compared with $CD34^+$ positive selection, the number of graft-contaminating T-cells is approximately tenfold higher in CD3/19-depleted grafts, requiring short-term posttransplantation immunosuppression with mycophenolate mofetil (MMF) [18].

A further refinement of graft manipulation relies on the negative selection of T lymphocytes carrying the α- and β-chains of the TCR and of B lymphocytes while retaining $\gamma\delta^+$-T-cells, NK cells, and other immune cells in the graft (Fig. 13.1). $\gamma\delta^+$-T-cells (also termed "innate-like" T-cells or "transitional" T-cells) belong to the adaptive arm of the immune system [57]. These cells are capable of recognizing their targets in an MHC-independent manner through activating receptors (among others, γδ-TCR, NKG2D, TLRs, DNAM-1) and display a pre-activated phenotype which allows rapid cytokine production (IFN-γ, TNF-α) and strong cytotoxic response upon activation. The fact that MHC molecules do not restrict $\gamma\delta^+$-T-cells, in contrast to $\alpha\beta^+$-T-cells, makes them unlikely to elicit G*v*HD, based on HLA alloreactivity. A number of preclinical and clinical observations point to their potentially beneficial role in cancer medicine. In fact, $\gamma\delta^+$-T-cell functions are heterogeneous, ranging from protection against intra- and extracellular pathogens to tumor cell killing, immune response modulation, and maintenance of tissue homeostasis [58–60]. Noteworthy, similar to NK cells, both the spontaneous and cytokine-induced antitumor activities of $\gamma\delta^+$-T-cells appear more prominent against hematologic cancers than against other types of malignancy [61].

In haploidentical graft manipulation, the selective removal of $\alpha\beta^+$-T lymphocyte offers the advantage of providing functional $\gamma\delta^+$-T-cells, boosting their potent antileukemic activity with that of alloreactive mature NK cells, thus lowering the risk of rapid leukemia relapse occurring in cases of partial responses to the conditioning regimen and/or in rapidly proliferating leukemias. The subsequent generation of alloreactive NK cells from grafted product assures a late and more durable protection [62].

Clinical results in pediatric patients with nonmalignant disorders who received haplo-HCT using this new method of partial TCD are promising, as the reported G*v*HD incidence is low without additional posttransplantation immune suppression, while immune reconstitution is improved, mainly by virtue of a surge in $\gamma\delta^+$-T-cells during the early posttransplantation period, which helps prevent life-threatening infections [63]. Most recently, the importance of early recovery of $\gamma\delta^+$-T-cells was

further demonstrated in 27 children with either malignant or nonmalignant disorders, illustrating that Vδ1 cells are specifically expanded in patients experiencing CMV reactivation and exhibit greater cytotoxic activity compared with those of children who did not experience reactivation. Additionally, Vδ2 cells that were expanded in vitro after exposure to zoledronic acid (ZOL), which lyses primary lymphoid and myeloid blasts, were found to exert potent G*v*L activity, which strongly indicates that treatment with ZOL could additionally enhance the G*v*L reactivity of donor Vδ after transplantation [64].

The Tübingen group recently published the retrospective analysis of immune recovery in a cohort of 41 pediatric patients, with acute leukemias, myelodysplastic syndrome (MDS), and nonmalignant diseases (n = 5), who received αβ-T- and B-cell-depleted allografts from haploidentical family donors. Conditioning regimens consisted of fludarabine or clofarabine, thiotepa, melphalan, and serotherapy with OKT3 or ATG-Fresenius. The patients received a median number of 14.9×10^6 $CD34^+$ progenitor cells/kg of recipient weight. The median number of residual αβ-T-cells was 16.9×10^3 cells/kg of recipient weight. As far as clinical outcome is concerned, primary engraftment occurred in 88%, and acute G*v*HD grades II and III–IV occurred in 10% and 15%, respectively. With a median follow-up of 1.6 years, 21 of the 41 patients were alive. Relapse was the major cause of death (n = 17). Patients with leukemia and MDS who received first haplo-HCT in CR1–CR3 showed a favorable 1-year event-free survival of 100%. However, the outcome of children not in CR remained dismal, with no survivors among patients with active disease [65].

A Russian transplant group analyzed the outcome of children with high-risk AML, who received transplantation from unrelated (n = 20) and haploidentical donors (n = 13) after $\alpha\beta^+$-TCR and $CD19^+$ depletion approach. The preparative regimen included treosulfan, melphalan, fludarabine, and antithymocyte globulin. In the haplo-HCT group, graft contained a median of 7.8×10^6/kg of $CD34^+$ and 35.8×10^3/kg of recipient weight of αβ-T-cells. Posttransplantation immune suppression included tacrolimus until day +30 and methotrexate (MTX) in 21 patients, tacrolimus in 5, MTX in 2, and no prophylaxis in 5 patients. Primary engraftment was achieved in all 33 patients. Cumulative incidence of acute G*v*HD grade II–III was 39 % (95% CI, 26–60). Cumulative incidence of chronic G*v*HD was 30% (95% CI, 18–50%), but it must be underlined that seven of ten patients who developed chronic G*v*HD previously received donor lymphocyte infusions. Although comparison of haploidentical and unrelated transplantation was not the primary goal of this study, data suggested that the engraftment rate, G*v*HD incidence, and viral reactivation rate did not differ between these groups. Of note, recipients of haploidentical grafts more commonly developed isolated skin G*v*HD, whereas gastrointestinal involvement was more common in HLA-MUD HCT. The cumulative incidence of relapse and TRM at 2 years was 31% (95% CI, 18–51) and 10% (95% CI, 4–26), respectively, in the entire cohort. Within subgroups, the cumulative incidence of relapse was 25% (95% CI, 11–53) in HLA-MUD and 40% (95% CI, 20–80) in haplo-HCT, whereas TRM was 17% (95% 7–41) and 0%, respectively. At 2 years, EFS and OS in subgroups were as follows: EFS MUD 60% (95% CI, 38–81) and haplo-HCT

59% (95% CI, 31–87) and OS HLA-MUD 65% (95% CI, 44–85) and haplo-HCT 73% (95% CI, 45–100) [66].

Another approach of selective TCD is based on removal of $CD45RA^+$ *naive* T lymphocytes while retaining functional $CD45RO^+$ memory T-cells (see also Fig. 13.1). The rationale for this strategy is based on experimental data demonstrating that mouse CD4 memory T-cells, as well as effector memory CD8 T-cells, are devoid of GvH reactivity [67]. A recent study presented results from 17 patients with poor-prognosis hematologic malignancies, who received haploidentical donor transplantation with $CD45RA^+$-depleted progenitor cell grafts following a novel RIC regimen without TBI or serotherapy. Significant depletion of $CD45RA^+$ T-cells and B- cells, with preservation of abundant memory T-cells, was achieved in all 17 products. Neutrophil engraftment was observed on median day +10 and full donor chimerism on median day +11 post-HCT. There was no infection-related mortality, and no patient developed acute G*v*HD despite infusion of a median of $>100 \times 10^6$ haploidentical T-cells. However, dosages of $CD45RA^+$-depleted cells varied greatly between patients, and 6/17 developed symptoms of chronic G*v*HD [68]. This finding may be explained by the fact that the $CD45RA^+$-depleted fraction contained both T-effector-memory cells and T-central-memory cells, without regard of preclinical data indicating that the latter subset is capable of inducing significant, albeit somewhat reduced, G*v*HD [69].

13.6 Unmanipulated Haploidentical HCT in Children

As already mentioned, literature has been largely silent on the use of unmanipulated haplo-HCT in children, while this strategy has been extensively investigated in adult patients. However, first reports of the experience with the two major unmanipulated haplo-HCT approaches in the pediatric population have been recently published [21–23].

The first approach, pioneered by the Johns Hopkins group, relies on the use of posttransplantation cyclophosphamide (PTCy) [70]. This method takes advantage of the early proliferation of both donor and recipient alloreactive cells that occurs in the first few days after transplantation. PTCy is given in the window of 72 h after unmanipulated HCT, causing *in vivo* depletion of both donor and recipient alloreactive cells, which promotes engraftment and decreases G*v*HD, while quiescent non-alloreactive T-cells are spared. Moreover, $CD34^+$ cells are protected from the cytotoxic effects of PTCy due to higher amounts of aldehyde dehydrogenase [71, 72]. Early studies, carried out in adults after nonmyeloablative (NMA) preparative regimen and using BM as the source of graft, showed 90% engraftment with very low incidence of both acute and chronic G*v*HD [73]. Subsequent studies on PTCy-based haplo-HCT employing myeloablative conditioning reported better DFS with no significant increase in G*v*HD or NRM [74, 75]. The use of PB graft source instead of BM showed similar outcomes in terms of engraftment and NRM with some increase in acute G*v*HD incidence [76, 77]. Overall, these studies have established PTCy-based haplo-HCT as a frontrunner when it comes to alternate donor

HCT in adults, to the extent that many argue in favor of PTCy-based haplo-HCT over HLA-MUD or UCB HCT [11, 78].

Despite these impressive results in adults, the experience with this approach in the pediatric setting is extremely limited. One study from Japan employed a modified PTCy-based approach on day +3 alone and G*v*HD prophylaxis with steroids and tacrolimus in 15 children, 9 of whom had advanced leukemias [79]. They reported a higher incidence of graft failure probably promoted by the low-intensity conditioning regimen used. Although 46% of the patients achieved CR, the long-term outcome remained poor with 11 of 15 patients experiencing disease progression/relapse and 2 of 15 suffering from fatal treatment-related complications. Recently, Jaiswal and colleagues reported the result of a pilot study with PTCY-based haploidentical PB transplantation in 20 children with advanced leukemias, 13 with refractory or relapsed AML and 7 with high-risk ALL in CR1 [22]. A MAC with fludarabine, Bu, and melphalan (Mel) was employed, and G*v*HD prophylaxis consisted of MMF for 14–21 days and cyclosporine (CsA) for 60 days with further 2 weeks of tapering. Rapid engraftment occurred in all patients, with 35% experiencing grade II–IV acute G*v*HD and 5% having mild chronic G*v*HD. The NRM was 20% at 1 year, and this was associated with grade III–IV G*v*HD. Noteworthy, severe G*v*HD occurred exclusively in children below the age of 10 years. This age group also experienced higher incidence of early alloreactivity in the form of hemophagocytic syndrome. This finding somehow contradicts the prevailing concept that G*v*HD occurs with increasing age rather than the other way around. The relative content of $CD34^+$ and $CD3^+$ cells was similar in both younger and older children; authors hypothesized that the possible cause of intense early alloreactivity in patients under the age of 10 years could be related to the reduced efficacy of PTCy (as result of the variable metabolism of the drug in this age group) in clearing alloreactive T-cells [22]. In the same year, an Italian retrospective multicenter study reported the outcome of 33 pediatric patients with high-risk hematologic malignancies treated with PTCy-based haplo-HCT after a NMA ($n = 19$) or a MAC ($n = 14$) regimen. Besides PTCy, G*v*HD prophylaxis consisted of MFF plus tacrolimus or cyclosporine A (CsA). All patients, except one, had an autologous recovery, engrafted. Grades II–IV and III–IV acute G*v*HD and chronic G*v*HD developed in 22% (95% CI, 11–42), 3% (95% CI, 0–21), and 4% (95% CI, 0–27) of cases, respectively. The 1-year OS rate was 72% (95% CI, 56–88), progression-free survival (PFS) rate was 61% (95% CI, 43–80), cumulative incidence of relapse was 24% (95% CI, 1–44), and TRM was 9% (95% CI, 3–6). In the univariate analysis on risk of relapse incidence, mother as donor ($P = 0.02$), female donor gender ($P = 0.04$), and female patient gender ($P = 0.02$) were significantly associated with a lower risk of relapse. Disease progression was the main cause of death. In this small cohort, particular benefit was shown for patients with advanced lymphoma transplanted with active disease. Indeed, although none of the five patients affected by lymphoma (three Hodgkin and two non-Hodgkin lymphoma) were in CR at time of transplantation, the 1-year OS rate of this subgroup was 80% [21].

The other strategy pioneered by the Peking University group, combined MAC, T-cell modulation with G-CSF-primed marrow and PB grafts, ATG and intensive

multi-agent G*v*HD prophylaxis. Outcome data on 1210 transplants performed in adults and children with mostly ALL and AML showed an impressive DFS of 67% and a NRM of 17%. The 100-day cumulative incidences of grade II and grade III acute G*v*HD were 40% (95% CI, 37%–42%) and 12% (95% CI, 10%–14%), respectively. The 3-year cumulative incidences of total and extensive chronic G*v*HD were 50% (95% CI, 47%–53%) and 21% (95% CI, 19%–24%). The RI was only 17% [80].

The same group reported on the outcome of 212 children with a median age of 15 years (range 3–18 years) with AML or ALL. Conditioning regimen consisted of cytarabine, Bu, Cy, and semustine. Rabbit ATG was administered on days −5 to −2. All children received CsA, MMF, and short-term MTX for G*v*HD prophylaxis. The authors reported 100% engraftment with a NRM of 15% in those transplanted in CR1 and CR2 but 25–40% in those beyond CR2. The cumulative incidences of grade III–IV acute G*v*HD and extensive chronic G*v*HD were 14.3% and 26.6%, respectively. The 5-year LFS in CR1, CR2, and beyond CR2 or non-remission were 68.9%, 56.6%, and 22.2% for ALL patients and 82.5%, 59.4%, and 42.9% for AML. The RI was 7.2% and 19% in CR1 for AML and ALL, respectively, but it was two- to fourfold higher beyond CR1 [23]. Although these results compare favorably with TCD approaches reported thus far, it must be emphasized that this strategy carries a higher incidence of both acute and, especially, of chronic G*v*HD, a complication which can have a particularly detrimental impact on children.

13.7 Future Strategies to Prevent Relapse

With significant improvement in NRM, disease relapse has become the most important cause of treatment failure in patients with malignancies undergoing haplo-HCT, similar to what is observed in HLA-matched donor HCT (see Chap. 19). In particular, the outcome of haplo-HCT in children with leukemia not in complete remission or beyond CR2 has been uniformly dismal [18, 27]. For this reason, the main challenge in the future of haplo-HCT and all other types of allogeneic HCT for pediatric hematologic malignancies is to explore novel strategies aimed at further boosting the G*v*L effect, without concomitantly increasing the incidence of G*v*HD or TRM. In this perspective, the recent burst of immunotherapy treatments for cancer, and specifically for hematologic malignancies, highlighted the potential role of haplo-HCT as a prelude to cell therapy [81]. Haplo-HCT, in fact, offers immediate availability of the same donor to collect or generate additional cells, such as T-cells or NK cells, theoretically not subjected to rejection and capable of enhancing the antitumor effects of the graft.

The adoptive transfer of unstimulated and *ex vivo* cytokine-activated NK cells in pediatric patients has been explored in the haplo-HCT context [82–84]. From these initial studies, it may be concluded that infusion of purified unstimulated NK cells either immediately after collection or after *ex vivo* activation with cytokines (e.g., IL-2 or IL-15) is feasible and safe (see Chaps. 3 and 4). Results on circulating $\gamma\delta^+$-T-cell reconstitution after $\alpha\beta$+-TCD haplo-HCT paved the way to novel approaches for patients affected by acute leukemia, based on *in vivo* administration of $\gamma\delta^+$-T-cell-stimulating compounds, such as aminobiphosphonates or synthetic phosphoantigens. Nonetheless,

although some encouraging responses have been reported, the actual clinical benefits and antileukemic efficacy of these approaches await further evaluation [64, 85].

An intriguing approach to accelerate the recovery of adaptive immunity and to promote antitumor activity relies on the use of suicide gene-modified T-cells. The administration of donor T-cells with a "safety switch" can help prevent relapse when administered earlier after transplantation, with minimal risk for G*v*HD, thanks to the possibility of triggering cell apoptosis in case of severe alloreactions (see Chap. 19).

The first approach of this kind was based on the insertion of the herpes simplex thymidine kinase suicide gene into T-cells (TK cells) to achieve *in vivo* susceptibility to ganciclovir. A phase I/II multicenter trial (TK007) in the adult TCD haplo-HCT setting showed that posttransplantation infusion of the modified T-cells enabled regulation of G*v*HD while promoting immune reconstitution [86]. Preliminary results of a multicenter randomized phase 3 clinical trial (the TK008 study) to assess the efficacy of TK^+ cells in the context of TCD haplo-HCT for leukemia confirmed safety and benefit, as manifested by improved survival, rapid immune reconstitution, and prevention of G*v*HD by suicide gene induction [87]. The Baylor group developed an alternative strategy by using T-cells engineered to express caspase 9 (iC9-T-cells), which can be induced by using a dimerizing agent, AP1903. These iC9-T-cells provided rapid immune recovery in ten pediatric patients (aged 3–17) who received haplo-HCT after TCD of the graft. In five patients who developed G*v*HD, iC9-T-cells were eliminated within 2 h after AP1903 administration, and G*v*HD rapidly resolved without a significant effect on antiviral immune reconstitution [88]. A clinical trial using this approach after $\alpha\beta^+$-TCD haplo-HCT in children with both nonmalignant and malignant disorders is ongoing.

Although engineering donor lymphocytes to express suicide genes has a security system against the development of severe G*v*HD, it provides a nontargeted yet broad antitumor effect. Advances in cell culture and manipulation technology have resulted in the ability to expand clinically relevant numbers of engineered T-cells that express chimeric antigen receptors (CARs), which can redirect T-cells to recognize a selected tumor antigen [89]. Autologous T-cells modified with CD19-targeted CAR T-cell constructs consistently demonstrated high antitumor efficacy in children with relapsed BCP-ALL when infused both before and after allogeneic HCT [81, 90]. Recently, the administration of donor-derived CD19-specific CAR T-cells early after haplo-HCT as adjuvant therapy to prevent disease relapse was proven to be safe and showed promising results in adult patients with advanced $CD19^+$ non-Hodgkin lymphoma and ALL [91]; it is reasonable to speculate that a similar approach will be applied in the future also in the pediatric setting.

Conclusions

Available data suggest that T-cell-depleted haplo-HCT is a suitable option to treat children with hematologic malignancies in the absence of an HLA-identical donor. Moreover, the current excellent results obtained with TCD haplo-HCT could challenge the current hierarchical algorithm, in which HLA-MUD and UCB are preferred to haploidentical donors. This is particularly true for children with leukemia receiving a $CD3^+/CD19^+$ TCD haplo-HCT while in complete

remission, for whom 5-year OS as high as 88% has been reported. By contrast, results of TCD approaches have been uniformly discouraging, even with $CD3^+$/$CD19^+$ negative selection, in patients who were not in CR at the time of transplantation. In this subgroup of patients, newer methods of graft manipulation, such as those based on depletion of $\alpha\beta^+$-T-cells, coupled with adoptive immunotherapy, might pave the way to greater successes and represent an exciting area of research. Alternatively, an unmanipulated haploidentical graft could represent an option in patients with active/resistant disease. However, the unmanipulated approaches employed so far have limitations, since preliminary data show unacceptable control of alloreactivity with PTCy in patients under the age of 10 years, and the Chinese experience with G-CSF-primed grafts and intensive posttransplantation immunosuppression carries a high incidence of chronic G*v*HD. To date, no prospective randomized trial comparing different strategies, cell doses, or graft manipulation methods exists, and therefore, common standards for haplo-HCT in pediatric hematologic disorders are still lacking. Despite that, it would not be unwise to postulate that, in the near future, haplo-HCT might become the preferred alternative option for children without an HLA-identical sibling. In this perspective, besides the development of further refinements to currently employed approaches, another priority in the field over the next few years should be the comparison of haplo-HCT with alternative donor sources, such as UCB and HLA-MUD, and even with matched sibling HCT.

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Part IV

Disease-Specific Adult Population

Haloidentical Transplants: Nonmalignant Diseases in Adults

14

Javier Bolaños Meade

14.1 Introduction

Haploidentical transplantation is becoming a common practice in recent times particularly after the introduction of post-transplant cyclophosphamide by the Johns Hopkins group [1, 2]. In the past, the excessive morbidity and mortality caused by the HLA-mismatched transplant made this intervention very high risk for treatment-related mortality, but nowadays it appears that the outcomes are similar to those of HLA-matched transplants, i.e., HLA-matched, related, or unrelated [3–5]. In fact, they appear to compare well with other alternative donors such as cord transplants [6]. As it can be seen on Table 14.1, there are many potential advantages in using haploidentical donors as alternative donors when needed. Therefore, given that the toxicity seen in haploidentical transplants is not different compared with other types of allogeneic transplant approaches, its use in hereditary nonmalignant disorders seems logical for further exploration. Here, the results of such approach in patients with hereditary nonmalignant disorders will be reviewed.

14.2 Sickle Cell Disease: Is Haploidentical Transplant an Option?

Sickle cell disease (SCD) is a great example on how haploidentical transplantation (haplo-HCT) has evolved to offer a cure to patients affected by this condition. This condition kills nearly half a million people annually. In 2010, there were more than 300,000 newborns with SCD [7]. For adults with SCD, the average annual cost of

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Table 14.1 Potential advantages of haploidentical donors as alternative donors

Almost every patient has a donor [16]
Given that the donor is a close relative, usually these are willing to proceed with donation
Transplant preparation is speedy, usually faster than with unrelated donors
Is possible to proceed with donor lymphocyte infusions as opposed to cord blood grafts [42]
Possible to select younger donors avoiding donors with clonal hematopoiesis due to advance age [41]
No restriction to one type of graft, both bone marrow and peripheral blood grafts can be obtained as opposed to cord blood graft
While there are harvesting expenses, no storage fees
Outcomes appear to be similar when compared to HLA-matched donors [3–5]

medical care exceeds 35,000.00 US dollars per year [8]. Most adults and many children develop a chronic debilitating condition, with over 30% of adults on disability and over 50% of patients unemployed [9]. Median survival is shortened by more than two decades, and quality of life (QoL) is severely impacted due to complications of chronic pain, narcotic dependence, stroke, renal failure, thrombosis, pulmonary hypertension, blindness, priapism, and infection.

In 1984, Johnson and coworkers reported a successful bone marrow transplant (BMT) of a child with leukemia and SCD who was cured of both disorders [10]. This was followed by several reports of myeloablative allogeneic BMT from HLA-matched sibling donors (MSDs) for children with SCD [11, 12]. These data established that SCD is a potentially curative disease following myeloablative allogeneic BMT from a healthy HLA-MSD. Unfortunately, BMT is only widely available in developed countries. Even in these countries, there are numerous obstacles such as donor availability, transplant-related morbidity and mortality, and engraftment difficulty in patients with SCD that limit the availability of BMT to only a small percentage of patients [13, 14]. In fact, in a recent study from the National Institutes of Health (NIH), only close to 10% of patients were able to proceed to BMT due to donor availability [14]. While curative, BMT is seldom used for these patients due to perceived toxicity and lack of suitable donors. As of 2013, there were 1238 BMT for SCD reported to the CIBMTR and EBMT-Eurocord [15]; these numbers seem minuscule when compared to the number of transplants performed for other indications, such as acute myeloid leukemia.

In the USA, there is no consensus about the indications for BMT in SCD, but many centers accept stroke as a reason to proceed to BMT. Others such as recurrent acute chest syndrome, frequent pain crises, red cell alloimmunization, and osteonecrosis are also considered by other researchers [14, 16]. The majority of the published series report on highly symptomatic SCD with advanced disease. Most pediatric hematologists agree that stroke or silent cerebral infarction is an absolute indication for BMT in children with SCD, especially given the recent data showing that red cell exchange transfusions are not as effective as previously thought in preventing secondary vascular events [17]. Recurrent acute chest syndrome and frequent vaso-occlusive crisis (VOC) despite hydroxyurea with good compliance are also considered to be good indications for BMT in children. Others feel that all

symptomatic children with SCD be transplanted as soon as possible if they have an HLA-MSD. In adult patients, common indications have included cerebrovascular disease, recurrent VOC despite hydroxyurea, osteonecrosis, red cell alloimmunization, and recurrent acute chest syndrome [14, 16]. While pulmonary hypertension is a known cause for morbidity and mortality in these patients, there is no agreement on whether it should be used as an indication to proceed to transplant, and at least in one study, these patients were excluded [16]. While BMT can be toxic therapy, it is clear that the morbidity associated with SCD is such that transplantation can be justified in a select group of patients.

It is important to mention that despite the perceived toxicities of BMT, patients are willing to consider transplantation given the severe complications of SCD. Chakrabarti and Bareford surveyed 30 adult patients with SCD about their feelings toward receiving a reduced intensity conditioning (RIC) BMT [18]. Sixty-two percent were willing to accept a 10% transplant-related mortality (TRM) and a third of patients even a 30% TRM. Most patients, 62%, were willing to accept a 10% risk of graft failure; 50% were willing to accept infertility, but only 20% considered chronic graft-*versus*-host disease (cG*v*HD) acceptable. In fact, 60% of those surveyed would consider joining a clinical trial of RIC BMT. These authors conclude that SCD patients are willing to consider the option of BMT despite the morbidity and mortality associated with the procedure. This is of course in adults; in children the decision is more complicated as parents are accepting, or not, the toxicities of BMT on behalf of something else.

Historically, myeloablative conditioning (MAC) regimens have been used to condition SCD patients for BMT with HLA-matched donors [12, 19–21]. Alternative donors represented by cord blood (CB) cells have been used too [22]. These studies demonstrate that BMT or CB from HLA-MSD following an MAC regimen in children with SCD is highly successful. Overall survival (OS) is expected to be over 90% with cure rates over 80% [19]. Given these results, HLA-MSD BMT after MAC should be considered the standard of care for children and young adults with severe SCD.

Nonmyeloablative (NMA) conditioning regimens have several theoretical advantages over MAC regimens in patients with SCD. First, patients with SCD, especially adults, often have significant end-organ damage (renal, pulmonary, liver, etc.), and such approach is less toxic compared to MAC BMT; thus, children and adults with mild to moderate end-organ toxicity would still be eligible for BMT. Second, most NMA regimens do not lead to gonadal failure. Lastly, acute toxicity with NMA conditioning regimens tends to be less. A potential drawback of NMA conditioning is higher rate of graft failure (GF) and mixed chimerism with subsequent GF. Jacobsohn and coworkers studied 13 pediatric patients with nonmalignant disorders who underwent a RIC BMT from an HLA-MSD [23]. Three out of four patients with hemoglobinopathies rejected the graft. These findings have been duplicated in other small studies [24, 25].

In 2009, Hsieh and coworkers has published the first highly successful series of NMA BMT from HLA-MSD in adults with SCD [14]. Twenty-three patients were initially reported, with ages ranging from 17 to 64 years. All are alive at 2 months to

7 years after BMT. The graft consisted of unmanipulated G-CSF-mobilized peripheral blood (PB) progenitor cells obtained from eight of eight (HLA-matched for A, B, C, and DRB1) MSDs. Three patients engrafted temporarily but lost their grafts between the second and third months post-transplant and had recurrent SCD. Twenty patients engrafted with mean myeloid chimerism of 97.5% (median 89%) and $CD3^+$ chimerism of 42% (median 49%). In 17 patients at 1 year or more post-transplant, five had $CD3^+$ chimerism >50% which allowed complete withdrawal of immunosuppression, and they have maintained stable mixed chimerism. No engrafted patient to date has developed any evidence of acute or chronic graft-*versus*-host disease (G*v*HD). This study is of great relevance because it suggested that NMA conditioning followed by HLA-MSD BMT can lead to high-level engraftment and alleviate signs and symptoms of SCD. A drawback of this approach is that only 10% of patients screened were able to proceed to BMT due to lack of a suitable HLA-MSD. A recent update from this group shows continued success with this approach [26]. The importance of this confirmatory trial is that it demonstrates that adult patients with severe SCD should be considered for BMT and that NMA transplants are possible.

While BMT is an effective therapy to cure SCD, the large majority of patients will lack an HLA-MSD. As mentioned, Hsieh and coworkers reported that out of 112 patients referred to their study, they were able to find HLA-matched potential donors for only 24, and of these, 4 were excluded for ABO incompatibility [14]. Therefore, it is clear that donors other than HLA-matched siblings are needed if BMT is to be more widely used to treat SCD. Unrelated BMT are seldom performed for SCD [27]. This is because the majority of patients with SCD in the USA are of African ancestry, and less than 20% of African-Americans can find HLA-MUDs in the registry. As of now, the published data using unrelated donors is very limited. Kharbanda and coworkers, for example, recently published on two children with SCD undergoing an unrelated BMT, both died [27]. However, the Blood and Marrow Transplant Clinical Trials Network has a study open (BMT CTN 0601) addressing this issue (clinicaltrials.gov NCT00745420). This study is exploring the use of unrelated donors on patients receiving a nNMA conditioning. This study is of great importance given the lack of data on BMT for SCD using unrelated donors, and the results are eagerly expected.

The use of cord blood (CB) as graft source has the potential to expand the donor pool; however, to date, results of cord blood transplant (CBT) in SCD have been disappointing.

Adamkiewicz and coworkers reported on seven children with SCD and stroke (HLA-matched four of six $n = 5$; five of six $n = 2$) [28]. Four patients received MAC regimens. One had primary graft failure (PGF), three engrafted, two with grade III–IV G*v*HD, and one with stable mixed chimerism. Three patients treated with RIC regimens failed to engraft. Ruggeri and coworkers published the results from registry data of 16 children receiving CBT for SCD OS and disease-free survival (DFS) were 94% and 50%, respectively. High PGF rates remained the main cause of treatment failure, occurring in seven children. They found that the total nucleated cell (TNC) dose correlated with outcome and that only cord units with a TNC dose

of >5 × 10^7/kg of recipient weight should be considered for CBT for patients with SCD. Recently the results of the umbilical cord arm of BMT CTN 0601 were reported [29]. Eight children with severe SCD underwent unrelated donor CBT following alemtuzumab, fludarabine, and melphalan combination regimen. Cyclosporine or tacrolimus and mycophenolate mofetil were administered for G*v*HD prophylaxis. The median recipient age was 13.7 years. The median precryopreservation TNC dose was 6.4 × 10^7/kg of recipient weight, and the median post-thaw-infused $CD34^+$ cell dose was 1.5 × 10^5/kg of recipient weight. All patients achieved neutrophil recovery. Three patients who were engrafted had 100% donor cells by day 100, which was sustained, and five patients had autologous hematopoietic recovery. Two patients developed grade II acute G*v*HD (aG*v*HD). Of these, one developed extensive cG*v*HD and died of respiratory failure. With a median follow-up of 1.8 years, seven patients are alive, and three of eight are alive without GF or disease recurrence. Based upon the high incidence of graft rejection after unrelated CBT, enrollment on this arm was closed and continues on the unrelated donor arm. As a consequence of such dismal results, CBT for patients with SCD is only recommended in the setting of clinical trials and should not be considered standard for these patients.

The use of HLA-haploidentical donors has enormous potential to expand the donor pool since parents and children are almost guaranteed to share at least one haplotype. Moreover 50% of full-siblings and 50% of half-siblings will share one haplotype. Historically, results of HLA-haploidentical BMT in patients with hematologic malignancies were associated with TRM in excess of 50% [30, 31]. However, the use of post-transplantation, cyclophosphamide (PTCy) for G*v*HD prophylaxis, has markedly improved the safety of HLA-haploidentical BMT (see Chap. 7) [1, 16, 32]. In fact, this approach has been used in both malignant and nonmalignant disorders.

Our group at Johns Hopkins published their initial results using haploidentical donors [16]. The regimen consists of antithymocyte globulin, fludarabine, cyclophosphamide, and total body irradiation, with G*v*HD prophylaxis with post-transplant cyclophosphamide, mycophenolate mofetil, and tacrolimus or sirolimus. We transplanted 17 out of 19 referred for BMT, demonstrating that the use of HLA-haploidentical donors markedly improves the donor pool for patients with SCD and makes BMT more widely available. Of the 17 patients with SCD, 14 received transplants from HLA-haploidentical donors and 3 from HLA-matched related donors. The median age was 30, with a range of 15–46 years of age. Eleven patients were engrafted including six patients that achieved full donor chimerism (all haploidentical) with the rest being mixed chimeras. At the time of the report, the median follow-up was 711 days (minimal follow-up of 224 days), ten patients were asymptomatic, and six patients were off immunosuppression. There was no mortality and no G*v*HD requiring treatment. The main problem was that 43% of the haploidentical transplant patients experienced GF. Other groups worldwide have adopted this approach, but their results have not been fully published at this time, and the BMT CTN is launching a multi-institutional study for patients with SCD undergoing haploidentical BMT. Preliminary results from the UK presented by De

La Fuente and coworkers on 13 children with SCD and 3 with beta-thalassemia show very high engraftment rates with low toxicities utilizing post-transplantation cyclophosphamide [33]. They utilized, however, a more intensive regimen than the one from Johns Hopkins.

HLA-haploidentical BMT is becoming widely used for other indications due to its proven efficacy and low toxicity. In SCD, the approach clearly expands the number of potential donors. Enrollment in clinical trials exploring this option is encouraged.

14.3 Immunodeficiencies and Haploidentical Transplantation

Other conditions in which there are substantial data on the use of haploidentical BMT are severe combined immunodeficiency (SCID) and other immunodeficiencies (see Chap. 12) [34–37]. Initial results were discouraging. Tsuji and coworkers retrospectively analyzed their results of 30 patients with three distinctive primary immunodeficiency diseases such as SCID (11 patients), Wiskott-Aldrich syndrome (11 patients), and X-linked hyper-immunoglobulin M syndrome (8 patients) who underwent hematopoietic BMT [37]. Until 1995, all donors were haploidentical relatives with T-cell depletion (TCD; eight patients). Since 1996, the donors were HLA-matched related, matched unrelated, and unrelated cord. Although one of the eight patients transplanted with TCD is alive with full engraftment, the other seven died. However, 18 of 22 patients transplanted without TCD are alive and well, including 6 of 8 transplanted from HLA-matched related donors, 7 of 7 from unrelated donors, and 5 of 7 from unrelated cords. The outcome of haploidentical transplants in this study is dismal with OS close to 25%, mainly due to infections.

Pai and coworkers collected data retrospectively from 240 children with SCID who had received transplants at 25 centers during a 10-year period culminating in 2009 [34]. Transplants were well tolerated with low incidence of complications. Survival at 5 years, freedom from immunoglobulin substitution, and $CD3^+$ T-cell and IgA recovery were more likely among recipients of grafts from HLA-MSD than among recipients of grafts from alternative donors. However, the survival rate was high regardless of donor type among infants who received transplants at 3.5 months of age or younger (94%) and among older infants without prior infection (90%) or with infection that had resolved (82%). Among actively infected infants without a HLA-MSD, survival was best among recipients of haploidenticalTCD transplants in the absence of any pretransplantation conditioning. Among survivors, reduced-intensity or myeloablative pretransplantation conditioning was associated with an increased likelihood of a $CD3^+$ T-cell count of more than 1000 per cubic millimeter, freedom from immunoglobulin substitution, and IgA recovery but did not significantly affect $CD4^+$ T-cell recovery or recovery of phytohemagglutinin-induced T-cell proliferation. The genetic subtype of SCID affected the quality of $CD3^+$ T-cell recovery but not survival. While the data is retrospective and includes a variety of

grafts and conditioning regimens (or none), it is very clear that children with SCID can benefit from haploidentical transplants (see Chap. 12).

14.4 Haploidentical Transplants Beyond Sickle Cell Disease and Immunodeficiencies

The data on the use of haploidentical donors for benign conditions other than SCD and immunodeficiencies is extremely limited as can be seen in Table 14.2. Our group initially published a successful transplant for two of the three patients with paroxysmal nocturnal hemoglobinuria (PNH), one of them also with SCD [32]. We also have transplanted one patient with systemic lupus erythematosus (SLE) and SCD with substantial improvement on the lupus activity, as part of our SCD trial [16]. We also presented preliminary results on patients with aplastic anemia (AA) [38]. Ten patients have been transplanted on this study. Eight patients received grafts from five of ten related donors. Six had failed ATG-containing regimens previously and two relapsed after PTCy. Two patients had inherited syndromes: one with Diamond-Blackfan anemia who received a graft from 5/10 related donor and one with telomeres less than the first percentile in length with a presumed familial syndrome who received a 9/10 unrelated graft. Median follow-up time was 17.5 months (range 5–49). The median age was 34 years with six patients greater than age 30 years, and 50% were males. The median time to neutrophil engraftment was 18 days. The median time to red cell engraftment was 25 days. The median time to platelet engraftment was 27.5 days. At the time of BMT, six patients had PNH clones, and all were eliminated. All patients are alive and well, fully engrafted with 100% donor chimerism in the blood and marrow. Two patients had grade II skin acute G*v*HD. These two also had cG*v*HD of the skin/mouth requiring systemic steroids; one patient was able to come off all immunosuppression by 15 months and the other by 17 months. Other studies are ongoing elsewhere.

Table 14.2 Results of selected haploidentical BMT studies for different conditions

	SCID [34]	AA [38]	SCD [16]
Number of patients	138	10	17
OS	74%	100%	100%
EFS	N/A	100%	57%
TRM	N/A	0%	0
Graft failure	24% at 5 years	0%	43%
Ages	Infants	Median 34	15–46
Acute G*v*HD	21%	20%	1
Chronic G*v*HD	16%	20%	0
Comments	Retrospective study	Small prospective study	Small prospective study

SCID severe combined immune deficiency, *AA* aplastic anemia, *SCD* sickle cell disease, *OS* overall survival, *EFS* event-free survival, *TRM* transplant-related mortality, *GvHD* graft-*versus*-host disease

Hickstein and coworkers reported at the American Society of Hematology meeting in 2015 on five patients receiving haploidentical transplants for GATA2 deficiency [39]. All patients were engrafted after an MAC and had resolution of their syndrome. The procedure was well tolerated.

Recently, Zecca and coworkers reported the outcome of 12 consecutive pediatric patients with Fanconi anemia (FA) who were given TCD, $CD34^+$positively selected cells from a haploidentical related donor after an RIC, and fludarabine-based conditioning regimen [40]. Engraftment was achieved in 9 of 12 patients, and the cumulative incidence of graft rejection was 17%. Incidences of grades II–IV acute and chronic G*v*HD were low. The conditioning regimen was well tolerated, with no fatal regimen-related toxicity and three cases of grade III regimen-related toxicity. The 5-year OS, event-free survival (EFS), and DFS were 83%, 67%, and 83%, respectively.

14.5 Expert Point of View

BMT is the only potential cure available for SCD, SCID, AA, and other nonmalignant conditions. The cure rate in children with SCD using an HLA-MSD following an MAC regimen is over 85% and in SCID reaches 90%. Unfortunately, most of these patients in need of a BMT are not eligible due to lack of a HLA-MSD. The recent reported success of HLA-haploidentical BMT in patients with SCD, SCID, and AA is encouraging, and promises to greatly improve the potential donor pool for patients with nonmalignant conditions particularly now that the data showing comparable results to matching donors are available. Another advantage of haploidentical BMT in these patients is that it can avoid the use of older donors that may be carrying already clonal hematopoiesis that can translate into therapy-related myeloid neoplasms in the recipient [41].

14.6 Future Directions

Exploring the use of alternative donors, such as haploidentical, unrelated, and CB, is of paramount relevance for the treatment of nonmalignant conditions (clinicaltrials.gov NCT00745420, NCT02224872, NCT02013375, NCT00152113, NCT01461837, NCT00977691, and of course NCT00489281). The use of haploidentical donors is being explored in autoimmune disorders such as SLE. Given that BMT has shown promise in this condition, alternative donors may be studied (clinicaltrials.gov NCT02080195). Patients should be enrolled on these and similar trials. It is very likely that in the near future, with emerging data, haploidentical BMT will become standard therapy offered for the care of patients with nonmalignant conditions.

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Haploidentical Transplants for Acute Myeloid Leukemia in Adults

15

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15.1 Introduction

Acute myeloid leukemia (AML), the most common acute leukemia in adults, accounted for an estimated 20,830 new diagnoses and 10,460 deaths in 2015 in the United States [1]. Although improved in recent years, the overall survival (OS) of adults with AML, particularly in older patients and those with intermediate and high-risk disease or therapy-related AML, continues to be low [1, 2]. Allogeneic hematopoietic cell transplantations (allo-HCTs) are cost-effective compared to chemotherapy alone [3], can improve survival, and may even offer a chance of cure in carefully selected patients with AML [4]. In recent years, therefore, the use of allo-HCT for AML and other hematologic malignancies has increased in the United States and worldwide; the survival of the patients undergoing allo-HCT has also improved as a result of advancement in transplant medicine and supportive care [5–9]. AML currently remains the most common indication for allo-HCT [10].

Timely availability of donors remains one of the major challenges to the success of allo-HCT. A HLA-matched sibling or unrelated donor cannot be identified or mobilized in time for up to 50% of patients with hematologic malignancies [11]. The availability of a HLA-matched unrelated donor is lower among patients from underrepresented minorities such as African Americans [11, 12] (see Chap. 19). In the United States, the median time from donor search to allo-HCT is more than 3 months [13]. Delays in allo-HCT may be associated with disease progression with

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deterioration in clinical status [14–16]. Haploidentical hematopoietic cell transplantation (haplo-HCT) allows eligible first-degree relative to be a donor and permits timely, easy, and cheap access to donor availability. Potential haplo-HCT donors include siblings, biological parents, or children. Each sibling has a 50% probability of sharing an HLA haplotype [11]. At Johns Hopkins, haplo-HCT technique has allowed identification of at least one HLA-haploidentical donor for 95% of patients; the average number of HLA-haploidentical donors per patient is 2.7 [17]. Additionally, being related to the patients, such donors are more likely to be available for second allo-HCT and donor lymphocyte infusion (DLI) if needed for graft failure and relapse in the future. Although earlier attempts at haplo-HCT were associated with higher treatment-related mortality (TRM) due to infections and graft-*versus*-host disease (G*v*HD), newer approaches have lowered the risk of such complications and improved outcomes. In this chapter, we attempt to review the various conditioning regimens used in haploidentical transplant setting, in addition, to review of data comparing HLA-matched transplants to haploidentical transplants in the setting of AML.

15.2 Approaches to Haploidentical Transplants

The use of traditional G*v*HD prophylaxis in haplo-HCT is associated with primary graft failure and hyperacute G*v*HD [18]. The T-cell depletion (TCD) strategies, utilized to reduce the risk of G*v*HD, resulted in an unacceptable rate of graft rejection, delay in post-transplant immune recovery, opportunistic infections [19], and possibly decreased graft-*versus*-leukemia (G*v*L) effect [19–21] (see Chaps. 2, 3, and 4). Current haplo-HCT techniques include the use of post-transplantation cyclophosphamide (PTCy) [22–26], TCD with megadoses of $CD34^+$ cells [27], intensified G*v*HD prophylaxis [28], and selective depletion of T-cells [29, 30]. The TCD requires graft manipulation and is performed only in a few centers, whereas the experience with intensified G*v*HD prophylaxis is limited mainly to China (see Chap. 5). In North America, PTCy has gained popularity because of its ease, cheap cost, and lack of graft manipulation (see Chaps. 7 and 8). Unlike alloreactive T-cell lymphocytes, quiescent memory lymphocytes and hematopoietic stem and progenitor cells express high levels of aldehyde dehydrogenase and are able to inactivate cyclophosphamide [31]. Hence, the use of PTCy is able to reduce alloreactivity, while relatively preserving immune reconstitution. In a retrospective study, the use of PTCy was associated with superior OS and progression-free survival (PFS) compared to conventional TCD strategy [32].

15.3 Conditioning Regimen for Adult Patients with AML Undergoing Haploidentical Transplants

Myeloablative conditioning (MAC) regimens are preferred in young patients with low hematopoietic cell transplantation-comorbidity index (HCT-CI), those with aggressive disease course or active disease at the time of allo-HCT, whereas

reduced-intensity conditioning/nonmyeloablative conditioning regimens are utilized in older adults, those with poor performance status, high HCT-CI, or relapsed disease after a prior autologous or allogeneic hematopoietic cell transplantation [33]. Geriatric assessment can also be valuable in predicting the risk of NRM and suitability for different intensity of conditioning regimens [34]. A phase III Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 0901 trial compared the outcomes by conditioning intensity in 272 patients with AML or myelodysplastic syndrome (MDS) with the following characteristics: age 18–65 years, HCT-CI score ≤4, and <5% marrow myeloblasts at the time of pre-transplant assessment. The use of myeloablative *versus* reduced-intensity conditioning (RIC) regimen resulted in higher NRM (15.8% *vs.* 4.4%, $p = 0.02$), higher grade II–IV acute G*v*HD (44.7% *vs.* 31.6% at 100 days, $p = 0.024$), lower relapse (13.5% *vs.* 48.3%, $p < 0.01$), and better relapse-free survival (67.7% *vs.* 47.3%, $p < 0.01$) and trend toward improved OS (77.4% *vs.* 67.7% at 18 months, $p = 0.07$) [35]. Hence, in AML patients younger than 65 years of age with HCT-CI score ≤4, MAC regimen is preferred in the setting of HLA-matched related or HLA-matched unrelated transplant. Haplo-HCT has been performed with MAC, RIC, and nonmyeloablative (NMA) regimens; however, the optimal conditioning regimen remains unclear. The Hopkins group pioneered the NMA regimen with fludarabine (Flu; 30 mg/m^2 on day −6 to −2), cyclophosphamide (Cy; 14.5 mg/kg day −6, −5), and total body irradiation (TBI; 2 Gy on day −1) (Flu/Cy/TBI) [36]. This regimen has been very well tolerated; however, a perceived higher relapse rate up to 50–55% [37, 38] prompted other transplant groups to explore more intense conditioning regimens, i.e., RIC and MAC [39], such as Flu (25 mg/m^2 on days −6 to −2), busulfan (Bu; 110 mg/m^2 on days −7 to −4), and Cy (14.5 mg/kg on days −3 and −2) (Flu/Bu/Cy) [22, 40]; thiotepa (Thio; 5 mg/kg on days −6 and −5), Bu (3.2 mg/kg intravenous on days −4 to −2), and Flu (50 mg/m^2 on days −4 to −2) (Thio/Bu/Flu) [41]; Flu (30 mg/m^2 on days −5 to −2) and TBI (3.3 Gy on days −8 to −6) (Flu/TBI) [41]; Flu (40 mg/m^2 on days −5 to −2), melphalan (Mel; 100–140 mg/m^2 on day −6), and Thio (5 mg/kg on day −7) (Flu/Mel/Thio) [42]; and Flu (25 mg/m^2 on days −7 to −5) and TBI (150 cGy twice a day on days −4 to −1) (Flu/TBI) [43]. The MAC regimens used to treat patients with AML are listed in Table 15.1. In a Center for International Blood and Marrow Transplant Research (CIBMTR) study discussed below, Bu/Cy (43%) and Flu/Cy/TBI (100%), respectively, were the most commonly used myeloablative and nonmyeloablative conditioning regimens. The Flu/Mel/Thio (22%) was the second most commonly used MAC [26]. A lack of head-to-head comparison and heterogeneous patient population precludes conclusions about the optimal regimen. Institutional preferences and the availability of radiation facility and expertise currently guide selection of conditioning regimen. TBI may have activity against chemoresistant disease and disease in sanctuary "immune privileged" sites. The early results of TBI-based MAC appear promising; however, potential long-term complications such as secondary malignancies, pulmonary toxicities, infertility, and cataract formation [44–47] should also be considered, particularly in patients with long life expectancy.

Table 15.1 Myeloablative conditioning regimens used in HLA-haploidentical transplantation with post-transplantation cyclophosphamide

Nr. of patients[a]	Conditioning regimen[b]	Graft source	Acute GvHD (II–IV)	NRM	Relapse rate	DFS	Reference
20	Flu/Bu/Cy	PB	30%	10% at 1 year	40% at 1 year	50% at 1 year	Solomon et al. [40]
50	Thio/Bu/Flu ($n = 35$) Flu/TBI ($n = 15$)	BM	12%	18% at 6 months	2% at 18 months	51% at 18 months	Raiola et al. [41]
53	Bu/Flu/Cy ($n = 18$) Flu/Cy/TBI ($n = 35$)	PB ($n = 18$) BM ($n = 35$)	30%	7% at 2 years	33% at 2 years	60% at 2 years	Bashey et al. [22]
27 (in CR)	Flu/Mel/Thio	BM	26%	9% at 4 years	24% at 4 years	66% at 4 years	Pingali et al. [42]
30	Flu/TBI	PB	44%	5% at 2 years	19% at 2 years	76% at 2 years	Solomon et al. [43]

The table is adapted from Shabbir-Moosajee and coworkers [39]
BM bone marrow, *Bu* busulfan, *Cy* cyclophosphamide, *DFS* disease-free survival, *Flu* fludarabine, *GvHD* acute graft-*versus*-host disease, *Mel* melphalan, *NRM* non-relapse mortality, *PB* peripheral blood, *CR* complete remission (by morphology), *TBI* total body irradiation, *Thio* thiotepa
[a]These studies included patients with acute myeloid leukemia as well as other myeloid malignancies such as myelodysplastic syndrome and acute lymphocytic leukemia
[b]Details of conditioning regimens are provided in the text

15.4 Outcomes with Haploidentical Transplants

The use of PTCy as GvHD prophylaxis has improved the outcomes of haplo-HCT [22–26]. In recent years, several studies have demonstrated excellent outcomes with such strategy; the results are largely comparable to those of HLA-matched unrelated and related donor HCT in retrospective analysis [22–26]. After a single institution study done at MD Anderson comparing outcomes of AML/MDS with different donors showing virtually identical result between haploidentical and HLA-matched unrelated donor transplants [23], in a larger CIBMTR study, Ciurea and coworkers compared the results of haplo-HCT with PTCy ($n = 192$) and eight out of eight HLA-matched unrelated donor HCT ($n = 1982$) in patients with AML. Haplo-HCT, compared to MAC HLA-matched unrelated donor HCT, resulted in a lower risk of 3-month grade II–IV acute GvHD (16% *vs.* 33%, $p < 0.0001$) and 3-year chronic GvHD (30% *vs.* 53%, $p < 0.0001$) and similar 3-year NRM (14% *vs.* 20%, $p = 0.14$), relapse rate (44% *vs.* 39%, $p = 0.37$), and OS (45% *vs.* 50%, $p = 0.38$) (Fig. 15.1). Haplo-HCT, compared to RIC HLA-matched unrelated donor HCT, resulted in a lower risk of 3-month grade II–IV acute GvHD (19% *vs.* 28%, $p = 0.05$), 3-year chronic GvHD (34% *vs.* 52%, $p = 0.002$), and 3-year NRM (9% *vs.* 23%, $p = 0.0001$), higher 3-year relapse rate (58% *vs.* 42%, $p = 0.006$), and similar 3-year OS (46% *vs.* 44%, $p = 0.71$) [26]. In a nonrandomized multicenter prospective study of AML in

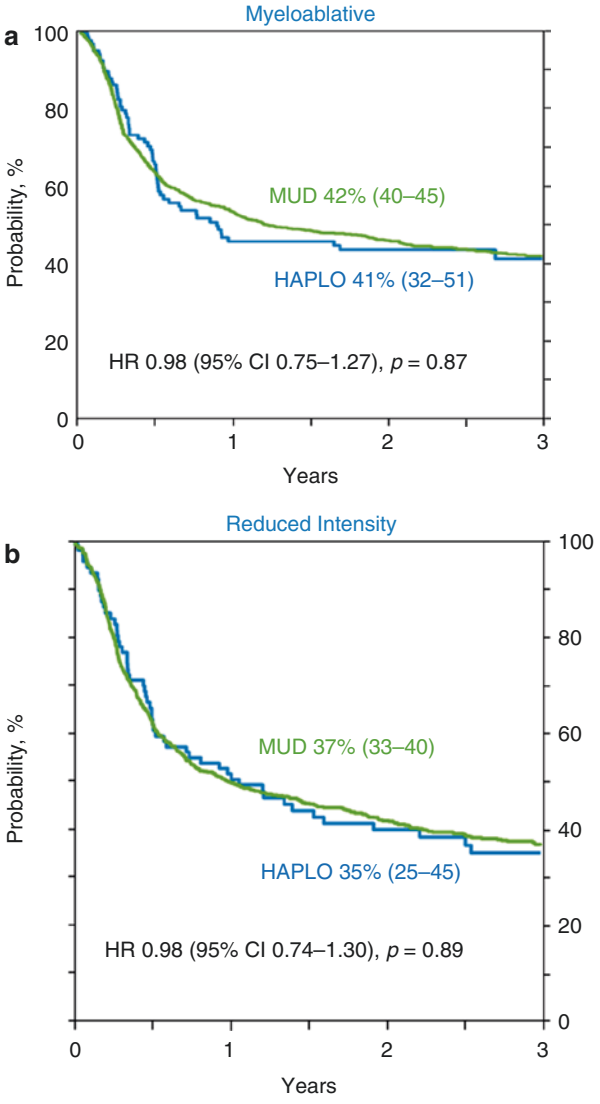

Fig. 15.1 Leukemia-free survival after HLA-haploidentical *versus* HLA-matched unrelated donor transplant using myeloablative (**a**) or reduced-intensity conditioning (**b**) regimens. Outcomes are adjusted for disease risk index, performance score, and secondary acute myeloid leukemia. Reference: Ciurea et al. [26]

remission, Wang and coworkers demonstrated similar 3-year disease-free survival (74% *vs*. 78%, $p = 0.34$), OS (79% *vs.* 82%, $p = 0.36$), relapse risk (15% *vs.* 15%, $p = 0.98$), and NRM (13% *vs.* 8%, $p = 0.13$) with haplo-HCT, compared to HLA-identical sibling HCT. Conditioning regimen was similar in the two groups; patients undergoing haplo-HCT also received antithymocyte globulin (ATG). The G*v*HD

prophylaxis regimen included cyclosporine A (CsA), mycophenolate mofetil (MMF), and short-term methotrexate but not PTCy [48]. In the absence of randomized trials, institutional preferences, urgency of transplant, timely availability of HLA-matched unrelated donors, donors' age, presence of donor-specific antibodies, and other factors may determine the choice between a haploidentical donor and HLA-matched unrelated donor. Older donor age may be associated with lower $CD34^+$ cell dose in the graft [49] and a risk of clonal hematopoiesis [50, 51] and subsequent donor-derived malignancy [52]. Hence, in certain circumstances, younger haploidentical donors may be preferred over older HLA-matched unrelated donors. Feasibility of haplo-HCT for older adults has also been studied [53–55]. Gaballa and coworkers utilized Flu/Mel RIC haplo-HCT in 30 patients aged ≥55 years, mostly with AML. This resulted in rates of grade II–IV acute G*v*HD of 30%, chronic G*v*HD of 10%, PFS and OS of 55%, and NRM of 21% at about 2 years [55]. Kasamon and coworkers at Johns Hopkins demonstrated good outcomes with the use of Flu/Cy/TBI NMA haplo-HCT (see Chap. 7). Older age was not associated with worse outcomes. For example, the NRM at 6 months for patients in their 50s, 60s, and 70s were 8%, 9%, and 7%, respectively ($p = 0.2$) [53]. In a small retrospective study, Blaise and coworkers demonstrated that the results of haplo-HCT in older patients may be better than HLA-matched unrelated donor HCT and similar to HLA-matched related donor allo-HCT [54]. Taken together, these studies indicate that haplo-HCT results in acceptable outcomes in older adults and serves as a good option for alternate donor allo-HCT (see Chap. 8).

15.5 Complications and Post-transplant Relapse

Haplo-HCT is associated with a risk of grade II–IV acute G*v*HD (15–40% at 3 months), chronic G*v*HD (15–35% at 1–3 years), and NRM (10–15% at 1–3 years) [22, 26, 38, 39]. Graft failure is an unacceptable complication; the risk increases with the presence of donor-specific anti-HLA antibodies at high titers and was identified as being more common in the middle-aged females with AML [56, 57] (see Chaps. 9 and 20). Graft failure, G*v*HD, infection, and organ failure can result in morbidity and mortality; however, disease relapse is the most common cause of deaths, particularly in high-risk patients and those undergoing RIC haplo-HCT. In the aforementioned CIBMTR study, MAC haplo-HCT was associated with a relapse rate of 44% at 3 years, where 3-year relapse rate was 58% with RIC haplo-HCT. Disease relapse accounted for three-quarter of all deaths [26].

The use of higher $CD3^+$ cell dose (up to 2×10^8 cells/kg of recipient weight) and early immunosuppression taper in the absence of G*v*HD may possibly reduce the risk of relapse [58]. Post-transplant maintenance strategy (e.g., with low-dose azacitidine [59] or FLT3-ITD inhibitors such as sorafenib and midostaurin) [60, 61], monitoring of minimal residual disease (MRD), preemptive therapy for MRD, or worsening donor chimerism are other emerging techniques to reduce the risk of relapse (see Chap. 19). The DLI with or without chemotherapy can be an effective strategy to prevent and, to some extent, treat post-transplant relapses [62]. However,

DLI can be associated with a risk of marrow aplasia and G*v*HD. Engineered donor lymphocytes with a safety switch (e.g., lymphocytes expressing herpes simplex virus-thymidine kinase suicide gene or inducible caspase-9 transgene) offer a potentially safer strategy, whereby T-cells can be eliminated if G*v*HD emerges. Other post-transplant cellular therapies such as infusion of natural killer (NK) cells or T-cells with chimeric antigen receptors (CARs) are being explored to improve leukemia eradication [19]. In some cases, however, AML relapses after haploidentical HCT may be associated with loss of expression of the HLA-mismatched human leukocyte antigen (HLA) haplotype in leukemic cells [63]. Acquired uniparental disomy of chromosome 6p in leukemic cells leads to loss of the HLA haplotype that differed from the donor's haplotype; consequently, mutant leukemic cells are not recognized by T-cells. Such leukemic relapses do not respond to DLI and require chemotherapy and second allo-HCT from a new donor with mismatched HLA from the first donor (see Chaps. 19 and 20). Although not routinely performed, HLA typing of leukemic cells at relapse and comparison with patient's and donor's HLA typing can detect loss of expression of the mismatched HLA haplotype in leukemic cells [63].

15.6 Expert Point of View

Haplo-HCT permits rapid and relatively cheaper access to donor in virtually every transplant-eligible patient, thus can reduce the risk of disease progression and clinical deterioration associated with delays in donor search [14–16]. In North America, haplo-HCT using PTCy is preferred over TCD techniques because of its ease, low cost, lack of graft manipulation, and possibly improved OS [32] (see Chaps. 19 and 21). The choice of conditioning regimen varies based on age, HCT-CI, performance status, and other patient-specific factors [33]; however, the results of BMT CTN 0901 trial indicates a lower risk of relapse and a higher probability of relapse-free survival with MAC in patients <65 years old with HCT-CI of ≤4 [35]. Hence, MAC may be preferred in young and fit AML patients undergoing haplo-HCT as well. Several Mel-, Bu-, or TBI-based MAC regimens have been developed with very good results [39]. As safety with these regimens has been demonstrated in this setting, a MAC regimen would be preferred at least for younger, fit patients with AML. Bu/Cy and Flu/Mel/Thio are the two most commonly utilized MAC regimens and may be preferred. Flu/Cy/TBI is very well tolerated and may be the preferred NMA conditioning regimen [26]. With advances in the techniques of haplo-HCT, the outcomes are comparable to HLA-matched unrelated and related donor HCT [22–26, 48]. As such, other factors such as urgency of transplant, timely availability of HLA-matched unrelated donors, donors' age, and presence of donor-specific antibody may play increasingly greater role in selection of HLA-haploidentical donor and HLA-matched unrelated donor. The use of PTCy has reduced the risk of G*v*HD and opportunistic infections; however, disease relapse continues to be an important cause of deaths, particularly in high-risk patients with AML. In the CIBMTR study, about 50% of the patients with AML relapsed following

haplo-HCT; disease relapse accounted for three-quarter of all deaths [26]. Close post-transplant monitoring of MRD, post-transplant maintenance strategy [59, 60], and preemptive therapy for MRD or worsening donor chimerism are emerging techniques targeted at reducing the risk of relapse and remain areas of active investigation. Additionally, the use of cellular therapy post-transplant may provide an improvement in relapse rate, much needed especially in patients with high-risk disease [64]. Finally, multidisciplinary team-based long-term follow-up clinic is critical to improve management of late effects such as G*v*HD, opportunistic infection, secondary solid malignancies, late-onset organ toxicities, psychosocial dysfunction, and financial toxicities [65]. Such survivorship management can reduce late mortality and enhance quality of health of transplant recipients.

15.7 Future Directions

The outcomes of haplo-HCT have significantly improved in last decade. A large number of mainly retrospective studies have demonstrated similar outcomes between HLA-haploidentical and HLA-matched HCTs [22–26, 48]; however, a randomized trial is necessary for confirmation. Although the BMT CTN 0901 trial indicates improved outcome with MAC in young and fit patients with AML [35], it remains to be established whether the results are similar for haplo-HCT that utilizes PTCy. Several institutions have developed a number of conditioning regimens, which needs to be compared. Even though the early results of TBI-based MAC regimens appear encouraging, concerns for long-term complications with TBI [44–47] mandate a long-term comparison conditioning regimens with and without TBI. Despite significant improvement in the outcomes of haplo-HCT, disease relapse in particular and also G*v*HD, graft failure, infections, and organ toxicities continue to be associated with mortality in more than half of patients after HCT [26, 56]. Identification of high-risk patients (e.g., the presence of high-risk cytogenetics, somatic mutations, active disease at the time of allo-HCT, MRD before or after allo-HCT [66–69]), post-transplant maintenance therapy [59, 60, 70], MRD monitoring (e.g., with the use of flow cytometry, genetic or molecular markers [71, 72]), preemptive therapy of MRD [73], and improved management of post-transplant relapses with the use of novel therapies (e.g., immune checkpoint inhibitors [74]) are key approaches to reduce the risk of deaths from post-transplant relapses (see Chap. 19). Post-transplant maintenance strategy using azacitidine [59] and sorafenib [60, 61, 70] have shown exciting results in AML patients, and larger studies are ongoing. Post-transplant cellular therapy such as infusion of natural killer cells or T-cells with chimeric antigen receptors, donor lymphocytes expressing herpes simplex virus-thymidine kinase suicide gene, or inducible caspase-9 transgene are emerging cellular therapy to reduce or treat post-transplant relapses [19]. Improving the survival of AML patients undergoing haplo-HCT requires improved understanding of tumor biology and transplant immunology, translational research, as well as high-quality trials via collaboration between multiple research centers, funding agencies, and pharmaceutical industry.

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Haloidentical Hematopoietic Cell Transplantation in Lymphomas

16

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16.1 Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) is a potentially curative salvage treatment for patients with lymphoproliferative disorders (LPDs). In the United States, it is estimated that about 19,790 patients with non-Hodgkin lymphoma (NHL) and 1150 patients with Hodgkin lymphoma died in the calendar year of 2015 [1, 2]. Progressive disease is a leading cause of mortality in patients with lymphomas [3]. Although allo-HCT appears to be an attractive treatment option for patients with advanced lymphomas, its widespread application is often limited by factors such as HLA-matched donor availability and the risk of post-transplant morbidity. Haploidentical HCT (haplo-HCT) expands the allo-HCT strategy to patients without an available fully HLA-matched adult donor while novel haplo-HCT techniques have improved the safety of this approach. Historically, in the haploidentical setting, severe graft-*versus*-host disease (G*v*HD), higher risk of non-relapse mortality (NRM), disease relapse, and delayed immune reconstitution were challenges despite extensive *in vivo* or *ex vivo* T-cell depletion aimed at reduced graft rejection and G*v*HD risk [4, 5]. More recently, several Asian centers have reported favorable outcomes of haplo-HCT utilizing T-cell replete grafts with intensive immunosuppression using antithymocyte globulin (ATG) [6]. A different strategy of T-cell replete haplo-HCT being increasingly used involves the administration of post-transplantation cyclophosphamide (PTCy), which mitigates the risk of G*v*HD by

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targeting alloreactive T-cells rapidly proliferating early after HLA-mismatched transplant while sparing regulatory T-cells and hematopoietic progenitor cells [7]. The near universal and ease of haploidentical donor availability have led to a rapid adoption of this strategy in patients with LPDs.

16.2 Indications for Allo-HCT in Lymphomas

While HLA-identical sibling remains the gold standard donor source in patients with lymphoid malignancies undergoing an allo-HCT, haploidentical related donors are increasingly being considered an option in those without HLA-identical siblings (and/or matched unrelated donors). In lymphoma, haploidentical grafts are predominantly used as a curative option in those with relapsed or refractory disease. Table 16.1 summarizes the common indications for haplo-HCT in lymphoma patients lacking an HLA-identical adult donor.

16.3 Assessment of Potential Recipient Prior to Allo-HCT

Patients are assessed for haplo-HCT using a multidisciplinary approach as described previously in literature [8]. Similar to the workup for all allo-HCT, patients should have adequate functioning of the following organ systems prior to transplant—cardiac (LVEF >45%), pulmonary (DLCO ≥50%, FEV1/FVC

Table 16.1 Indications for haploidentical transplantation in lymphomas

Disease	Indication
Hodgkin lymphoma	1. Relapse after autologous HCT 2. Primary refractory disease 3. High-risk disease in CR2 or beyond
Follicular lymphoma	1. Relapse after autologous HCT 2. Primary refractory disease 3. Duration of CR1 less than 6 months 4. Transformed lymphoma
DLBCL	1. Relapse after autologous HCT 2. Primary refractory disease 3. Double hit or triple hit lymphoma 4. High-risk features such as CNS involvement 5. Duration of CR1 <6 months
Mantle cell lymphoma	1. Relapse after autologous HCT 2. Primary refractory disease 3. Duration of CR1 <6 months
Mature T-cell lymphoma	1. Primary refractory disease 2. Relapse after autologous HCT 3. CNS involvement 4. High-risk histology such as *gamma-delta* lymphoma or adult T-cell lymphoma

≥60%), renal (serum creatinine ≤1.5 mg/mL), hepatic (serum bilirubin <2 times the upper limit of normal), and adequate performance status (Karnofsky scale ≥70). Pre-transplant disease assessment could be done using imaging modalities such as CT or PET scans. Although chemotherapy sensitivity of lymphoma at the time of transplant is associated with favorable outcomes, it is not mandatory as the benefits of haplo-HCT have been demonstrated even in patients with active disease at the time of transplant [9, 10]. In addition, the efficacy and feasibility of haplo-HCT extends to all age groups including older adults using reduced-intensity regimens [11]. Hence, as long as organ function, performance status and comorbidity scores are favorable, chronological age by itself should not be considered a contraindication to allogeneic transplantation, including haploidentical HCT.

Scoring systems such as hematopoietic cell transplant comorbidity index (HCT-CI) that have been validated for HCT in lymphoma could be used as a tool to assess patients and predict their mortality and survival [12]. Donors for haplo-HCT are usually first-degree relatives such as biological parents, biological children, and full or half-siblings. Donor selection criteria include medical fitness, age >18 years (younger donors preferred over older donors), donor-specific HLA antibody (DSA) status (see Chaps. 9, 10, and 20), and no evidence of active malignancy or infection such as human immunodeficiency virus (HIV). Other selection parameters include gender matching, major ABO matching, and CMV IgG serostatus matching. Approach to determine the ideal donor for haplo-HCT is an area of active research (see Chaps. 5 and 10) [13]. A study by Wang and coworkers suggested that younger age and male gender were associated with lower NRM, and father donors (compared to maternal donors) and children donors (compared to sibling donors) were associated with lower incidence of acute G*v*HD [14] (see Chap. 5). Similarly, the role of factors such as killer immunoglobulin-like receptor (KIR) ligand mismatch and non-inherited maternal antigen (NIMA) mismatch is also being explored as predictive factors for transplant outcomes [15] (see Chap. 10).

16.4 Conditioning Strategies and Regimens for Haploidentical Transplants

The most commonly modern conditioning strategy for haplo-HCT in lymphoma is reduced-intensity/nonmyeloablative conditioning (RIC/NMA), although myeloablative conditioning (MAC) has been described in a few studies [16, 17] (see Chap. 11). It is unclear if there is any advantage of MAC over RIC/NMA in haplo-HCT for lymphoma due to the lack of clinical studies in this area. In the setting of HLA-matched donor allo-HCT for lymphoma, several studies failed to demonstrate an advantage of MAC over RIC/NMA conditioning [18–21]. There is less data in haplo-HCT for lymphoma; hence, more research is needed to address the optimal conditioning strategy for haplo-HCT for this group of diseases.

16.4.1 Myeloablative Strategy

The use of MAC for haplo-HCT (see Chap. 11) in lymphoma even in fit patients has not so far been studied well due to the concern for high transplant-related mortality. Two studies have described this strategy. In a retrospective study by Bashey and coworkers which included 23 patients receiving haplo-HCT using PTCy as G*v*HD prophylaxis, two MAC approaches were employed—total body irradiation (TBI)-based and non-TBI-based approach [16]. In the non-TBI-based approach, patients were treated with the following agents: fludarabine (25 mg/m^2 intravenous once daily on days −6 to −2), busulfan (110–130 mg/m^2 IV once daily on days −7 to −4), and cyclophosphamide (14.5 mg/kg IV once daily on days −3 and −2 and 50 mg/kg once daily on days +3 and +4) (Flu/Bu/Cy). In the TBI-based approach, patients received fludarabine (30 mg/m^2 once daily on days −7 to −5) and TBI (150 cGy twice daily on days −4 to −1, total dose 1200 cGy) (Flu/TBI). Granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood (PB) grafts were the source in all these transplants. In another study by Brammer and coworkers, a MAC strategy using melphalan 140 mg/m^2 and fludarabine (40 mg/m^2/day intravenous over 4 days for a total of 160 mg/m^2). This strategy was shown to be associated with higher gastrointestinal toxicity, hence subsequently discontinued, and lower doses of melphalan (100 mg/m^2) were recommended [17]. Moreover, the Flu/Bu/Cy was also discontinued due to concerns of higher incidence of BK virus cystitis (see Chaps. 11 and 20).

16.4.2 Reduced-Intensity and Nonmyeloablative Strategies

Most of the clinical studies with the PTCy platform have employed RIC/NMA strategy for haplo-HCT (Table 16.1 and Table 16.2). The standard regimen used in this setting includes the following agents: fludarabine (30 mg/m^2 IV, days −6 to −2, renally adjusted), cyclophosphamide (14.5 mg/kg intravenous, days −6 and −5), and TBI (200 cGy, day −1), followed by infusion of allografts. T-cell replete unmanipulated haploidentical source from the bone marrow (BM) is the most common type of graft used with a few exceptions where PB grafts were used [Table 16.1]. G-CSF is administered from day +5 until neutrophil recovery to ≥1000 cells/mL (Fig. 16.1) (see Chap. 11).

An alternative RIC regimen has also been reported in a study by Brammer and coworkers [17] using the following agents: melphalan 100 mg/m^2 on day −6, fludarabine 40 mg/m^2 on days −5 to −2, and 2 Gy TBI on day −1. Thiotepa (5 mg/kg) was utilized in place of TBI (on day −6 and melphalan on day −7) for patients with prior lymphomatous involvement of the central nervous system (CNS). Additionally, patients with CD20$^+$ disease received rituximab 375 mg/m^2 on days −13, -6, +1, and +8.

Table 16.2 Summary of selected studies using haploidentical transplantation in lymphomas

Study	Histology	Type of graft	Type of conditioning	GvHD prophylaxis	Acute GvHD	Chronic GvHD	OS	PFS	NRM	Relapse
Kanate et al. [24]	B-cell NHL, T-cell NHL and Hodgkin's	Bone marrow (n = 172) and PB (n = 13)	Flu/Cy/2 Gy TBI	PTCy, CNI, MMF	Day +100 Grade II–IV 27% Grade III–IV 8%	3-year chronic GvHD—9%	3-year OS—60%	3 year PFS—47%	3-year NRM—17%	3-year relapse—36%
Raiola et al. [9]	Hodgkin's	Bone marrow (n = 26)	Flu/Cy/2 Gy TBI	PTCy, CNI, MMF	Day +100 Grade II–IV 24%	3-year chronic GvHD—9%	3-year actuarial survival 77%	3-year PFS 63%	4%	31%
Burroughs et al. [25]	Hodgkin's (haplo n = 28)	Bone marrow (n = 28)	Flu/Cy/2 Gy TBI	PTCy, CNI, MMF	Grade II–IV 43% Grade III–IV 11%	2-year extensive chronic GvHD—35%	2-year OS 58%	2-year PFS 51%	2-year NRM 9%	2-year relapse 40%
Bashey et al. [16]	Hodgkin's (n = 11) NHL (n = 12)	PBHC for MAC, BM graft for NMA	NMA Flu/Cy/2 Gy TBI MAC Flu/Bu/Cy or Flu/TBI	PTCy, CNI, MMF	6 months Grade II–IV 41% Grade III–IV 17%	2-year moderate-severe chronic GvHD—31%; severe GvHD 7%	2-year OS 57%	2-year DFS 54%	2-year NRM 17%	2-year relapse 21%

(continued)

Table 16.2 (continued)

Study	Histology	Type of graft	Type of conditioning	GvHD prophylaxis	Acute GvHD	Chronic GvHD	OS	PFS	NRM	Relapse
Kasamon et al. [11]	NHL (n = 148) Hodgkin's (n = 7)	Bone marrow (except one patient)	Flu/Cy/2 Gy TBI	PTCy, CNI, MMF	180 days Grade II–IV 33% Grade III–IV 3%	1-year chronic GvHD 10%	3-year OS 47%	3-year PFS 39%	1-year NRM 12%	3-year relapse 39-40%
Kanakry et al. [10]	T-cell NHL (haplo n = 22)	Bone marrow (except one patient)	Flu/Cy/2 Gy TBI	PTCy, CNI, MMF	Grade II–IV 16%	19% at 1 year	2-year OS 43%	2-year PFS 40%	1-year NRM 8%	1-year relapse 34%
Castagna et al. [29]	NHL (n = 17) Hodgkin's (n = 27)	Bone marrow	Flu/Cy/2 Gy TBI	PTCy, CNI, MMF	Grade II–IV 25.6%	5.2%	2-year OS 71%	2-year PFS 63%	2-year NRM 16.3%	18.7%
Luznik et al. [32]	NHL (n = 10) Hodgkin's (n = 14)	Bone marrow	Flu/Cy/2 Gy TBI	PTCy, CNI, MMF	Day +200 Grade II–IV 34% Grade III–IV 6%	1-year extensive chronic GvHD—5% with two doses of PTCy	2-year OS 36%	2-year EFS 26%	1-year NRM 15%	1-year relapse 51%

Brammer et al. [17]	NHL (n = 11) Hodgkin's (n = 7)	Bone marrow	Flu/Mel/TBI or Thiotepa	PTCy, CNI, MMF	Grade II–IV 51%	27.2% (three cases)	2-year OS 54%	2-year PFS—57% for Hodgkin and 51% for NHL	1-year TRM 19%	3-year relapse 27%
McCurdy et al. [22]	NHL (n = 152) Hodgkin's (n = 37)	Bone marrow	Flu/Cy/2 Gy TBI	PTCy, CNI, MMF	Day +180 Grade II–IV 32% and Grade III–IV 4%	2-year chronic GvHD 13%	3-year OS 50%	3-year PFS 40%	Day +180 NRM 8%	3-year relapse 46%

PB peripheral blood, *CNI* calcineurin inhibitor, *NHL* non-Hodgkin lymphoma, *Flu* fludarabine, *Cy* cyclophosphamide, *PTCy* post-transplant cyclophosphamide, *MMF* mycophenolate mofetil, *Mel* melphalan, *TBI* total body irradiation, *GvHD* graft-*versus*-host disease, *NMA* nonmyeloablative, *MAC* myeloablative conditioning, *NRM* non-relapse mortality, *TRM* transplant-related mortality, *PFS* progression-free survival, *EFS* event-free survival, *DFS* disease-free survival, *OS* overall survival

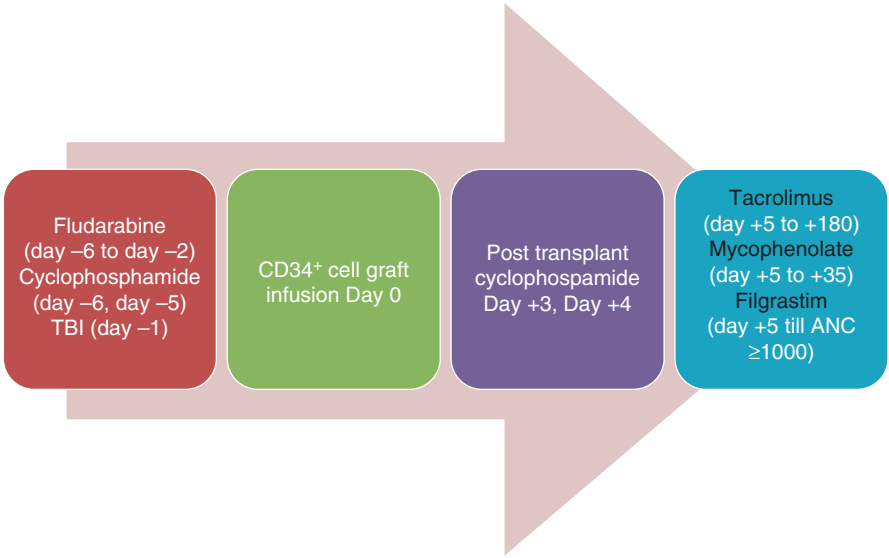

Fig. 16.1 Most common RIC/NMA conditioning and G*v*HD prophylaxis for haploidentical transplantation

16.5 G*v*HD Prophylaxis: Post-transplant Cyclophosphamide

The most common G*v*HD prophylaxis used in the United States for haplo-HCT in lymphoma is PTCy along with a calcineurin inhibitor (CNI) such as tacrolimus and mycophenolate mofetil (MMF) (see Chap. 7). The typical regimen consists of starting immune suppression on day +3 after transplant with high-dose cyclophosphamide (50 mg/kg IV, days +3 and +4), mycophenolate mofetil (days +5 to day +30 or 35), and tacrolimus (initiated day +5, targeted to a level of 5–15 ng/mL). In the absence of G*v*HD or failing hematopoietic graft, tacrolimus is usually stopped after day +180.

16.6 Outcome Data

16.6.1 Neutrophil and Platelet Recovery

Compared to HLA-matched related donor (HLA-MRD) and HLA-matched unrelated donor (HLA-MUD) allo-HCT, haplo-HCT is associated with a relatively delayed neutrophil and platelet recovery. The use of bone marrow grafts and myelosuppression with high-dose cyclophosphamide is thought to be the contributing factor. With the routine use of G-CSF after transplant, the duration of engraftment has been reasonably shortened. The median time for neutrophil recovery is 16–18 days and platelet recovery is 24–26 days [9, 11, 16, 22]. Graft failure has been considerably reduced in the recent era and varies between 2.5% and 8.2% [10, 11, 16, 22, 23] (see Chap. 9).

16.6.2 GvHD

Acute and chronic GvHD had been a major problem with haplo-HCT before the advent of immunosuppression with PTCy. However, current studies indicate that the incidence of acute GvHD is comparable to HLA-matched HCT, and incidence of chronic GvHD is lower with haplo-HCT compared to HLA-matched allo-HCT, likely due to the use of post-CY. In a study by Bashey and coworkers, the incidence of grade III–IV acute GvHD was 17% with haplo-HCT as compared to 16% in HLA-MUD HCT and 9% in HLA-MRD HCT ($p = 0.05$) [16]. The 2-year incidence of moderate-severe chronic GvHD was significantly lower with haplo-HCT (31%) as compared to HLA-MUD (47%) and HLA-MRD HCT (44%) ($p = 0.004$). A recent large registry analysis by Center for International Blood and Marrow Transplant Research (CIBMTR) also showed that patients who underwent haplo-HCT had a significantly lower risk of grade III–IV acute GvHD [24]. In this study, the GvHD risk was higher in HLA-MUD without ATG (RR 2.87; 95% CI, 1.52–5.4; $p = 0.001$) and HLA-MUD with ATG (RR 2.45; 95% CI, 1.23–4.87; $p = 0.01$). Also, the 1-year risk of chronic GvHD was lower in haplo-HCT arm (HLA-MUD HCT without ATG had a RR 5.85; 95% CI, 3.96–8.64; $p = 0.0001$; and HLA-MUD HCT with ATG had a RR 3.64; 95% CI, 2.37–5.60; $p < 0.0001$). Other studies also suggested a lower incidence of acute GvHD and chronic GvHD with haplo-HCT, as summarized in Table 16.2.

16.6.3 Non-relapse Mortality and Relapse

With the advent of RIC strategy and careful patient selection, the NRM with haplo-HCT has been lower compared with historical data. Retrospective analysis of the CIBMTR database by Kanate and coworkers [24] showed that the 1-year NRM with haplo-HCT was 11% (95% CI 7–17%) and not significantly different from HLA-MUD HCT with or without ATG. The cumulative incidence of relapse/progression after haplo-HCT ranges from 18.7% to 51% [Table 16.2]. Notably, studies such as the CIBMTR data analysis also showed that incidence of disease relapse at 3 years is not significantly different between haplo-HCT (36%, 95% CI, 29–43%), HLA-MUD without ATG (28%, 95% CI, 24–33%), and HLA-MUD with ATG (36%, 95%CI, 29–43%) ($p = 0.07$) in patients with lymphoma [24].

16.6.4 Infection

One of the significant side effects of intense immunosuppression is increased incidence of infectious complications. However, with the advent of antimicrobial prophylaxis and periodic screening of allo-HCT recipients, the morbidity and mortality due to infections are anticipated to be decreasing. In haplo-HCT studies for lymphoma, the incidence of infectious complications had been variably reported. A study by Raiola and coworkers showed higher incidence of CMV reactivation (42%), asymptomatic EBV DNA detection in blood (23%), and hemorrhagic cystitis due to BK virus infection (23%), HHV-6 pneumonia (4%), bacterial infections

(4–8%), and invasive fungal infection (8%) [9]. In the study by Castagna and coworkers, viral, bacterial, and fungal infections were observed in 59%, 44%, and 12% of the patients [29]. Cautious monitoring of these patients for viral reactivation and appropriate prophylactic antimicrobials per local institutional protocol is essential to reduce the infectious complications of haplo-HCT (see Chap. 20).

16.7 Survival Outcomes: Haploidentical HCT *vs.* HLA-Matched Donor HCT in Adults

Haplo-HCT provides a significant improvement in the survival of patients with advanced lymphoma who had been treated with multiple prior lines of chemotherapy. Notably, survival after haplo-HCT was found to be comparable to patients who underwent HLA-matched donor HCT in several studies mentioned below. In a study by Burroughs and coworkers which included heavily pretreated patients with Hodgkin lymphoma, the 2-year PFS was significantly better with haplo-HCT (51%) compared to HLA-MUD (29%) and HLA-MRD (23%) HCT with a similar 2-year OS (58% *vs.* 58% *vs.* 53%, respectively; p = NS) [25]. Another single center study by Bashey and coworkers [16] which included 23 patients with lymphoma showed a 2-year OS rate of 57% for haplo-HCT, 59% for HLA-MUD HCT, and 72% for MRD HCT with no significant difference in survival between haplo-HCT and HLA-MUD HCT. Table 16.2 summarizes the OS and PFS from various studies with haplo-HCT for lymphoma.

Two registry-based studies using the CIBMTR database have compared the outcomes of haplo-HCT against HLA-MUD and HLA-MRD HCT for lymphoma. In the first study by Kanate and coworkers haplo-HCT was compared to HLA-MUD HCT with or without ATG. The PFS and OS in this study were not significantly different between haplo-HCT and HLA-MUD HCT with or without ATG (3-year OS 60% *vs.* 62% *vs.* 50%, respectively) [24]. In a different study by Karmali and coworkers haplo-HCT was compared to HLA-MRD HCT, and no significant difference in PFS or OS were seen [26]. In contrast, an analysis from the EBMT registry by Dietrich and coworkers suggested that patients who underwent haplo-HCT for NHL had a worse OS compared to HLA-MRD HCT (HR 1.9, CI 1.5–2.5, $p < 0.0001$) [27]. However, in another large EBMT study by Martinez and coworkers for Hodgkin lymphoma, this group found that the PFS and OS were not significantly different between haploidentical and HLA-MRD and unrelated donor HCT, while better than cord blood transplants (CBTs) [28]. Heterogeneity of these results warrants prospective studies to compare the outcomes of haplo-HCT and conventional HLA-matched donor HCT for lymphomas.

16.8 Disease-Specific Data with Haploidentical Transplants

16.8.1 B-Cell NHL

In recent years, studies have increasingly explored the role of haplo-HCT in patients with B-cell NHL. A large retrospective analysis of the CIBMTR database by Kanate and coworkers [24] had a subset of patients with B-cell NHL-follicular lymphoma

(n = 28), DLBCL (n = 66), and mantle cell lymphoma (n = 24). The cumulative incidence of relapse/progression at 3 years was 36% with haplo-HCT, 28% in HLA-MUD without ATG, and 36% in HLA-MUD with ATG groups (p = 0.07). Based on histology, the respective 3-year PFS and OS were 66% and 70% in follicular lymphoma, 44% and 58% in DLBCL, and 32% and 60% in mantle cell lymphoma. Outcomes of other retrospective and prospective studies for haplo-HCT in B-cell NHL are summarized in Table 16.2.

For practical purposes, haplo-HCT is a reasonable treatment option for patients with B-cell NHL who relapse after prior autologous HCT and when no HLA-matched donor is identified. For patients with primary refractory NHL or relapsed/refractory with persistent disease after salvage chemotherapy, haplo-HCT could be considered either after autologous HCT or as alternative allogeneic transplant when an HLA-MRD is not available, or a HLA-MUD will take too long to obtain while risking disease progression, as autologous transplantation in this setting has been traditionally associated with very poor outcomes. More research is needed to explore the ideal timing and patient population who would benefit from earlier haplo-HCT.

16.8.2 T-Cell NHL

T-cell lymphomas often have variable response to chemotherapy and relapse after autologous HCT. There is a growing interest in exploring the role of allo-HCT in the management of T-cell lymphoma for its graft-*versus*-lymphoma (G*v*L) effect. Two retrospective studies have demonstrated feasibility of haplo-HCT in T-cell NHL. In the first study by Kanakry and coworkers, 44 patients with T-cell NHL underwent allo-HCT of which 22 patients underwent haplo-HCT [10]. Median age of this cohort was 60 years. The estimated PFS at 2 years was 40% (95% confidence interval, 26–55%) and OS was 43% (95% CI, 28–59%). Notably, this study also suggested that allo-HCT at first complete remission (CR1) was more beneficial as compared to its utilization beyond CR1. In the haplo-HCT cohort, cumulative incidence of relapse was 34% with a 1-year NRM of 11%. In another large retrospective CIBMTR analysis by Kanate and coworkers 24 patients with mature T-cell/NK cell lymphoma were included in the haplo-HCT arm and demonstrated a 3-year PFS of 32% (14–54%) and 3-year OS of 36% (16–60%) [24]. Overall, these studies suggest effectiveness of haplo-HCT in T-cell lymphoma. Decision to proceed with haplo-HCT in these studies was based on high-risk factors such as a history of primary induction failure, central nervous system involvement, or very high-risk histology such as *gamma-delta* subtypes or adult T-cell leukemia/lymphoma.

16.8.3 Hodgkin Lymphoma

Although majority of patients with Hodgkin lymphoma are cured in the current era, about 10–20% of patients with refractory disease or relapse after prior autologous HCT may be candidates for haplo-HCT. Several studies evaluated outcomes of

patients with HL receiving a haplo-transplant with PTCy, starting with the above-mentioned studies by Burroughs and coworkers [25] and Martinez and coworkers [28]. Another study by Raiola and coworkers included 26 patients with advanced Hodgkin lymphoma who had failed prior autotransplant and demonstrated a 3-year actuarial survival and disease-free survival of 77% and 63%, respectively, and a relapse rate of 31% [9]. Hence, haplo-HCT remains as a valuable management option for Hodgkin lymphoma with high-risk features such as primary refractory disease or relapse after prior HCT.

16.9 Relapse After Haploidentical Transplantation: Management Issues

A common method of managing relapse after haplo-HCT is the use of donor lymphocyte infusion (DLI) both preemptively with loss of donor chimerism and at evidence of clinical relapse. In a study by Raiola and coworkers, DLI was started for relapse at a dose of 1×10^3/kg $CD3^+$ cells and increased every 1–2 months up to 1×10^7/kg [9] (see Chap. 19). Of the six patients who received DLI, five patients responded. A retrospective series by Zeidan and coworkers included 40 patients (11 patients with lymphoma) who underwent DLI for relapse after haplo-HCT and demonstrated a 30% complete remission rate and 25% incidence of acute G*v*HD [30]. The efficacy of DLI after haplo-HCT reiterates the existence of G*v*L effect in this setting. In addition, strategies such as drug therapy maintenance (brentuximab) or infusion of natural killer cells (NK cells) had been studied in the prophylaxis as well as management of relapse after haplo-HCT for hematological malignancies [31].

16.10 Expert Point of View

Haplo-HCT is an important alternative donor option for the management of lymphoma. A simplified algorithm highlighting the role of haplo-HCT in the management of lymphoma is depicted in Fig. 16.2. Encouraging results have been demonstrated with a uniform RIC/NMA strategy using fludarabine, cyclophosphamide, and TBI followed by G*v*HD prophylaxis with PTCy, CNI, and mycophenolate. It might be prudent that in the absence of HLA-MSD, haplo-HCT could be a reasonable alternative to unrelated donor transplant, as the former is associated with lesser risk of G*v*HD, similar relapse risk, NRM, and survival, especially if proceeding fast to an allo-HCT is needed. The histology of lymphoma also appears to play a role in the risk of relapse after haplo-HCT with diffuse large B-cell lymphoma and mantle cell lymphoma having a tendency to relapse early (<7 months) and Hodgkin lymphoma tending to relapse later (>7 months) [24]. The optimal timing of haplo-HCT in the therapeutic armamentarium of lymphoma is still uncertain, especially in the era of targeted therapies. It is important to note that these results are predominantly based on retrospective studies in patients with lymphoma. Hence, there might

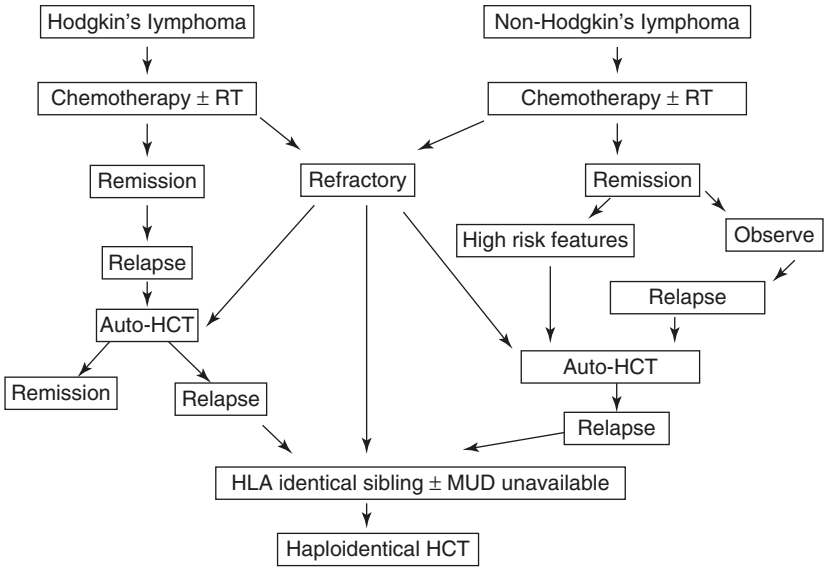

Fig. 16.2 Overview of management approaches for lymphomas

be confounding factors such as institutional preference of donor selection, better performance status, lower comorbidities, and others. However, these encouraging results pave way for considering randomized clinical trials to validate these findings and to find the optimal timing and patient population who would derive the maximum benefit from haplo-HCT.

16.11 Future Directions

Relapse/progression of lymphoma continues to be a major problem after haploidentical HCT (see Chap. 19). An approach which has been studied is the use of post-transplant maintenance therapy with agents such as rituximab. In a study by Kanakry and coworkers, 69 patients who received haplo-HCT for lymphoma were treated with post-transplant rituximab for 8 weeks from day +30 and noted to have improved 1-year PFS and OS—70% and 83%, respectively—with a 20% incidence of relapse [23]. Another strategy which is currently being studied (NCT02169791) is the augmentation of NK cell alloreactivity after haplo-HCT using a proteasome inhibitor MLN9708. This is postulated to sensitize tumor cells to NK cytotoxicity and protect against G*v*HD. Additionally, more strategies such as preemptive NK cell DLI after haplo-HCT (NCT01386619) and post-transplant regulatory T-cell infusion (NCT01050764) are currently being explored to improve the outcomes of haplo-HCT for lymphoma (see Chaps. 4 and 19). Together, efforts to reduce relapse, decrease G*v*HD, and improve survival would enable more patients to derive the benefit of haplo-HCT that could lead to a cure of lymphomas.

16.12 Clinical Pearls

- Haplo-HCT is an upcoming important strategy in the management of relapsed/refractory lymphoma when HLA-matched related donor is not immediately available.
- PTCy has formed an important platform of mitigating the risk of G*v*HD after haploidentical transplant.
- Retrospective studies suggest lesser risk of G*v*HD, similar risk of relapse, NRM, and survival with haploidentical transplant as compared to HLA-matched unrelated donor transplant.
- Prospective studies are needed to determine the optimal timing and patient population who would derive the maximum benefit from haplo-HCT.

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Haloidentical Transplants for Myeloproliferative Neoplasms

17

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17.1 Introduction

Myeloproliferative neoplasms (MPNs) comprise several clonal hematologic disorders that are thought to arise from transformation in a hematopoietic stem cell. The main clinical features of these diseases are the overproduction of mature, functional blood cells and a long clinical course. The 2008 WHO classification divides these diseases into two broad categories—classical and MPN variants [1]. The classical MPNs are chronic myeloid leukemia (CML), primary myelofibrosis (PMF), polycythemia vera (PV), and essential thrombocythemia (ET), whereas MPN variants include systemic mastocytosis (SM), chronic neutrophilic leukemia (CNL), and chronic eosinophilic leukemia (CEL). Other MPNs are chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia, and atypical BCR-ABL-negative CML.

Transplantation for patients with CML has now changed from upfront therapy to patients who have failed to achieve hematologic remission to several tyrosine kinase inhibitors (TKIs), are intolerant to TKIs, or have progressed to accelerated or blasts phase disease, while indication for transplantation for myelofibrosis has been guided more recently by DIPSS-plus scoring system.

Patients with PV or ET have in general better outcomes with long clinical course and may be considered for transplantation after progression to secondary myelofibrosis or acute leukemia. Except treatment for CML using TKIs, which have

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changed the approach to treatment for this disease, there are limited effective drug treatment options for other MPNs.

Allogeneic hematopoietic cell transplantation (allo-HCT) remains the only curative therapy available for patients with MPNs and is mostly performed for PMF or secondary myelofibrosis with high DIPSS-plus score, advanced (or refractory) CML, and CMML [2, 3], traditionally with HLA-matched donors [4–8]. However, in general, only approximately 30% of the patients will have an HLA-matched related donor (HLA-MRD), while with respect to older population mostly affected by these diseases, this is an overestimate. Unrelated donor availability varies widely with the race of the recipient ranging from approximately 60–70% for the Caucasian patients to approximately 30% of the Hispanics and less than 20% of African-Americans and Asians [9]. Haploidentical hematopoietic cell transplantation (haplo-HCT) related donors can extend transplantation to almost all patients in need, as more than 90% of patients will have an HLA half-matched donor readily available for transplantation irrespective or recipient's race [10]. In addition, the use of haploidentical donors may be performed much faster, similar with HLA-MSD transplants. Moreover, while outcomes for CML with HLA-matched unrelated donors (HLA-MUD) may be similar with HLA-MRD transplants, outcomes for other MPNs may not be the same.

Although results with busulfan-based conditioning for patients with myelofibrosis treated with unrelated donors were very encouraging [11], results with melphalan-based conditioning have been more disappointing for this group of patients. Recently, Myeloproliferative Disorder Research Consortium (MPD-RC) reported results of a phase II clinical trial using fludarabine plus melphalan conditioning (FM140 regimen) in patients with HLA-matched related or HLA-matched unrelated donors (MUD) [12]. While patients with an HLA-MSD had excellent outcomes, patients treated with a HLA-MUD had significantly worse outcomes with lower engraftment rate (75% *vs.* 97%), higher incidence of severe grade III–IV acute G*v*HD (12% *vs.* 21%), higher non-relapse mortality (NRM, 22% *vs.* 59%), and subsequent worse overall survival (OS, 32% *vs.* 75%, $p < 0.001$), after median follow-up of 25 months [12].

Other alternative donors have been under investigation and several retrospective studies [13–15] showed similar disappointing engraftment rate and higher treatment-related mortality (TRM) leading to worse outcome using umbilical cord blood (UCB) source compared to HLA-matched transplants. Consequently, exploring haploidentical donors, for patients without HLA-matched donors, remains an important objective in order to extend transplantation to most patients with curative intent.

17.2 Haploidentical Transplantation for Chronic Myelogenous Leukemia

Haploidentical hematopoietic cell transplantation (haplo-HCT) with post-transplantation cyclophosphamide (PTCy) has been successfully used for patients with hematological malignancies requiring a transplant that do not have an HLA-matched donor [16–18]. Multiple single institution and registry studies have shown

safety of this approach and comparable outcomes with HLA-matched transplants [19–22]. All studies so far have in fact shown lower incidence of acute and chronic G*v*HD with comparable treatment-related mortality (TRM) with HLA-MUD transplants. However, very few patients with MPNs have been treated with a haploidentical transplant so far.

Huang and coworkers [23] initially reported results on 93 CML patients with a median age of 29 years treated with T-cell replete unmanipulated haplo-HCT who lacked an HLA-matched related donor. All patients received a myeloablative, busulfan plus cyclophosphamide conditioning regimen (Bu-Cy2) [22]. Day 100 and 1-year TRM post-transplant were only 8.7% and 20.7%, whereas 4-year event-free survival (EFS) for patients in second chronic phase (CP2), accelerated phase (AP), and blast phase (BP) were 85%, 73%, and 61.5%, respectively [22]. Interestingly, a high survival rate was obtained especially in patients with AP and BP owing probably to the high intensity of the conditioning regimen in a younger population, while a higher incidence of G*v*HD was observed. A concerning higher incidence of severe acute G*v*HD and chronic extensive G*v*HD may be associated with continuous TRM with longer follow-up in this group of patients.

Relapse disease represents a major cause of mortality in these patients. The same group subsequently reported in 97 patients with CML in which KIR-ligand mismatch was analyzed in donor-recipient pairs that patients with class I ligands for the donor-inhibitory KIR gene exhibited significant decrease in molecular and hematologic relapse rates ($p = 0.003$ and $p = 0.015$, respectively). The relapse risk was reduced in patients with HLA-C1C2 or HLA-C2C2 who accepted donors with KIR2DS1 or in patients with HLA-Bw4 who accepted donors with KIR3DS1, compared with the remaining transplants ($p = 0.009$ and $p = 0.009$, respectively). In addition, the presence of class I ligand in the recipients of donor-activating KIR contributed to a decreased relapse rate in patients lacking class I ligand in the recipient of donor-inhibitory KIR ($p = 0.04$ and $p = 0.03$, respectively) [24]. Patients with resistant mutations, especially T315I, have no good treatment except transplantation. The same Chinese transplant group evaluated outcomes of patients with CML carrying such mutation (a third of patients had HLA-haploidentical donors) and found that transplantation was able to overcome the negative prognosis of this mutation for patients in chronic and accelerated phases (80% and 73% progression-free survival [PFS] at 2 years, respectively); however, none of the patients in BP survived long term [25]. The same group compared outcomes for patients with advanced CML using haploidentical and HLA-MSD transplants and found comparable overall and PFS at 3 years (51.1% for haploidentical and 47.5% for HLA-matched sibling transplants), with similar relapse and TRM between the two groups [26].

More recently, haplo-HCT with PTCy-based G*v*HD prophylaxis is gaining worldwide acceptance and has been associated with a lower incidence of acute and mostly chronic G*v*HD (see Chaps. 7 and 8). The MD Anderson group reported the first experience with PTCy-based G*v*HD prophylaxis for patients with advanced CML treated with HLA-haploidentical donors. Results from the first ten patients with melphalan-based conditioning were presented to the American Society for Blood and Marrow Transplantation (ASBMT) Annual Meeting in 2014. The median number of prior medical therapies including TKIs was 3 (three). Preparative regimen used consisted of melphalan 140 mg/m^2 (one patient had 100 mg/m^2), thiotepa 5–10 mg/kg,

and fludarabine 160 mg/m^2 total dose. All but one patient received bone marrow (BM) graft. All patients were engrafted with neutrophils after a median of 18 days, with 100% donor cells on chimerism, and achieved remission post-transplant. Acute grade II–IV G*v*HD occurred only in 30% (no grade III–IV acute G*v*HD was observed), and overall chronic G*v*HD occurred in 37.5% of evaluable patients, with no severe chronic G*v*HD. Four patients relapsed and died; three of them were not in chronic phase at the time of transplant. Remarkably, no patient died of TRM. About 71% of patients transplanted in second CP remained in molecular remission at the last follow-up. After a median follow-up of 22 months, median PFS was reached; six patients (60%) were alive; five are in complete molecular remission and one with low level PCR positivity [3]. These results from a small number of patients showed safety of haplo-HCT for this group of patients and potential good long-term outcomes due to low incidence of acute and chronic G*v*HD.

The same group updated results for patients with advanced CML (beyond CP1) and performed a comparison of transplantation with different donors for 207 patients with different donors. About 81% of the patients had ≤5% BM blasts at transplant, 65% had t(9;22) by cytogenetics, and 94% of the patients were not in complete or major molecular remission at transplant. Donors were HLA-matched siblings (38%), HLA-matched unrelated (36%), HLA-haploidentical (9%), HLA-mismatched unrelated (8%), cord blood (5%), and one HLA-antigen-mismatched related donor (3%), with similar pre-transplant characteristics except race of the recipient (significantly higher proportion of non-Caucasians in the haploidentical and cord blood transplant groups *vs.* other groups). The median follow-up for the whole group was 20.1 months. The OS and PFS at 1 and 5 years for the entire group were 63% and 49% and 45% and 34%, respectively. In univariate analysis, patients with ≤5% BM blasts at transplant ($p = 0.007$), those who receive myeloablative conditioning ($p = 0.06$), and those who had a haploidentical donor ($p = 0.023$) had decreased risk of progression and/or death ($p = 0.033$) and longer PFS. In multivariable analysis for PFS, ≤5% BM blasts at transplant ($p = 0.011$ *vs.* 5–20% blasts and $p = 0.002$ *vs.* >20% blasts) and donor type (haploidentical *vs.* HLA-matched transplants $p = 0.13$, HR 1.83, haploidentical *vs.* MMUD 0.018, HR 3.83, UCB $p = 0.02$, HR 5.23) retain statistical significance.

Moreover, as previously seen, haplo-HCT had similar incidence of grade II–IV acute G*v*HD at day 100 post-transplant (24% *vs.* 29% HLA-MRD *vs.* 41% HLA-MUD, $p = 0.44$), no grade III–IV acute G*v*HD (0% *vs.* 15% HLA-MRD *vs.* 12% HLA-MUD, $p = 0.24$), and lower incidence of all and extensive chronic G*v*HD (1-year CI of extensive chronic G*v*HD was only 7% for haploidentical *vs.* 30% for MRD and 24% for HLA-MUD transplants). When G*v*HD was taken into consideration in addition to relapse, significant better G*v*HD-free, relapse-free survival (GRFS) was noted for haploidentical transplant patients compared with HLA-matched donor transplants. Multivariable analysis for GRFS showed that ≤5% BM blasts at transplant (*vs.* 5–20% BM blasts, $p = 0.007$, HR 1.99) and haploidentical donor (*vs.* HLA-matched donors, $p = 0.029$, HR 2.25) were associated with significantly better GRFS (Fig. 17.1) [27]. These results confirm for patients with CML also that, in addition to what was previously shown for patients with AML [21] and lymphoma [24], transplant

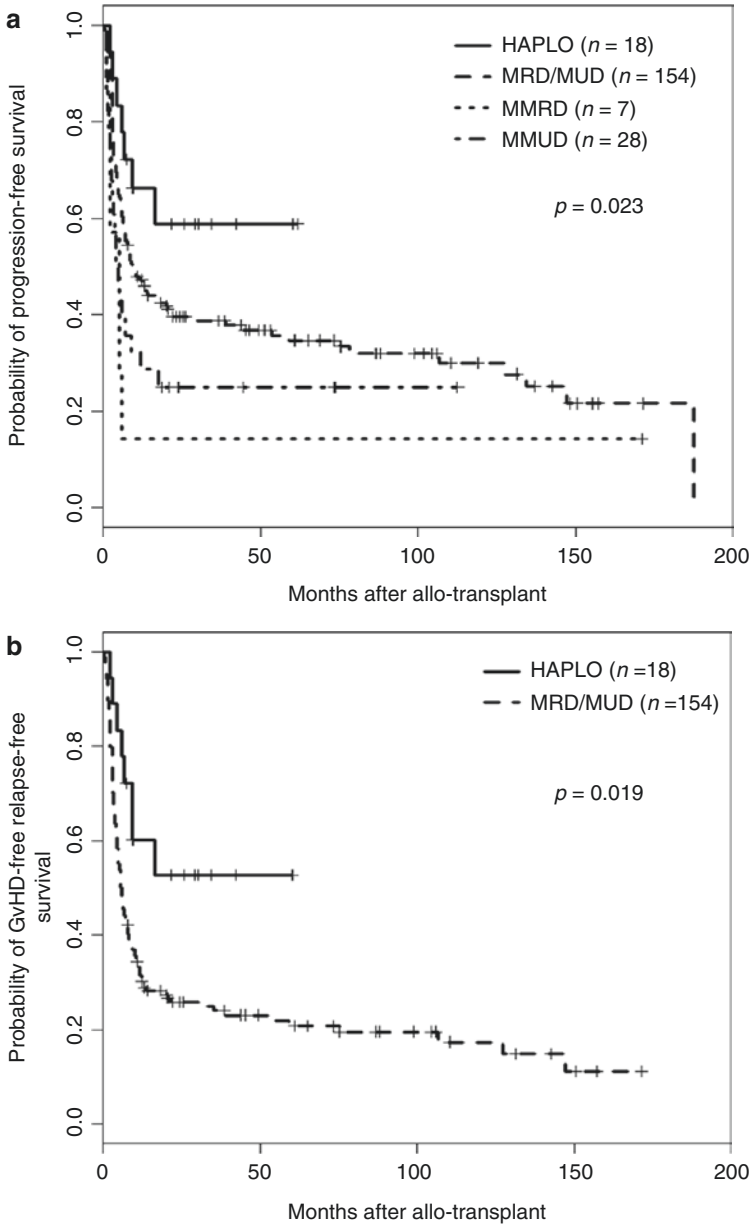

Fig. 17.1 PFS (**a**) and G*v*HD-free, relapse-free survival (GRFS) (**b**) for patients with advanced CML transplanted with a HLA-haploidentical *vs.* HLA-matched (related and unrelated) donors

outcomes with haploidentical donors performed with PTCy are not worse than HLA-matched transplants and are the first to show better GRFS for haplo-HCT compared with HLA-matched donor transplants.

17.3 Haploidentical Transplantation for Myelofibrosis

Myelofibrosis, either primary (PMF) or secondary myelofibrosis is a BCR/ABL1-negative MPN for which transplantation remains the only curative option. Transplantation for clonal MF is usually performed in more advanced stages of the disease in patients with a DIPSS-plus beyond 1, until recently almost exclusively with an HLA-matched donor. Multiple studies documented outcomes of patients treated with HLA-matched donors; however, the experience with alternative donor transplants is very limited [13, 14]. Results with UCB transplantation (UCBT) from two reports show lower engraftment rate (57%) [14] and higher TRM at 2 years (35% and 64%, respectively, in the two reports). These results suggest that engraftment might be more difficult to achieve with CBT.

PTCy may not only mitigate the risk of G*v*HD but also favor engraftment in haploidentical transplants. Multiple studies in haploidentical transplant patients performed with PTCy in other hematological malignancies showed high engraftment rates and low incidence of acute and chronic G*v*HD, suggesting that this approach could be explored in patients with myelofibrosis, for which primary engraftment has posed more challenges as compared with other hematologic malignancies due to the extensive BM fibrosis.

While still a small number of patients with clonal MF have been treated to date with haploidentical donors and PTCy-based G*v*HD prophylaxis, early results are encouraging. In a case-series of 95 patients undergoing allo-HCT for myelofibrosis between 2001 and 2014 published by Bregante and coworkers [28], a total of 49 patients were included in the "alternative donor" group (26 patient with HLA-MMUD and 23 patients with a haploidentical donor); most of patients were conditioned with thiotepa, busulfan, and fludarabine (TBF) regimen and received BM grafts [28]. All patients receiving haplo-HCT had PTCy-based G*v*HD prophylaxis regimen. Engraftment was 92% with median time to engraft of 21 days (range 15–50). One-year TRM in the alternative donor group was reported to have improved from 56% to 31% when comparing outcome of transplants before and after 2010. When survival of patients who received transplants from HLA-MSD compared with that for those who received alternative donor grafts (HLA-MMUD + haploidentical donors) in the two periods, actuarial survival in the 2000–2010 period was 45% *versus* 21% ($p = 0.02$) and in recent years (2011–2014) was 72% *versus* 69% ($p = 0.6$), respectively [28], a significant improvement for patients treated with haploidentical donors. Authors concluded that outcome of allografts in patients with myelofibrosis has improved in recent years due to reduction of both TRM and relapse. Improvement was most significant in alternative donor transplantations, with modifications in donor type and conditioning regimen.

A small number of patients with myelofibrosis (n = 4) received a melphalan-based reduced intensity conditioning (RIC) haplo-HCT with PTCy at MDACC. The median age was 61 years. Two patients had primary and two had secondary myelofibrosis. All patients progressed to AP or BP. All patients received chemotherapy/JAK2 inhibitors prior to transplantation, and all achieved morphologic remission (<5% blasts) prior to transplant. Conditioning regimen consisted of fludarabine and melphalan (FM100) with thiotepa 5 mg/kg or 200 cGy total body irradiation (TBI). All patients had PTCy, tacrolimus, and mycophenolate mofetil (MMF) for G*v*HD prophylaxis and a BM graft. All patients engrafted the donor cells with 100% donor chimerism on day 30 post-transplant. Median time to neutrophil and platelet engraftment was 24 and 44 days. None developed grade II–IV acute or chronic G*v*HD. One patient with advanced disease died of TRM and one of relapsed disease. Two patients (50%) were alive and disease free after 6 months and 2 years post-transplant (Ciurea SO, personal communication). Although only a small number of patients have been treated to date, these results have been encouraging and showed feasibility of this approach.

17.4 Expert Point of View

Haplo-HCT for MPNs can now be performed with lower TRM and reliable engraftment, especially for patients with myelofibrosis, who traditionally had a higher rate of graft failure. PTCy appears to attenuate not only the alloreactivity in the graft-*versus*-host but also in the host-*versus*-graft direction and facilitate more reliable engraftment of donor cells. While CML patients are younger and can tolerate more intense conditioning, myelofibrosis patients tend to be older, and myeloablative conditioning can be associated with prohibitive TRM. Disease control at the time of transplant (morphologic remission) continues to be an important goal for MPNs also, and haplo-HCT is now a reliable graft source for patients without HLA-matched donors with outcomes almost similar to HLA-matched donors.

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Haloidentical Transplants: Immune Reconstitution With and Without Augmentation Strategies

18

Antonio Di Stasi and Leo Luznik

18.1 Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) is a lifesaving procedure for many neoplastic and nonmalignant hematologic disorders [1, 2]. Following myeloablative and reduced-intensity conditioning regimens therapy, patients undergo a period of marrow aplasia and neutropenia lasting 2–3 weeks. Important factors that determine the speed of engraftment are the total nucleated cell (TNC) dose and/or $CD34^+$ cell dose in the hematopoietic graft [3]. In the case of haploidentical hematopoietic cell transplantation (haplo-HCT), the donor is HLA matched at only one haplotype.

As in HLA-matched transplantation, T-cell recovery after haplo-HCT relies on peripheral expansion of T-cells infused with the graft and the thymic reconstitution mediated by the precursors emerging from the donor progenitor cells. The reconstitution of T-cells in thymus is a slower process in adults, which usually explains a slower reconstitution in adults compared with children. $CD4^+$ T-cells reconstitute later than $CD8^+$ T-cells and depend more on thymic generation of $CD4^+CD45RA^+$ naive T-cells with resulting inverted $CD4^+/CD8^+$ ratio earlier after allo-HCT [4]. Increasing age is associated with thymic atrophy and loss of function, additionally aggravated by intensity of conditioning regimen, infections, and/or G*v*HD,

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predisposing to delayed immune reconstitution and an increased risk for opportunistic infections, which has opened the door to thymus regeneration therapies [5].

The B-cell compartment representing the humoral immunity has a typical slower recovery rate, as compared with T-cell compartment. Delayed recovery of memory B-cells results in decreased levels of circulating immunoglobulins, impaired immunoglobulin class switching, and a loss of required complexity in immunoglobulin gene rearrangement patterns, with increased susceptibility to infection especially with encapsulated bacteria.

The use of HLA-haploidentical donors has extended the applicability of allo-HCT for patients without HLA-matched donors. Much has been learned about immune recovery posttransplant by changing how haplo-HCT has been performed over time. Initially, unmanipulated T-cell-replete haplo-HCT performed with conventional G*v*HD prophylaxis has been associated with intense alloreactivity in the graft-*versus*-host and host-*versus*-graft reactions with high rate of graft rejection and hyperacute G*v*HD [6]. Administration of $CD34^+$ selected grafts after T-cell-depleted (TCD) haplo-HCT is associated with control of bidirectional alloreactivity between the donor and recipient and rapid neutrophil recovery (median of 11–12 days); however, considering the high degree of HLA disparity and the absence of T-cells from the graft, a higher rejection rate up to 20% has been observed, usually mitigated by combination of intense conditioning, i.e., MAC and large doses or "megadoses" of $CD34^+$ selected cells (see Chaps. 1–3) [7]. The early outcomes of extensive TCD have been associated with a profound immunodeficiency in the first year post allo-HCT, which coupled with major HLA mismatching in haplo-HCT may cause extremely high rates of infectious complications, especially viral and fungal, and high non-relapsed mortality (NRM) and likely contributed to a higher incidence of disease relapse posttransplant [8–10].

These findings have contributed to better understanding of limitations of extensive TCD in this form of transplantation and made a major shift in how haplo-HCTs are performed. Attempts to overcome this major limitation of very delayed immune reconstitution have been made by administering T-cells early posttransplant to stimulate immune reconstitution in conjunction with regulatory T-cells to prevent G*v*HD [10] (Chap. 3), by administration of a modified DLI in which alloreactivity is controlled by photodepletion [11] (Chap. 6), or by a suicide gene incorporated in the T-cells [12, 13] (Chaps. 3 and 19), partial αβ TCD [14, 15] (Chap. 3), and posttransplant cyclophosphamide (PTCy; Chap. 7) application for patients receiving a MAC HLA-haploidentical bone marrow (BM) graft [16, 17].

18.2 Immune Reconstitution Profile and *In Vitro* T-Cell Depletion

18.2.1 Extensive Depletion of T-Cells from the Graft

Extensive TCD of the haploidentical graft, obtained primarily by $CD34^+$ selection, has been associated with rapid expansion of natural killer (NK) cells in the first few

months posttransplant despite T- and B-cells being very low or nonexistent [18]. The rapid recovery of NK cells after haplo-HCT is based on an expansion of the cytokine-producing $CD56^{bright}$ NK subsets. Also, monocyte engraftment is rapid, with normal values observed approximately 2 weeks posttransplant. In TCD haplo-HCT, a significant delay in T-cell subset recovery posttransplant was noted with low absolute numbers and function extending well beyond 6–12 months posttransplant. The NK cells, which mediate antitumor effects, are phenotypically and functionally immature in the first 3–6 months posttransplant with *low* killer immunoglobulin receptor (KIR) expression, *high* NKG2A expression, and inverted $CD56^{bright}$/$CD56^{dim}$ ratio as well as decreased killing potential against K562 cell line [19–21]. These findings suggest a deficient antitumor effect early posttransplant, not only after haploidentical transplantation but all forms of allo-HCTs. It is to be noted that higher NK cell numbers noted in some patients have been associated with lower relapse rate and improved survival posttransplant [22, 23].

Although the factors affecting the more prompt recovery and the active functional status remain elusive, for example, regarding NK cells, factors that can affect outcome include the state of maturation of early recovering NK cells, the number of receptor-ligand mismatch pairs, the presence of inhibitory KIRs on the donor NK cells, and the absence of corresponding KIR ligand in the recipient's HLA repertoire (a receptor-ligand model) rather than being based on the ligand-ligand mismatch model (see Chap. 10).

In addition, this decreased antitumor effect in the first 3–6 months post all-HCT, lymphopenia, and low thymic function expose the patient to viral or fungal infections leading to high treatment- related mortality (TRM), especially in TCD haplo-HCT [8, 24, 25]. A polyclonal T-cell repertoire comparable to age-matched controls is achieved at about 4–6 years after allo-HCT, through de novo thymic production, which usually is delayed in older patients and/or in patients with G*v*HD. Monitoring immune reconstitution of cellular subsets and performing assay to evaluate the functionality of those cells (e.g., pathogen-specific interferon enzyme-linked immunosorbent assay, ELISpot) would allow the identification of patients at risk for such complications. Clave et al. found in pediatric recipients of $CD34^{+}$ selected haplo-HCT that a T-cell receptor signal joint excision circle (TREC), a surrogate marker of post-thymic naïve T-cell emigrants, value below the limit of detection was associated with an increased risk of disease relapse [26]. However, some of those assays require a fine expertise and should be subjected to validation and standardization. Several reports have correlated the absolute lymphocyte count (ALC) at day +30, and overall an ALC $\geq$300/μL has a significant impact on TRM and survival [24, 27]. The ALC is a simple and easy parameter to obtain and might help guide in tailoring adoptive immunotherapy strategies but should be validated in prospective studies [28].

Invariant natural killer T-cells (iNKT), a subset of NK cells, play a role in both tumor surveillance and G*v*HD, with their early reconstitution predictive of both acute G*v*HD and survival. de Lalla et al. [29] found that in 33 pediatric patients receiving TCD haplo-HCT for hematological malignancies, iNKT cell recovery (both the number and function) to normal ranges was associated with maintenance

of remission, whereas iNKT cells failed to reconstitute in all eight patients who had disease relapse [30]. Therefore, their rate of depletion during TCD graft processing and their fate during immune reconstitution are worth investigating. *Ex vivo* expansion of all the abovementioned cellular subsets is feasible and under active investigation.

These considerations clearly suggest that strategies to boost immune reconstitution may exert a significant impact on the morbidity, mortality, relapse, and survival after haplo-HCT. Strategies to overcome the limitations arising from extensive TCD aiming to enhance immune reconstitution posttransplant have been developed: (1) a selective T-cell removal with depletion of αβ T-cells, $CD8^+$ T-cells, or alloreactive T-cells [31] or (2) *in vivo* allo-depletion using a T-cell-replete graft followed by PTCy [32] or (3) administration of genetically modified donor T-cells with lower potential to generate G*v*HD [33]. These strategies to enhance immune reconstitution will be discussed later in the chapter. A diagram showing the most widely used graft processing and post-grafting modulation strategies for haplo-HCT is presented in Fig. 18.1.

18.2.2 Immune Reconstitution Profile and Selective T-Cell Depletion

Depletion of αβ T-cells and/or B-lymphocytes has been performed with the rationale to reduce the risk of G*v*HD while preserving the anti-infectious and antitumor activity of remaining cell subsets, such as monocytes, dendritic cells (DCs), γδ T-cells, and NK cells. In a retrospective analysis, a better recovery of γδ T-lymphocytes has been associated with an increased 5-year leukemia-free survival (LFS) and overall survival (OS) [54% *vs.* 19% ($P < 0.0003$) and 71% *vs.* 20% ($P < 0.0001$), respectively], with no increase in the incidence of G*v*HD ($P = 0.96$) [34] (Chap. 3).

Evaluation of immunologic reconstitution after αβ TCD haplo-HCT showed that the early T-cell recovery in this form of transplant is based mainly on γδ T-lymphocytes, which represent the predominant T-cell subset until day 45–60 posttransplant [35]. In a prospective evaluation of immunologic reconstitution after αβ T-/B-cell-depleted haplo-HCT, Airoldi and colleagues showed that after an initial expansion of γδ T-lymphocytes, the αβ population gradually increased over time with higher αβ cell numbers seen in patients who had higher numbers of γδ T-cells infused [15]. Among the γδ T-cell subsets, Vδ2 predominates in the first month posttransplant, when both Vδ2 and Vδ1 are predominantly of central memory subsets. When compared with $CD34^+$ selected grafts, recipients of αβ TCD haploidentical transplants had higher numbers of γδ T-cells early posttransplant [15]. The γδ T-cells obtained from the peripheral blood of transplanted patients were able to kill primary leukemia blasts with cytotoxicity enhanced by zoledronic acid. Moreover, Vδ1 appeared to preferentially expand in patients with CMV reactivation [15].

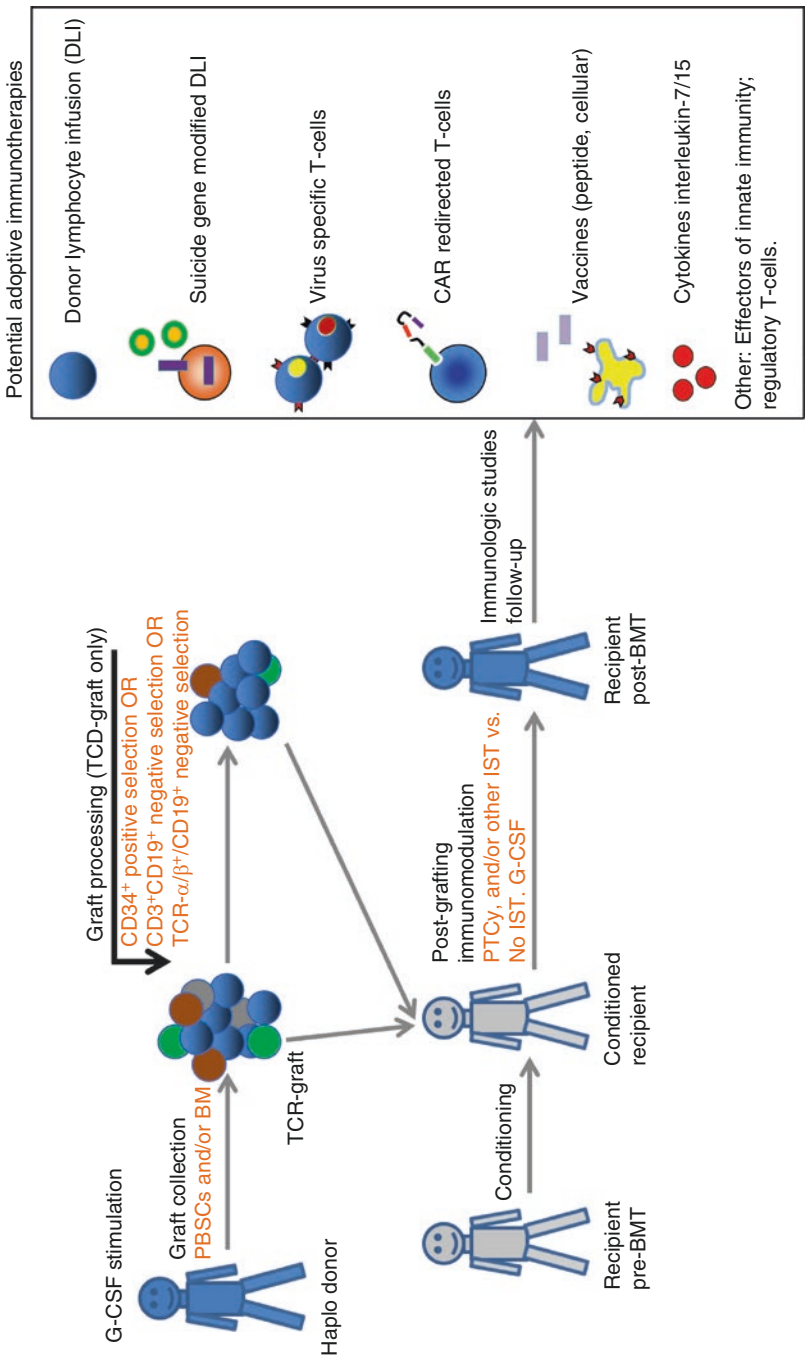

Fig. 18.1 Haploidentical hematopoietic cell transplant modalities in regard to graft processing, post-grafting immunomodulation, and most investigated adoptive immunotherapy strategies to boost immune reconstitution. *G-CSF* granulocyte colony-stimulating factor, *TCD* T-cell deplete, *TCR* T-cell replete, *PTCy* posttransplant cyclophosphamide, *CAR* chimeric antigen receptor

Clinical trials are also evaluating the impact on immune reconstitution and clinical outcomes of αβ TCD add-back after T-cell-replete haplo-HCT performed with PTCy-based G*v*HD prophylaxis (clinicaTrials.gov NCT02193880) (see Chap. 4).

18.3 Immune Reconstitution Profile and *In Vivo* T-Cell Depletion

18.3.1 Posttransplant Cyclophosphamide

Cyclophosphamide, an alkylating agent, applied early post allograft, has been shown to prevent skin allograft rejection in murine models [36] and had been appreciated as potentially useful in controlling G*v*HD after allo-HCT [37] (see Chaps. 7 and 8). More recently, application of cyclophosphamide after haplo-HCT has allowed the use of a T-cell-replete bone marrow graft with effective control of G*v*HD and a lower incidence of infectious complications, and it is gaining widespread acceptance due to the low cost (see Chap. 7), lack of sophisticated devices to manipulate the graft, and rapid applicability (see Chap. 19). Preclinical experiments first, and clinical trials later, have shown that PTCy administrations result in a "partial *in vivo* TCD," of both anti-host T-cells and recipient anti-donor alloreactive T-lymphocytes. For haplo-HCT using T-cell-replete grafts, median time to neutrophil recovery is approximately 18–19 days with a bone marrow graft [38, 39] and about 1–2 days sooner with peripheral blood graft [40], with a lower rejection rate compared with TCD haplo-HCT.

Several studies examined immune reconstitution after haplo-HCT with PTCy-based G*v*HD prophylaxis. Data from the MD Anderson Cancer Center (MDACC) showed that patients treated with a T-cell-replete haplo-HCT with PTCy had improved outcomes as compared with patients receiving complete TCD via CD34 selection [8]. This study [8] analyzed 65 adult patients with hematologic malignancies who received T-cell-replete graft and PTCy-based G*v*HD prophylaxis ($n = 32$) or TCD graft with $CD34^+$ selection ($n = 33$) haplo-HCT. Patients receiving T-cell-replete haplo-HCT had improved outcomes with lower 1-year NRM of 16% *versus* 42% ($P = 0.02$) and better 1-year OS and progression-free survival (PFS) of 64% *versus* 30% ($P = 0.02$) and 50% *versus* 21% ($P = 0.02$), respectively, while the incidence of grades II–IV acute G*v*HD, 20% *versus* 11% ($P = 0.20$), and less chronic G*v*HD (7% *versus* 18% ($P = 0.03$) [8] was not different. Data from Ciurea et al. [8] showed that improvements in immunologic reconstitution of T-cell subsets were associated with a significant lower NRM attributed to infectious complications (9% and 24%, respectively ($P = 0.01$)) as compared to recipients of TCD haplo-HCT. Patients in the TCD group were 5.6 times more likely to develop an invasive fungal infection within 6 months after transplant [8].

With PTCy, donor stem cells and memory T-lymphocytes are spared, owing to their quiescent nature as well as high level of aldehyde dehydrogenase, an enzyme that allows the cell to be resistant to various drugs, including cyclophosphamide, contrarily to NK cells, or naïve T- and B-lymphocytes [41]. Persistence of stem

cells reconstitutes donor hematopoiesis after haplo-HCT, while memory T-lymphocytes defend the host against posttransplant infections, or potentially disease relapse. Cieri and colleagues also showed that after haplo-HCT with PTCy, memory stem T-cells can also differentiate directly from naïve precursors infused within the graft and that the extent of their generation might correlate with interleukin-7 (IL-7) serum levels [42]. Regulatory T-cells in mouse and human models are resistant to PTCy-induced cytotoxicity. This is due to increased expression of aldehyde dehydrogenase enzyme upon allogeneic stimulation in a lymphopenic environment [43, 44].

More recently, data from different centers and from CIBMTR have reported comparable outcomes between haplo-HCT and HLA-matched donor transplant [16, 45–49] (Chap. 19). In addition, immune reconstitution of T-cell subsets ($CD4^+$ or $CD8^+$, naïve, memory T-lymphocytes, NK cells, and B-lymphocytes) appeared to be similar with HLA-matched donor transplants when similar groups of patients were compared who received haplo-HCT [46]. Di Stasi et al. evaluated immune reconstitution in a cohort of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) patients treated with the same conditioning regimen and HLA-matched related, HLA-matched unrelated, and haplo-HCT with PTCy and showed that with the exception of higher absolute numbers of $CD4^+$ and $CD8^+$ on day 30 posttransplant seen in HLA-matched related donor transplants only, patients who received a haploidentical graft had remarkably similar immune reconstitution of T-cell subsets in the first 6 months posttransplant with normal $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and $CD56^+$ numbers seen in all three groups by 6 months posttransplant [46].

However, when comparing immune reconstitution in 71 patients undergoing MAC haplo-HCT with PTCy, mycophenolate mufti (MMF), and tacrolimus G*v*HD prophylaxis with 73 patients undergoing MAC HLA-matched allo-HCT with PTCy (days +3 and +4) as sole G*v*HD prophylaxis, the Johns Hopkins group showed that after PTCy-based G*v*HD prophylaxis, there is comparable reconstitution of NK- and B-cells, while early recovery of $CD4^+$ and $CD8^+$ T-cell lags after haplo-HCT when compared to recovery after HLA-matched transplants. The authors reported that this delay can potentially be attributable to the addition of MMF and tacrolimus, which may be mitigated by discontinuation of MMF at day 35, resulting in equivalent $CD4^+$ T-cell and $CD8^+$ T-cell numbers by 3 and 6 months, respectively [30].

18.4 Strategies to Enhance Immune Reconstitution After Haploidentical Transplantation: Do They Work?

18.4.1 Donor Lymphocyte Manipulation and Infusion

Under selected circumstances, donor lymphocyte infusion (DLI) is an effective strategy for conferring antiviral and antitumor immunity following allo-HCT. However, to avoid G*v*HD, one strategy has been to stimulate donor T-cells with host stimulators and remove activated T-cells in virtue of their overexpression

of activation markers, such as $CD25^+$. In a previous experience from colleagues at Baylor College of Medicine, haploidentical donor T-cells had been depleted of >90% $CD25^+$ alloreactive precursors *in vitro* by stimulating them with recipient *Epstein-Barr virus* (EBV)-transformed B-lymphoblastoid cell lines (EBV-LCLs) [50], followed by the application of an anti-CD25 immunotoxin conjugate. Allo-depleted expanded T-cells were infused at two dose levels (10^4 and 10^5 cells/kg of recipient weight/dose) into recipients of CD34 selected (i.e., TCD) haplo-HCT. Patients receiving 10^5 cells/kg of recipient/dose showed significantly improved T-cell recovery at 3, 4, and 5 months after haplo-HCT compared with those receiving 10^4 cells/kg of recipient/dose ($P < 0.05$). Accelerated T-cell recovery occurred as a result of expansion of the effector memory population ($P < 0.05$). T-TRECs were not detected in recovering T-cells in dose level 2 patients, indicating they were likely to be derived from the infused allo-depleted cells. Spectratyping of the T-cells at 4 months demonstrated a polyclonal Vβ repertoire. Using tetramer and ELISpot assays, *Cytomegalovirus* (CMV) and EBV-specific responses were detected in four of six evaluable patients at dose level 2 as early as 2–4 months after transplantation, whereas such responses were not observed until 6–12 months in dose level 1 patients. The incidence of significant acute (2 of 16 patients) and chronic G*v*HD (2 of 15 patients) was low. The amount of cells infused was subsequently escalated to 10^6 cells/kg of recipient weight without evidence of G*v*HD. Although this approach reconstituted antiviral immunity, relapse remained a major problem, and six patients transplanted for high-risk leukemia relapsed and died of the disease. Higher T-cell doses could be useful to reconstitute antitumor immunity and to provide an antitumor effect, since the estimated frequency of tumor-reactive precursors is 1–2 logs less than the frequency of viral-reactive precursors. However, in some patients, these doses of cells were still sufficient to trigger G*v*HD even after allo-depletion, as confirmed in studies with CD25 allo-depletion performed in other centers [31].

18.4.2 Suicide Gene Modification of Donor T-Cells

Suicide gene modification of DLI has enabled the administration of high dose of T-cells with a goal to better fight viral infections and to exert antitumor immunity, with the ability to eliminate them in case of adverse events, such as G*v*HD, or other off-target effects. A suicide gene is a genetically encoded molecule that allows selective destruction of adoptively transferred cells. Suicide gene addition to cellular therapeutic products can lead to selective ablation of gene-modified T-cells, preventing collateral damage to contiguous cells and/or tissues.

Two suicide gene approaches have been validated in the clinical setting to date: the inducible caspase-9 (iC9) and the herpes simplex thymidine kinase (HSV-TK) safety switches (also see Chap. 19).

18.4.2.1 Inducible Caspase-9 Suicide Gene

The iC9 consisted of a *FKBP12-F36V* domain linked to Δcaspase-*9*, which is *caspase-9* without its physiological dimerization domain. The FKBP variant is

optimized to bind a chemical inducer of dimerization (CID), or AP1903. Administration of this nontherapeutic small molecule results in cross-linking and activation of the proapoptotic target molecules. In the first inhuman clinical trial, patients who had undergone CD34 selected haplo-HCT for hematological malignancies were administered escalating doses (1×10^6 to 1×10^7 cells/kg of recipient weight) of iC9-modified allo-depleted T-cells from day +30 onward. The iC9-modified T-cells expanded and were detected in the peripheral blood as early as 7 days after infusion and persisted in surviving patients. The engrafted iC9-modified T-cells included both $CD4^+$ and $CD8^+$ T-cells and predominantly had an effector-memory or central-memory phenotype. Four of ten patients developed acute G*v*HD grades I–II of the liver and/or skin. The administration of a single dose of 0.4 mg/kg of recipient weight AP1903 resulted in the apoptosis of ≥90% of iC9-modified T-cells within 30 min, followed by the rapid (within 24 h) and permanent abrogation of G*v*HD. Remarkably, residual iC9-modified T-cells were able to re-expand, containing antifungal- and antiviral-specific precursors, and had a polyclonal TCR Vβ repertoire, without recurrence of G*v*HD or onset of chronic G*v*HD. Both $CD4^+$ and $CD8^+$ T-cells were detected long term in the peripheral blood. However, the CD4:CD8 ratio of $CD3^+CD19^+$ T-cells was initially low and progressively declined with time, from 0.3 to 0.11 in median value at 2 and 9 months, respectively, with the ratio falling further to 0.09 in median value at 24 months. The donor graft is intensively depleted of T-cells (4–5 logs), donor T-lymphocytes may not appear for 6 months or longer after such haplo-HCT, and the delay may be even longer for the $CD4^+$ subset. In this study, however, the early rise in the infused $CD3^+CD19^+$ T-cells was rapidly followed by recovery of endogenous $CD3^+CD19^{neg}$ T-cells compared with patients who underwent haplo-HCT without T-cell add-back at concurrent points posttransplant. At the time of T-cell infusion, $CD3^+CD19^-$ cells were 36 ± 19 cells/μL but had increased by a mean 25-fold within 6 months and 42-fold by 12 months. Thus, the mean absolute counts of $CD3^+CD19^{neg}$ were greater than 500 per μL at 3 months after iC9-T-cell infusion (5 months posttransplantation), in contrast to delays of up to 12 months posttransplant in patients without T-cell add-back. The phenotype of the recovering endogenous $CD3^+CD19^-$ cells was, however, quite distinct from that of the infused $CD3^+CD19^+$ T-cells, and the populations retained this difference with time. Thus, $CD3^+CD19^{neg}$ cells were predominantly $CD4^+$ rather than $CD8^+$ T-cells, compared with their $CD3^+CD19^+$ counterparts. Moreover, 49% and 44% of the $CD3^+CD19^{neg}$ cells expressed markers typical of naive T-cells at 6 and 12 months, respectively. As anticipated, numbers of NK cells and B-lymphocytes also normalized over time, but there was no evident acceleration relative to previously reported values at any given time after haplo-HCT [13, 51]. Zhou and colleagues have recently confirmed these findings in patients treated with iC9-DLI without prior allo-depletion step [52]. This group infused 12 haplo-HCT patients with increasing numbers of allo-replete haploidentical T-cells expressing the iC9 T-cells to determine whether the iC9-T-cells produced immune reconstitution and if any resultant G*v*HD could be controlled by administration of a CID (AP1903); all patients receiving $>10^4$ allo-replete iC9-T lymphocytes/kg achieved rapid reconstitution of immune responses toward five major pathogenic

viruses and concomitant control of active infections. Four patients received a single AP1903 dose. CID infusion eliminated 85–95% of circulating $CD3^+CD19^+$ T-cells within 30 min, with no recurrence of G*v*HD within 90 days. In one patient, symptoms and signs of G*v*HD-associated cytokine release syndrome (CRS-hyperpyrexia, high levels of pro-inflammatory cytokines, and rash) resolved within 2 h of AP1903 infusion. One patient with varicella zoster virus meningitis and acute G*v*HD had iC9-T-cells present in the cerebrospinal fluid, which were reduced by >90% after CID. Notably, virus-specific T-cells recovered even after AP1903 administration and continued to protect against infection. Hence, allo-replete iC9-T cells can reconstitute immunity after transplant and administration of CID can eliminate them from peripheral blood and the central nervous system (CNS), leading to rapid resolution of G*v*HD and CRS. The approach may therefore be useful for the rapid and effective treatment of toxicities associated with infusion of engineered T- lymphocytes.

18.4.2.2 HSV-TK Suicide Gene

The largest study of suicide gene engineered DLI after haplo-HCT was published in 2009 by the Milan group [12]. This phase I and II study enrolled 50 patients with high-risk hematologic malignancies out of which 28 were eligible for HSV-TK DLI. Infusions started at day 28 after transplant and continued monthly up to a total of four infusions, with doses ranging from 0.9 to 40 × 10^6 cells/kg of recipient weight. After HSV-TK DLI, no G*v*HD prophylaxis was administered. Patients with TK-cell engraftment promptly achieved $CD3^+$ counts of 100 cells/μL or more at median of 75 days (range 34–127) from transplantation and 23 days (13–42) from TK-cell infusion, whereas patients without engraftment remained immunodeficient. Immune reconstitution seemed dependent on dose of TK-cells: median infusion to achieve immune reconstitution was 11 × 10^6/kg. The $CD8^+$ T-cells recovered more rapidly than did $CD4^+$ lymphocytes. At immune reconstitution, TK-cells formed a high proportion of circulating lymphocytes, whereas at later time points, the percentage of TK-cells progressively decreased, also due to expansion of untransduced lymphocytes. Reconstitution was recorded only in those patients with TK-cell engraftment. At immune reconstitution nearly all circulating T-lymphocytes had an effector-memory phenotype similar to that of infused cells; 6 months later, naive and central memory T-lymphocytes appeared. At 1 year, the distribution of T-cell subsets normalized. The T-cell repertoire conferred by TK-cells progressively developed from being oligoclonal to being polyclonal, and, by 1 year, it was indistinguishable from that of healthy individuals.

Antivirus-specific immune responses analyzed at immune reconstitution were significantly higher than at baseline before TK-cell infusion, with normalization of antiviral responses and reduced cumulative incidence of NRM in TK-treated immune-reconstituted patients: 14% (infectious mortality 9%) *vs.* 60% in non-immune-reconstituted patients. Ten of twenty-two immune-reconstituted patients developed acute G*v*HD, and one had chronic G*v*HD. All ten patients were treated with ganciclovir at 5 mg/kg twice daily for 2 weeks and obtained complete resolution of G*v*HD. Some patients required transient courses of other immunosuppressants,

including steroids, cyclosporine, and mycophenolate mofetil (MMF) which were added for treatment of chronic G*v*HD. There were no cases of ganciclovir resistance, no progression from acute to chronic G*v*HD, and no G*v*HD-associated deaths in this study. Contrary to the iC9 suicide gene, almost completely human derived, HSV-TK proved immunogenic in immune-competent patients with limited persistence of HSV-TK cells [53]. Additionally, ganciclovir-resistant truncated HSV-TK forms have been observed [54]. In both the HSV-TK and iC9 studies, infusion of suicide gene modified cells aided non-gene modified T-cell immune reconstitution [12, 13], as a consequence of IL-7 secretion by gene modified cells [55]. The lack of further acute G*v*HD in these studies might suggest either complete elimination of alloreactive cells or development of peripheral tolerance. Additionally, the incidence of chronic G*v*HD was low in the HSV-TK T-cell studies and absent in the iC9 trial [13, 51]. Although while performing TCD haplo-HCT the safety and feasibility to infuse suicide gene-modified T-cells without immunosuppressive treatment have been demonstrated, one preclinical strategy has been to generate T-cells that are resistant to posttransplant immunosuppression, including tacrolimus, MMF, or corticosteroids, as nicely reviewed elsewhere [56]. Considering that the activation of a suicide gene to treat G*v*HD has the potential to eliminate tumor-reactive T-cells, tumor antigen-specific T-cells could obviate this side effect.

It is interesting to note that both the *in vitro* allo-depletion with targeted antibodies, *in vivo* allo-depletion strategy using PTCy, and cell elimination with the iC9 suicide gene led to the preferential elimination of alloreactive cells while sparing at least some anti-infectious precursors. The same pattern is anticipated also in studies using *ex vivo* photodepletion. The photosensitizer dibromorhodamine accumulates in activated T-cells but not in resting T-cells leading to the elimination upon visible light exposure (514 nM). The elimination of activated T-cells is attributed to the inhibition of the P-glycoprotein pump. An interim analysis of 13 patients treated on phase 2 clinical trials showed promising results in $CD34^+$ T-cell-depleted haplo-HCT, without any G*v*HD prophylaxis.

18.5 Administration of Regulatory T-Cells (T_{regs})

T_{regs} are a subset of T-cells (see Chap. 4) whose function is to suppress immune responses and maintain self-tolerance. A transcription factor called FoxP3, a member of the fork head family of transcription factors, is critical for the development and function of T_{regs} and is used as one of the main marker to identify them. Natural T_{regs} are derived from the thymus and are characterized by the co-expression of $CD4^+$ and high expression of $CD25^+$ and FoxP3. Induced or adaptive T_{regs} are generated in peripheral lymphoid organs following adequate antigenic stimulation in the presence of cognate antigen and specialized immunoregulatory cytokines such as transforming growth factor β (TGFβ), interleukin-4 (IL-4), and/or interleukin-10 (IL-10). One interesting approach is to infuse T_{reg} cells, freshly isolated from the donor T or expanded *ex vivo*, with the goal of suppressing G*v*HD in recipients of allo-HCT.

Proof of safety and efficacy in the haplo-HCT setting comes from two recent investigations, where freshly isolated donor T_{regs} were infused on day −4 and conventional T-cells (T_{cons}) were infused on day 0 together with CD34 selected cells, in the absence of pharmacological G*v*HD prophylaxis in patients with high-risk acute leukemia, after TBI-based conditioning [57, 58] (see Chap. 4).

In the first reported experience, at a median follow-up of 11.2 months, no patients developed chronic G*v*HD, and 2 of 26 evaluable patients developed ≥grade II acute G*v*HD. Furthermore, the T_{regs} infused did not inhibit expansion of co-infused T_{cons} allowing for rapid and sustained T-cell subpopulation reconstitution with a reduced incidence of CMV reactivations [57]. The authors recently updated the results of this clinical experience [30, 58], conducted on patients with a median age of 39 years. Fifty-six percent (n = 29 of 52) of patients received 30–35 mg/kg of cyclophosphamide from days −8 and −7, and 44% (n = 23 of 52) patients were given alemtuzumab or thymoglobulin (ATG) instead, 21 days before transplantation, to prevent interference with T_{reg}-T_{cons} adoptive immunotherapy. Sustained full donor engraftment was achieved in the majority of patients (96%, n = 50 of 52).

Only 6 of 41 patients (15%) developed ≥grade II acute G*v*HD. In two patients, G*v*HD responded rapidly to a short course of immunosuppression, and only one of these patients developed chronic G*v*HD.

There was a rapid, sustained increase in peripheral blood T-cell subpopulation recovery. $CD4^+$ and $CD8^+$ counts reached 100/mL at a median of days 40 (range, 25–150) and 45 (range, 18–100) after transplantation, respectively. Compared with historical TCD haplo-HCT controls, $CD4^+$ and $CD8^+$ specific for opportunistic pathogens such as *Aspergillus fumigatus*, *Candida albicans*, *CMV*, adenovirus *(AdV)*, herpes simplex virus *(HSV)*, varicella zoster virus *(VZV)*, and toxoplasmosis emerged significantly earlier (at each time point $P < 0.0001$). Overall, at a median follow-up of 4 years (range, 7–58 months), the cumulative incidence of NRM was 40%, and DFS was 58% (n = 30 of 52). In patients receiving anti-T-cell antibodies or lower dose of cyclophosphamide, NRM was 23% and DFS was 70%. Only 5% (n = 2 of 41) evaluable patients relapsed. These patients had evidence of MRD at the time of transplantation as they were both NPM^+FLT3^+ and had received a transplant from non-NK alloreactive donors. Multivariate analysis identified T_{reg}-T_{con} adoptive immunotherapy as the only predictive factor associated with a reduced risk of relapse (relative risk, 0.06; 95% CI, .02–.35; P = 0.02). The authors interpreted that T_{regs} interfered with the pathophysiology of acute G*v*HD and permitted co-transplantation of enough T_{cons} to eradicate MRD, thus eliminating the usual 30–35% incidence of posttransplantation high-risk acute leukemia relapse without increase in incidence of acute G*v*HD, and were associated with improvement in immunologic reconstitution. Those results are encouraging, and at the same time, a complex *ex vivo* T_{reg} expansion was not required; however, they will need to be confirmed in larger series and/or in other allo-HCT settings. Furthermore, they pave the way for future studies to exploit the G*v*L effect of T_{cons} that recognize minor histocompatibility antigens in HLA-matched transplants, ideally addressing questions such as the impact of T_{regs} coadministration on antigen-specific antitumor responses.

A novel strategy under investigation involves the administration of mesenchymal stromal cells (MSCs), in virtue of their immunomodulatory activity, with report of initial safety and activity data. This therapy can be given without the need for donor-recipient HLA matching [59].

Overall, strategies to control alloreactivity are potentially able to exert a profound impact on the immune reconstitution, reduction of NRM, and relapse rate after allo-HCT. This is particularly relevant for those that allow avoidance of posttransplant immunosuppression, which can have off-target effects and may dampen the immune system.

18.6 Administration on NK Cells

NK cells have potent antitumor and are capable of eliminating virally infected cells, properties that have made them a focus for cancer immunotherapy. Isolation of NK cells form PB and more recently *ex vivo* expanding of this T-cell subset has renewed the promise of the development of effective NK cell therapeutic approaches with or without transplantation. Initial work by Ruggeri et al. suggested utility of exploring how best to use NK cell alloreactivity in this setting [60]. Because most studies could infuse only a limited number of NK cells obtained through the apheresis procedure [61, 62], subsequent studies attempted to expand NK cells *ex vivo* using IL-2 and/or IL-15 have shown limited clinical success [63].

Using a different approach, MDACC group showed that NK numbers and function can be increased by *ex vivo* expansion using feeder cells and APCs expressing mb-IL21. Administration of these NK cells in the early posttransplant period has been proven very safe with no adverse effects [64]. In addition, a very low relapse rate has been noted in the phase I clinical trial when compared with a similar cohort of patients treated without NK cells. This trial demonstrates a lack of major toxicity attributable to third-party NK cell infusions delivered in combination with an HLA-compatible allogeneic transplantation. The infusion of haploidentical alloreactive NK cells was well tolerated and did not interfere with engraftment or increase the rate of G*v*HD after allo-HCT. Durable complete remissions occurred in five patients at high risk for disease recurrence. Moreover, a significantly lower incidence of viral reactivation was seen in this group of patients, in line with previous observations. This approach is being further developed in a phase I and II trial with *ex vivo* expanded NK cells to increase the NK cell dose with the objective of reducing relapse and improving the outcome of allo-HCT for AML and MDS. In addition, several clinical trials are now ongoing to determine the efficacy of NK cell therapy before and after transplant.

18.7 Administration of Antigen-Specific T-Cells

One additional strategy involves the administration of antigen-specific mature T-cells for anti-infectious or antitumor purposes. These strategies have also been adopted prophylactically or preemptively in allo-HCT settings with the goal of reducing relapse rates after transplant.

18.7.1 Add-Back of Virus-Specific T-Cells

Reactivation of latent viruses such as CMV, EBV, HSV, and VZV may cause symptomatic disease. Therefore, it is a routine strategy at many institutions to perform routine monitoring of CMV, EBV, AdV, and human herpesvirus-6 (HHV-6) viremia in recipients of alternative (TCD, cord, and haploidentical) donor transplants. CMV may cause pneumonia, colitis, or retinitis, and EBV can cause posttransplant lymphoproliferative disease (PTLD). HHV-6 infections are generally encountered early posttransplant and can lead to engraftment delays or failure or altered mentation [65]. Polyoma BK virus and/or AdV can lead to hemorrhagic cystitis, associated with significant and prolonged morbidity and in-hospital stay [66]. Although the infusion of unmanipulated graft followed by novel strategies to contain the risk of G*v*HD allows faster immune recovery with a reduced risk of infections, the aforementioned viral infections continue to be a common cause of morbidity and mortality. Treatment dose of currently used antimicrobial drug therapies is frequently associated with toxicities, especially renal insufficiency and pancytopenia. Therefore, an effort has been undertaken to improve anti-infectious immunity after allo-HCT. Giving DLI could be considered for the treatment of EBV viremia or EBV-associated PTLD; however, for other indications such as relapse, it may result in the potential fatal complication of G*v*HD, even when an allo-depletion procedure is performed. The donor seropositivity is also relevant for expanding *ex vivo* virus-specific T-cells, albeit some protocols are under investigation promoting the generation of antigen-specific cells from naïve precursors. In the haplo-HCT setting, clinically investigated strategies for fighting viral pathogens include (1) the generation and add-back of viral antigen-specific T-cells, recognizing up to 12 immunogenic antigens from five viruses, (2) the selection with reinfusion of antigen-specific cells, and (3) off-the-shelf partially HLA-matched virus-specific T-cells. In aggregate, virus-specific T-cells result in a high rate of virological control, a decrease in the use of antiviral therapies, without increasing the rate of acute or chronic G*v*HD [67]. Antifungal-specific T-cells have also been expanded *ex vivo* with control of *Aspergillus* infection after haplo-HCT.

18.7.2 Add-Back of Tumor Antigen-Specific T-Cells

Treatment of G*v*HD can potentially affect the risk of tumor relapse, as although graft-*versus*-tumor (G*v*T) effect in the absence of G*v*HD has been reported, there is overlap between the two mechanisms, considering the HLA disparity both on normal and cancer tissues, and also because minor histocompatibility antigens (mHAgs), responsible of G*v*HD and G*v*T effect, can be expressed on both hematopoietic and non-hematopoietic tissues. Larger trials are needed to confirm the antitumor efficacy of iC9-modified DLI, and antitumor immune

reconstitution data are lacking from patients treated with the HSV-TK DLI, although in the latter, longer follow-up showed that all patients in remission 3 years after transplant remained so in the following years (longest follow-up 9 years) [12, 68]. Additional indirect evidence suggesting a G*v*T effect was the finding of de novo loss of mismatched HLA expression on leukemic blasts in one patient at time of relapse (see Chaps. 19 and 20). Although antitumor-specific T-lymphocyte precursors are present in the blood of transplant donors and are transferred to patient after transplant or DLI, clinical responses responses have been transient potentially because most tumor-associated antigens are aberrantly expressed self-proteins resulting in low-affinity T-cell receptors as a consequence of the thymic negative selection process or because T-cells undergo activation-induced cell death in patients with high tumor burden. Clinical trials of tumor antigen-specific cytotoxic T-lymphocytes or mHAg cytotoxic T-lymphocytes generated *ex vivo* and infused in recipient of HLA-matched related donor HCT showed transient persistence and activity, with serious adverse events reported when targeting mHAgs [56]. The use of cytokine (IL-7, IL-15, IL-21) deserves further investigation to augment the magnitude and persistence of the T-cell responses. Additional interventions aimed at boosting antitumor immunity that deserve further attention in haplo-HCT include vaccination with tumor antigens, the infusion of CAR (or T-cell receptor) gene-modified T-cells redirected toward tumor-associated antigens. Several clinical trials with CAR or T-cell receptor-redirected T-cells are ongoing in patients with hematologic malignancies. In order to minimize the risk of G*v*HD, it is worth considering to engraft a CAR molecule on the surface of virus-specific T-cells, which has been proven feasible with resulting clinical benefit, and the co-expression of a suicide gene for safety may still be desirable.

Conclusions

Allo-HCT is a potentially lifesaving procedure, and the use of HLA haploidentical donors has enabled the majority of eligible and suitable patients to find a donor. Improvement in donor selection and G*v*HD prophylaxis strategies resulted in lower rates of G*v*HD and graft failure. Outcomes of HLA-haploidentical transplants have improved, now approaching outcomes of HLA-matched transplantations, owing at least in part to improvements in immunologic reconstitution as compared with T-cell-depleted haploidentical transplants. Many strategies are under investigation aimed at identifying biomarkers and milestones for immune reconstitution, which are critical for anti-infectious and antitumor immunity. Areas of active investigation focus on improved control of undesirable alloreactive reactions while enhancing antitumor effects and elimination of posttransplantation immunosuppression. Improvements in immunologic reconstitution posttransplant hold the key to further improving the transplant outcomes, both for treatment-related mortality and prevention of disease relapse, and haplo-HCT represents the prime setting to investigate that.

18.8 Key Points

- Extensive depletion of T-cells from the HLA-haploidentical graft is associated with profound and prolonged immunodeficiency posttransplant.
- PTCy after a T-cell-replete graft retains quiescent memory T-cells and is associated with better immune reconstitution posttransplant.
- Approaches to improve immune reconstitution in the first 6 months posttransplant with modified T-cell subsets are being explored.

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19 Prevention and Treatment of Relapse After HLA-Haploidentical Hematopoietic Cell Transplantation

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19.1 Introduction

Haploidentical hematopoietic cell transplantation (haplo-HCT) from a first-degree-related HLA-haplotype-mismatched donor (children, parents, siblings) offers a transplant option to patients without an HLA-matched donor [1]. Historically, outcomes with unmanipulated T-cell replete haploidentical transplants in the 1970s, using *conventional* graft-*versus*-host disease (G*v*HD) prophylaxis methods, were associated with unacceptably high non-relapse mortality (NRM) from intense bidirectional alloreactivity with the development of graft rejection and hyperacute G*v*HD (see Chaps. 1 and 2) [2–4]. Moving forward, such haplo-HCT-related complications, i.e., graft rejection and hyperacute to acute G*v*HD, were tackled using T-cell depletion (TCD) techniques developed in the 1980s, but relapse and NRM remained high due to slow immunological recovery and infections (see Chap. 2) [5–8]. The seminal observations by Santos and coworkers in 1966 showed that G*v*HD induced in rats could be eliminated by using high-dose posttransplant cyclophosphamide (PTCy); this strategy paved the way to a safer haplo-HCT platform [9, 10]. The PTCy selectively eliminates alloreactive donor and recipient T-cells but spares the quiescent hematopoietic progenitor and stem cells due to high levels of

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aldehyde dehydrogenase, an enzyme responsible for cellular resistance to cyclophosphamide [11] (see Chap. 7). Overall, PTCy approach is associated with a faster immunological recovery relative to T-cell depletion (TCD) approaches in addition to being a relatively less expensive strategy that does not require cumbersome graft manipulation (see Chap. 18) [12]. Several retrospective studies have reported similar outcomes after haplo-HCT and HLA-matched-related or HLA-matched-unrelated hematopoietic cell transplantation (HCT) [13–17]. Improvements in NRM using PTCy have left disease relapse as the main cause of treatment failure, a similar problem encountered with transplants from HLA-matched-related and HLA-matched-unrelated donors [13]. While, in general, etiology of relapses after haplo-HCT may overlap with other types of allogeneic transplants, unique relapse mechanism with loss of heterozygosity (LOH) is observed only after haplo-HCT (also see Chap. 20).

19.2 Why Recommend Haploidentical Transplant?

There are several advantages for using haploidentical donors, for instance, immediate availability of the graft with no additional registry cost; this is in contrast to additional costs associated with maintaining an unrelated donor registries. Haplo-HCT is becoming increasingly instrumental in developing nations with limited resources which have access issues to unrelated donor registries [1]. Timely procurement of acceptable graft especially in high-risk patients is another major advantage given comparable outcomes (albeit retrospective) using alternative hematopoietic graft sources. Moreover, HLA-mismatched haploidentical-related donors may expand the availability of hematopoietic grafts for patients in ethnic minorities; donors for such group of patients are underrepresented in the national registries. Currently, approximately 30% of Caucasians, 70% of Hispanics, and 90% of African-Americans do not have HLA-matched unrelated donors (MUD) in the worldwide registries [18]. Furthermore, family HLA-haploidentical donors have rapid turnaround time and, if needed, availability for lymphocyte infusion and second haploidentical graft. The former approach can be done as a prophylactic or preemptive or at disease relapse in select group of individuals even in haplo-HCT setting.

19.3 Strategies for Prevention of Relapse After Haploidentical Transplant

19.3.1 Conditioning Regimen Intensity, Selection of a Transplant Donor and Graft

19.3.1.1 Conditioning Regimens in Haplo-HCT

Allogeneic hematopoietic cell transplantation (allo-HCT) provides potential advantage over high-dose chemotherapy (HDT) and autologous hematopoietic

cell rescue with its immunologically induced G*v*T effect. However, the intensity of the conditioning regimen remains an important aspect in certain group of patients and diseases, i.e., young adults with advance disease who have not had prior autologous transplant or young adults with acute leukemias. One approach to lowering the incidence of disease relapse after allogeneic transplant is by way of increasing the intensity of the conditioning regimen; however, such approach is not possible in all patient groups and requires careful clinical and psychosocial examination of an individual patient. Initial haplo-HCT trials with PTCy used non-myeloablative conditioning (NMA) regimen, i.e., fludarabine, cyclophosphamide, and low-dose TBI; this regimen is associated with a low NRM of about 4% at day 100 after transplant; however, the rate of disease relapse remains high of about 58% at 2 years, especially in patients with myeloid malignancies [10]. The importance of conditioning intensity has been clarified in HLA-matched transplant setting by a phase III study which randomized adult patients with AML and MDS to myeloablative conditioning (MAC) *or* reduced intensity conditioning (RIC) regimen, and the study was terminated early due to better survival observed in MAC cohort (NCT01339910). Subsequently, several trials explored higher intensity, somewhere in between NMA and MAC conditioning, i.e., RIC or MAC regimens in haplo-HCT, and showed that a lower disease relapse rate can be achieved with acceptable NRM rates in selected group of patients [19–23]. A recent review of conditioning regimens used in haplo-HCT provides an overview of commonly used regimens in the setting of PTCy-based G*v*HD prophylaxis, summarized in Table 19.1 [22]. In a nonrandomized comparison between NMA and melphalan-based conditioning (RIC regimen), lower relapses with improved survival were observed with the latter approach [25, 28]. One-year progression-free survival (PFS) was 60% and 48% for patients treated with melphalan-based conditioning and NMA conditioning, respectively, while the 1-year NRM was higher (21% *vs.* 7%, respectively) and the relapse rate was much lower (19% *vs.* 45%, respectively). These data suggest melphalan-based conditioning may result in improved survival and requires further examination in a prospective trial.

19.3.1.2 Gender and Bone Marrow vs. Peripheral Blood Preference in Haplo-HCT

Female donor of HLA-mismatched transplants to male recipient and the use of peripheral blood (PB) graft can potentially enhance the G*v*L effect; however, in general such donors and grafts are associated with greater and higher G*v*HD risk and may result subsequently in higher TRM [29, 30]. Whether PB grafts are associated with lower relapse rates and better outcomes compared to bone marrow (BM) grafts remains to be established in haplo-HCT setting. A retrospective registry study on behalf of Center for International Blood and Marrow Transplant Research (CIBMTR) is currently underway analyzing outcomes with both types of graft sources in HLA-mismatched haplo-HCT setting.

Table 19.1 Conditioning regimens used in HLA-haploidentical transplantation

Reference	Conditioning regimen	G*v*HD prophylaxis regimen	Graft source	aG*v*HD (II–IV)	NRM	Relapse rate	OS
Luznik et al. (2008) [10]	Flu/Cy/TBI	PTCy/Tacro/MMF	BM	34% at 200 days	15% at 1 year	58% at 2 years	46% at 1 year
Solomon et al. (2015) [24]	TBI/Flu	Tacro/MMF/PTCy	PB	43%	3% at 2 years	24% at 2 years	78% at 2 years
Solomon et al. (2012) [20]	Bu/Flu/Cy	Tacro/MMF/PTCy	PB	30%	10% at 1 year	40% at 1 year	69% at 1 year
Gaballa et al. (2016) [25]	FM/TBI or thiotepa	PTCy/Tacro/MMF	BM	33% at 1 year	21% at 1 year	19% at 1 year	70% at 1 year
Raj et al. (2014) [23]	Flu/Cy/TBI	PTCy/Tacro/MMF	PB	53% at 1 year	23% at 2 years	28% at 2 years	48% at 2 years
Raiola et al. (2013) [26]	Thiotepa/Bu/Flu or TBI/Flu	PTCy/MMF/CsA	BM	12%	18% at 6 months	26%	62% at 1.5 years
Wang et al. (2012) [27]	Cytarabine, busulfan, Cy, Me-CCNU	ATG, CsA, MMF, and MTX	Combined	49% at 100 days	13% at 3 years	15% at 3 years	79% at 3 years

MTX methotrexate, *CsA* cyclosporine, *MMF* mycophenolate mofetil, *Tacro* tacrolimus, *TBI* total body irradiation, *Flu* fludarabine, *Bu* busulfan, *Cy* cyclophosphamide, *FM* fludarabine/melphalan, *OS* overall survival, *NRM* non-relapse mortality, *aGvHD* acute graft-*versus*-host disease, *PB* peripheral blood, *BM* bone marrow, *NR* not reported

19.3.2 Identification of NK Cell Alloreactivity in Haploidentical Transplants: Does It Matter?

Donor selection based on natural killer (NK) cell alloreactivity (donor-*versus*-recipient) may influence outcomes especially in TCD transplant setting but needs prospective trial conformation before widespread use in clinical practice (also see Chap. 10). Several studies in transplantation have shown the importance of NK cells, in particular killer immunoglobulin receptor (KIR) haplo-typing, in affecting transplant outcomes through a number of postulated mechanisms. KIR incompatibility at both the phenotypic and genotypic level has been demonstrated using several models, including the ligand-ligand model and the receptor-ligand model. The presence or absence of certain KIR receptors (and haplotypes), as well as the overall number of certain types of KIR receptors or haplotypes present, has also been shown in some studies to impact transplant outcomes. In the TCD haplo-HCT setting, there have been favorable observations (decrease in disease relapse) of NK cell alloreactivity with KIR incompatibility [31]. Ruggeri and coworkers demonstrated that in patients (n = 112) with high-risk AML, the KIR-ligand incompatibility (n = 51) resulted in significantly less relapse rates compared to KIR-ligand compatibility (n = 61) [31]. The outcomes in TCD haplo-HCT from NK-alloreactive donors were associated with a significantly lower relapse rate in patients transplanted in complete remission (3% *vs.* 47%) ($P > 0.003$), better event-free survival (EFS) in patients transplanted in relapse (34% *vs.* 6%, $P = 0.04$) and in remission (67% *vs.* 18%, $P = 0.02$), and reduced risk of relapse or death (relative risk *vs.* non-NK-alloreactive donor, 0.48; 95% CI, 0.29–0.78; $P > 0.001$). In another study by Chen and colleagues, the impact of *activating KIR* (aKIR) and *inhibitory KIR* (iKIR) on OS, relapse-related mortality (RRM), and acute G*v*HD was prospectively studied in 84 adults with high-risk hematologic malignancies receiving RIC regimen with TCD haplo-HCT. In this clinical model, freedom from RRM is dependent on G*v*L effect. Patients were divided into myeloid (n = 49) and lymphoid (n = 35) malignancy groups. KIR-ligand and ligand-ligand models were studied in both graft-*versus*-host and rejection directions and statistically correlated with outcome measures. In the myeloid group, OS was higher ($P = 0.009$), and RRM was lower ($P = 0.036$) in patients missing HLA-C group 2 ligand to donor iKIR. The OS was higher if patients had >1 missing ligand ($P = 0.018$). In lymphoid malignancy, missing ligand to donor KIR had no impact on OS or RRM. However, OS was better with donor aKIR 2DS2 ($P = 0.028$). There was a trend toward shorter OS in recipient with KIR 2DS1, 2DS5, and 3DS1, although sample sizes were too small to provide inferential statistics. These results suggest that the absence of appropriate HLA ligands in the recipient to donor iKIR may induce G*v*L without acute G*v*HD in myeloid malignancy patients undergoing TCD haplo-HCT. Similar observations of NK cell alloreactivity have not been observed in other studies [32–37]. Nonetheless, it remains of great interest to improve haplo-HCT outcomes and improve donor selection based on KIR alloreactivity [37, 38].

19.3.3 Posttransplant Interventions to Prevent Relapse

Disease relapse remains the major cause of treatment failure posttransplant which lends support to investigate posttransplant interventions to prevent relapses. Numerous strategies and agents are being explored. An ideal strategy would have zero to minimal toxicity without potentiating G*v*HD, while maintaining or "strengthening" remissions. Herein we discuss commonly employed posttransplant interventions that have been used with variable success in allogeneic transplant setting.

19.3.3.1 Hypomethylating Agents After Allogeneic Transplant

Posttransplant azacitidine may enhance the G*v*L effect by inducing the cytotoxic T-cells and lowering the G*v*HD rates by increasing the numbers of regulatory T-cells (T_{regs}) [39]. A phase I trial enrolled 45 patients with high-risk AML or MDS (67% of the patients were not in remission) to receive low-dose azacitidine at escalating doses after transplant [40]. This trial identified a dose of 32 mg/m^2 to be the safe and effective with reversible thrombocytopenia being the dose-limiting toxicity (DLT). However, 53% of patients eventually developed disease recurrence. The results of a randomized clinical trial by M.D. Anderson Cancer Center (MDACC) of azacitidine (*vs.* best supportive care) after allogeneic transplant in patients with AML and MDS (NCT00887068) are awaited. Romidepsin, a histone deacetylase inhibitor, is also being investigated as a prevention strategy after allogeneic HCT in patients with T-cell lymphomas (NCT02512497). None of these studies are specific to haplo-HCT; however, there is no apparent reason not to be used in the haploidentical transpalnt setting.

19.3.3.2 Monoclonal Antibodies and Targeted Agents

Brentuximab Vedotin After Allogeneic Transplantation

Brentuximab vedotin (BV) has remarkable single-agent activity in relapsed refractory Hodgkin lymphoma [41]. Its use as a "bridge therapy" to allogeneic transplantation in patients who failed autologous transplant has been reported [42]. The use of BV maintenance after allogeneic transplantation is being explored albeit mostly in HLA-matched donors (NCT02098512, NCT02169505) including recipients of haplo-HCT.

Rituximab After Allogeneic Transplantation

For patients with CD20$^+$ lymphoma, rituximab maintenance has been proposed. Kanakry and coworkers reported their experience with posttransplant rituximab as maintenance therapy after haplo-HCT for patients with B-cell lymphomas in a phase II study after a NMA regimen [43]. Donor selection was prioritized to the presence of FCGR3A-158 polymorphism. After a median follow-up of 2.6 years, the cumulative incidence of relapse was 20% at 12 months; however, donor selection based on FCGR3A-158 polymorphism did not influence PFS. The 12 month PFS and OS were 71% and 86%, respectively, which compared favorably to historical outcomes of about 50% 1-year PFS. Increased risk in infections was not apparent with posttransplantation rituximab [10]. These results are encouraging and pave the way for the further investigation.

19.3.3.3 Tyrosine Kinase Inhibitors After Allogeneic Transplantation

The tyrosine kinase inhibitors (TKIs), e.g., imatinib, dasatinib, or ponatinib, are being utilized to prevent relapses in patients with BCR/ABL1-positive acute lymphoblastic leukemia (ALL) and chronic myeloid leukemia (CML) [44–46]. Besides lack of standardization of such approach, the duration of therapy also remains unknown and is based on institutional preferences and individual patient condition; nonetheless at least 1 year of maintenance is generally an accepted practice [47, 48]. At least 10% of advanced CML patients will have persistent minimal residual disease (MRD) at day +30, and in such group of patients, preemptive (not prophylactic) TKI is a practical consideration, but how this approach compares to DLI administration is unclear [49]. The choice between TKI and DLI is largely dependent upon careful study of disease condition prior to and after transplant, comorbid conditions, transplant platform, G*v*HD status, and side effect profiles of available TKIs and DLI.

19.3.3.4 FLT3-ITD Inhibitors After Allogeneic Transplantation

Another well-defined molecular target is FMS-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD$^+$) which is associated with unfavorable outcomes in patients with normal karyotype AML. Consolidative allo-HCT is advised in such group of patients, and it would not be unreasonable to consider preventative strategies if the target, i.e., FLT3-ITD$^+$, can be inhibited to prevent relapse prior to and after transplant. Data suggests that FLT3-ITD inhibitors, i.e., sorafenib, after allogeneic transplant may reduce the risk of relapse without a significant increase in GvHD [50, 51]. Midostaurin is another multi-targeted small-molecule FLT3-ITD inhibitor that might have similar to superior role after transplant compared to sorafenib [52]. Several trials are currently investigating the utility of FLT3-ITD inhibitors as maintenance therapy posttransplant (sorafenib, NCT01398501; midostaurin, NCT01883362; crenolanib, NCT02400255; quizartinib, NCT01468467) and will likely become a valuable strategy for patients with FLT3-ITD$^+$ AML [53].

19.3.3.5 Cellular Therapies After Allogeneic Transplant

Advantage of haplo-HCT over other alternative donor options is donor's timely availability to provide T-cells for posttransplant cellular therapy. Several approaches are currently under investigation and are summarized in Table 19.2 [54].

Donor Lymphocyte Infusion(s)

Historically, DLI has been used with HLA-matched transplants with varying degrees of success in AML and MDS, while most effective in patients with early relapse of CML [55]. Similarly, haploidentical donor lymphocyte infusion (DLI) is a reasonable approach to treat early (molecular relapse) or frank relapse after haplo-HCT. However, in the setting of haplo-HCT, there is a higher risk of inducing severe acute G*v*HD after conventional dose of DLI used after HLA-matched transplants. Consequently, the DLI (CD3$^+$ cells/kg) dose in haplo-HCT is reduced by at least 1 (one) log [56]. The evidence is still scant, but at least one study suggested that the rate of acute G*v*HD in such group of patients was not higher than HLA-matched

Table 19.2 Posttransplant interventions after HLA-haploidentical transplantation to lower the risk of disease relapse

Approach	Rationale and advantages	Disadvantages and current status
Unmodified donor lymphocyte infusion (DLI)	• Possibly augment the graft-*versus*-leukemia effect	• Limited experience with haploidentical transplants • Potential to flare G*v*HD at conventional doses of $CD3^+$ cells (see text) • Non-specific with risk of G*v*HD and limited efficacy
Engineered donor lymphocytes with a safety "suicide" switch	• Possibly augment the graft-*versus*-leukemia effect • Safety switch allows T-cell suicide in case of unwarranted G*v*HD development	• Clinical efficacy not demonstrated—under investigation • Non-specific
T-cells with chimeric antigen receptors (CAR T-cells)	• T-cells engineered to recognize specific antigens (e.g., CD19) • Low G*v*HD potential	• Clinical efficacy after haploidentical transplants not demonstrated—under investigation • CRS potential likely low
Infusion of *ex vivo* expanded NK cells	• Potential graft-*versus*-tumor effect, likely without G*v*HD	• Clinical efficacy has not been demonstrated—under investigation • No adverse effects

donor sources especially when lower starting dose of $CD3^+$ T-cells is administered [56]. Among 40 patients who received unmodified haploidentical DLI (1×10^6/kg $CD3^+$ T-cells), acute G*v*HD was noted in 25% (grades III–IV aG*v*HD in 15%). About 30% of patients had a complete remission with a median duration of response of about 12 months. In this study, majority of patients received cytoreductive therapy prior to the DLI, which is important management step in rapidly progressive and bulky relapses. Another study of 42 patients used escalating doses of haploidentical DLI and reported similar findings with about 33% of AML patients responding. The grade II and III acute G*v*HD reported was 10–15%. Interestingly, higher response rates were observed in leukemic patients with molecular relapse (45%) and in patients with Hodgkin lymphoma (70%) [57]. Approach of unmanipulated HLA-haploidentical DLI has not been fully worked out in the preemptive or preventive settings due to concerns of escalating the risk for acute G*v*HD. However, incorporation of a safety switch to T-cells (discussed later) will provide the opportunity to infuse a safer product in the early posttransplant period, but many hurdles remain for such novel strategy to come into fruition including requirement of sophisticated cell processing laboratory and cost.

Evasion of the donor's immune system is a common theme of relapse after allo-HCT; one such relapse mechanism unique to haplo-HCT is loss of heterozygosity (LOH) or uniparental disomy (UPD), i.e., genomic loss of the recipient's mismatched HLA haplotype (see Chap. 20) [58–61]. Vago and coworkers reported

acquired uniparental disomy of chromosome 6p in leukemic cells in 5 out of 17 relapsed patients, a mechanism of tumor escape from the selective pressure of a patient-specific G*v*L reaction. Interestingly, mixed lymphocyte cultures revealed that T-cells ($CD3^+$ cells) from the donor and the patient after transplantation did not recognize the mutant leukemic cells, whereas the pretransplant leukemic cells were efficiently recognized and killed by donor's T-cells [59, 62, 63]. Loss of the patient-specific HLA haplotype is easy to diagnose (HLA typing of leukemic clone) and has important implications to select appropriate treatment for relapse disease especially if DLI is being considered (see Chap. 20).

Genetically Modified T-Cells as DLI

The use of inducible suicide genes or *safety switch* can "turn off" donor T-lymphocytes if adverse effects, such as extensive G*v*HD, are encountered. This concept was originally introduced to boost posttransplant immune recovery after TCD grafts [64]. These lymphocytes were genetically modified to express herpes simplex virus thymidine kinase suicide gene, which can induce apoptosis if exposed to antiviral drug, ganciclovir. However, ganciclovir may not be the ideal drug for this setting due to its role to control CMV seroconversion after transplant. Most recently, another similar concept has been introduced using DLI engineered to express an inducible caspase-9 (iC9) transgene [65]. This gene can be induced by a synthetic dimerizing drug that leads to rapid T-cell death if excessive G*v*HD is observed. This approach is currently being investigated in several clinical trials. A similar approach was used in pediatric patients who received an α/β-selected TCD haplo-HCT for neoplastic or nonmalignant disorders in a phase I and II trial to accelerate immune recovery posttransplant (NCT02065869). In this trial, donor derived T-cells, which were transduced with a new iC9 suicide gene (BPX-501), were administered within 14 days after haplo-HCT. A dimerizer drug was available to subjects who develop severe G*v*HD not responsive to 7 days of standard of care treatment. In a preliminary report, immune recovery was more robust compared to a matched historic control of patients receiving α/β-selected TCD haplo-HCT with none of the patients developing severe G*v*HD that would require administration of the dimerizer drug [66]. The iC9 cell-suicide system may facilitate the implementation of cellular therapy approaches in future studies.

Chimeric Antigen Receptor T-Cells

While DLI offers a nonspecific G*v*L effect, the introduction of chimeric antigen receptor T-cell (CAR T-cell) constructs engineered to express a chimeric receptor with an intracellular domain that activates the T-cell to induce cytotoxicity and an extracellular domain that can recognize a specific antigen on tumor cells (e.g., CD19 on lymphoid cells) offers a targeted approach which might prove more effective if eliminating tumor targets. These approaches have been successful in variety of diseases and are in various stages of development. Maude and coworkers reported on 30 relapsed/refractory ALL patients treated with autologous CAR T-cells expressing CD19 (CTL019) and showed complete remission of 90% [67]. Interestingly, CTL019 cells proliferated *in vivo* and were detectable in the PB, BM, and cerebrospinal fluid

(CSF) of patients who responded favorably. However, the median follow-up in this study was only 6 months and longer follow-up is needed. Haploidentical CAR T-cells are currently being investigated at MDACC early after haplo-HCT preemptively in patients with high-risk lymphoid malignancies to prevent disease relapse. A preliminary report from a phase I trial showed safety of this approach in 12 patients who received "adjuvant" CAR T-cells produced using the "Sleeping Beauty" system and infused after allo-HCT to target MRD in patients with ALL or lymphoma [68]. Eight haplo-HCT patients with advanced lymphoid malignancies were treated in this study with no added toxicity usually seen using CAR T-cell therapy in non-transplant setting and encouraging results. Although the median follow-up was short, the majority of patients remained alive and in remission following the CAR T-cell infusion. Long-term efficacy of this approach remains to be determined.

NK Cell Therapy After Transplant

NK cells are part of the innate immune system and are normally involved in identifying and killing tumor cells or virally infected cells. NK cells recognize and target "foreign" cells that *lack* one or more HLA class I alleles specific to the inhibitory receptors (killer immunoglobulin-like receptors, KIRs) [69]. This makes them an ideal cellular therapy approach in the posttransplant setting, not only because they possess anti-tunor properties, but also because they eliminate both residual T cell thus favoring engraftment and dendritic cells preventing GVHD. Moreover, and unlike T-cells, they don't rely on HLA recognition to provide antitumor properties and therefore may be used as an "off-the-shelf" cellular product in the future. In the non-transplant setting, haploidentical NK cell infusions with subcutaneous interleukin-2 (IL-2), administered after an immunosuppressive regimen, were able to induce complete hematologic remission in some patients with high-risk AML. In addition, haploidentical NK cells showed evidence of *in vivo* expansion and persistence [70]. NK cell lines (NK-92 cells) and NK cells derived from cord blood (CB) are being tested in clinical trials, and results are eagerly awaited (NCT01729091, NCT02280525) [71, 72]. Infusing high doses of NK cells appears to be important to obtain a therapeutic benefit and *ex vivo* expansion of NK cells seems to be needed to obtain a stronger antitumor effect. Currently, there are several approaches to expand NK cells *ex vivo*; however, most effective method appears to be using mb-IL21 method. High doses of NK cells can be generated in this fashion, and this approach is being tested in several clinical trials including after haplo-HCT (NCT01904136) in patients with high-risk myeloid malignancies at MDACC [73].

Other Potential Cell Therapy Products Under Investigation: The Invariant NK T-Cells and CAR NK T-Cells

Recently, scientific observations about invariant NK T-cells (iNKT) have been made that can potentially lead to expansion of cellular therapeutic armamentarium. These iNKT cells are a unique population of T-lymphocyte, which have both innate and adaptive immune properties, and are important mediators of immune response and tumor surveillance [74]. An interesting observation by Malard and coworkers

showed that recipients of HLA-matched grafts with a higher quantity of iNKT cells had better outcomes due to less disease relapse and less severe chronic G*v*HD [75]. This interesting finding may lead to future trials investigating cellular therapy with iNKT cells to prevent disease relapse after haplo-HCT.

The success with CAR T-cells in patients with ALL propelled an interest in testing the same concept with NK cells. While development of CAR T-cell therapy is revolutionizing the hematology field, a major limitation is long-term B cell aplasia not seen with NK cell therapy. Such would not be the case with CAR NK T-cells, which can survive in patients for several weeks, but do not persist for longer periods of time [70, 76].

19.4 Treatment of Relapse After Transplant

Outcomes of patients who relapse after allogeneic transplants remain, in general, poor. The approach to management of patients with disease relapse after haplo-HCT includes tapering of immunosuppression, debulking therapy (if circumstances warrant and allow it), DLI (in the absence of G*v*HD after cessation of immunosuppression), and a second transplant (if relapse happened >6 months after transplant). For second transplant, RIC regimen is recommended due to high TRM in such group of patients [77]. Recently, Tischer and coworkers reported a 45% 1-year OS and 33% 1-year relapse-free survival after 17 months of follow-up in 20 patients undergoing a second haplo-HCT for relapsed AML or ALL [78]. The selection of the same or alternative donor for second transplant is an area of controversy, and depends on many variables, i.e., disease type, duration of remission with first transplant, prior history of G*v*HD, etc.

The data on withdrawal of immunosuppression after haplo-HCT to tackle disease relapse is limited [79, 80]. Patients who show no improvement after withdrawal of immunosuppression and do not develop acute G*v*HD could be considered for DLI, chemotherapy re-induction, demethylating agents, or targeted agents (see above). Younger patients, in good physical condition with no active infections and at least 6 months out from the first transplant, may be considered for a second transplant, preferably with a different conditioning, reduced-intensity regimen and a different haploidentical donor and an expected long-term survival of approximately 20%.

19.5 Expert Point of View

Haplo-HCT has evolved from a very high-risk procedure to a widely acceptable alternative donor transplant option with similar toxicity and outcomes compared to HLA-matched donor transplants in retrospective studies. Over time, a dramatic decrease in TRM has been noted, making prevention of relapse the focus of improvement. Approaches to transplantation using HLA-matched donors as well as unique aspects of haploidentical transplants will provide the basis for enhancing the

antitumor effects of the graft to prevent relapses without added toxicity. Cellular therapy represents an exciting direction, which, to this point, appears safe in clinical early trials with very promising results.

19.6 Key Points

- Outcomes after haplo-HCT have dramatically improved over the last decade since the introduction of PTCy.
- Disease relapse has emerged as the most common cause of treatment failure.
- Treatment after disease relapse is suboptimal and requires better strategies.
- The use of cellular and drug therapy shows promise to reduce disease relapse after transplant.

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Unique Complications and Limitations of Haploidentical Hematopoietic Cell Transplant

20

Hermann Einsele, Stephan Mielke, and Matthias Hermann

20.1 Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) from an one human leukocyte antigen (HLA) haplotype matched first-degree relative donor (haploidentical donor) is increasingly applied to treat patients with advanced and high-risk hematological malignancies who lack an HLA-matched related and unrelated donor [1]. Historically, infectious complications and high relapse rates of the malignant disease were the main limitations after haplo-HCT leading to a highly compromised overall survival (OS) [2]. Complications such as acute G*v*HD (aG*v*HD) and primary graft failure (PGF) were significantly reduced by profound *in vivo* and *ex vivo* T-cell depletion (TCD) of the allograft [3] providing the basis of principle feasibility of this approach.

However, this strategy of recalled as "historical," "standard," or "naked" haploidentical stem cell transplantation came with significant drawbacks: first is the delayed immune reconstitution with an increased and long-term risk of infectious complications. Second, due to the rather advanced stage of the disease at the time of haploidentical transplantation and the delayed immune reconstitution, a significant risk of disease relapse remains. The relapse of acute leukemia especially after haplo-HCT is often associated with loss of the mismatched HLA on the leukemic cell. Finally, with delayed immune reconstitution in place, intense alloreactivity also in host-*versus*-graft (H*v*G) direction is associated with an increased risk of graft loss and higher risks of infectious complications.

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20.2 Graft Failure After "Standard" Haploidentical Transplant

Engraftment failure is observed in approximately 1–4% of patients following allo-HCT using HLA-matched related and unrelated donors and about 20% in umbilical cord blood (UCB) or TCD haplo-HCT [4, 5]. The most common cause of graft failure is the immunologic reaction of the residual host immune effector cells against donor cells, the so-called graft rejection. Graft rejection following haplo-HCT is largely caused by H*v*G reaction mediated by host T- and/or NK cells that survived the conditioning regimen [1].

However, antibody-mediated graft rejection (otherwise known as humoral rejection) has been increasingly recognized as a mechanism of primary graft failure (PGF).

20.2.1 Graft Rejection Mediated by T- and/or NK Cell Reactivity

The resistance to engraftment of an allogeneic hematopoietic graft was thought to be mediated primarily by residual recipient T-lymphocytes, which is increased if there is a genetic disparity between the donor and the recipient. In addition, the graft failure depends on the status of the host anti-donor reactivity. This makes recipients of an HLA-mismatched and haploidentical cell transplantation more susceptible to the development of graft rejection when compared with HLA-matched allo-HCT.

In clinical studies, especially in patients with severe aplastic anemia (SAA), a higher risk of graft failure was found due to the presence of resistant anti-donor cytotoxic T-cell populations sensitized to donor MHC antigens through repeated blood transfusions. A high number of transfusions of blood products were identified to be associated with a higher incidence of graft rejection and death [6].

In haplo-HCT the use of myeloablative conditioning (MAC) chemotherapy and high-dose post-transplant cyclophosphamide (PTCy), now commonly used to prevent G*v*HD, can diminish these cellular-mediated immune reactions as both human T-cells and NK cells are highly sensitive to cyclophosphamide [7]. In addition to T- and NK cell-mediated graft rejection (cellular rejection), antibody-mediated rejection (humoral rejection) occurring either by antibody-dependent cell-mediated cytotoxicity or complement-mediated cytotoxicity has been described.

20.2.2 Antibody-Mediated Graft Rejection

Antibody-mediated graft rejection has been a major obstacle and well-recognized cause of rejection and organ dysfunction in solid organ transplants. The risk of antibody-associated graft rejection following allo-HCT depends on antigen density on the target cell and capacities of the antibody Fc domain. While many types of preformed antibodies can be detected in alloimmunized stem cell transplant recipients, only antibodies against donor HLA antigens have been shown to have clinical significance [8–10].

Allosensitization is a common problem in both solid organ and allo-HCT [11, 12]. Approximately 50% of all patients requiring a transplant can become allosensitized and develop anti-HLA antibodies, and up to 30% of the patients might have donor-specific anti-HLA antibodies, which can mediate organ rejection or graft failure [13, 14]. A clear association between anti-HLA antibodies and graft failure in recipients of an allogeneic hematopoietic cell graft in particular HLA-mismatched transplantations has been demonstrated. Different mechanisms by which anti-HLA antibodies may cause graft failure are discussed [15].

The activation of the complement cascade has been shown in allosensitized recipients of solid organ transplantation and has been also described in animal models of allo-HCT [16, 17]. The classical pathway of the complement cascade is activated when the antigen-antibody complex binds C1q and initiates activation of other complement components resulting in the formation of membrane attack complex, which in turn induces lysis of the target cell with apoptosis [18].

The anti-HLA antibodies that target donor HLA antigens present on the surface of the donor-derived hematopoietic progenitor cells and antigen-antibody complexes can bind C1q and thus activate the complement cascade and cause destruction of the donor cells resulting in allograft rejection. C1q testing was developed to assess complement cascade activation in allosensitized recipients of solid organ transplants [19, 20]. In allo-HCT setting, Ciurea and coworkers showed that anti-HLA antibodies are associated with engraftment failure (see Chap. 9). This group analyzed 122 haploidentical transplant recipients tested prospectively for anti-HLA antibodies. Retrospective C1q testing was done on 22 allosensitized recipients. Twenty-two of 122 patients (18%) had donor-specific anti-HLA antibodies, 19 of which were females (86%). Seven patients with donor-specific anti-HLA antibodies (32%) rejected the graft [15]. Of the nine patients, who tested positive for C1q in the initial samples, five patients remained C1q positive at time of transplant [all with high anti-HLA antibodies levels (median 15,279, range 6487–22,944)] and experienced engraftment failure, while four patients became C1q negative pre-transplant and all engrafted the donor cells [15]. In conclusion, patients with high donor-specific anti-HLA antibodies levels and complement-binding antibodies (C1q positive) appear to be at much higher risk of primary graft failure. They concluded that C1q should be assessed in patients with donor-specific anti-HLA as prior to transplant and that reduction of anti-HLA antibodies to non-complement binding levels might prevent engraftment failure.

20.2.3 Documentation of Anti-HLA Antibodies

Preformed donor-specific anti-HLA antibodies (DSA) circulating in the peripheral blood (PB) at the time of transplant have been shown to be correlated with graft rejection and decrease survival in solid organ transplantation [13, 21–23]. Therefore, lymphocyte crossmatch tests have been developed for prediction of graft rejection [24, 25] and became mandatory in solid organ transplant according to the American Society for Histocompatibility and Immunogenetics (ASHI). In setting of allo-HCT,

there has been reported that a positive crossmatch for anti-donor lymphocytotoxic antibody associated strongly with graft failure, mainly in HLA-mismatched or HLA-haploidentical transplantation patients [12, 26]. Although a lymphocyte crossmatch is an effective tool to evaluate alloimmunization and potential donor-recipient incompatibility, the procedure is labor intensive and may detect non-HLA antibodies, which may not be associated with transplant outcome since there is no data to confirm the importance of these antibodies to date [1]. More recently, DSA have been reliably detected using single antigen beads in a Luminex assay. Sensitivity and specificity have increased significantly and allowed a more effective screening of allosensitized recipients. This has been particularly useful in screening haploidentical donors especially for multiparous middle-aged females who are at higher risk of becoming allosensitized through pregnancies. Detection of DSA in this setting has allowed early treatment of these patients to prevent primary graft failure, a dreaded complication of transplantation.

20.2.4 Prevention of Donor-Specific Anti-HLA Antibody-Mediated Graft Failure in Haploidentical Transplant Setting

Preformed antibodies present at the time of graft infusion are unaffected by standard transplantation conditioning regimens or T-cell or B-cell immunosuppressive or modulatory strategies given in the peri-transplantation period. To reduce the risk of graft failure, a number of studies have reported beneficial effects of a variety of interventions used to reduce total anti-HLA antibody load, predominantly by using a combined approach [27]. Each procedure on its own, plasmapheresis, intravenous immunoglobulin (IVIg), cyclophosphamide, polyclonal anti-lymphocyte antibodies, monoclonal antibodies to $CD20^+$ B lymphocytes (rituximab), and proteasome inhibitor directed against allo-antibody-producing plasma cells, has been described in recipients of a solid organ transplant. Their effectiveness as a single agent is modest [14, 28–31]. These treatment modalities also have been used to desensitize anti-HLA antibodies before haplo-HCT and HLA-mismatched allo-HCT [1].

Maruta and coworkers confirmed that repeated high-volume plasmapheresis does not effectively eliminate preformed anti-HLA antibodies [32]. Ciurea and coworkers for the first time used a combined approach using plasmapheresis, IVIg, and rituximab with some success: out of the first four patients treated with this approach, successful engraftment could be induced in 50% of the patients (two out of four), but graft failure due to persistence of anti-HLA antibodies occurred in the other two patients. In the two successfully treated patients, a significant reduction in the antibody levels could be achieved followed by successful engraftment of the donor cells, whereas the other two patients maintained high levels of anti-HLA antibodies and experienced PGF (see Chap. 9) [33].

Another strategy, which has been successfully applied also in a small number of patients, was the combination of plasmapheresis, rituximab, antibody adsorption

with platelets, and administration of the proteasome inhibitor, bortezomib. Reduction of anti-HLA antibodies was achieved in one of two patients, however, both engrafted. Some of the most impressive reductions of the levels of anti-HLA antibodies were achieved by the application of 40 units of platelet transfusion from healthy donors selected to express the HLA antigens corresponding to the anti-HLA antibodies [34]. The MD Anderson group used an irradiated buffy coat, in addition to plasma exchanged, rituximab and IVIg, to desensitized patients especially with C1q positive, deemed to have highest risk [35]. A different approach was developed by the Johns Hopkins group from solid organ transplants, using a combination of repeated plasmapheresis, IVIg, and immunosuppressive medications. This group treated 15 patients who received an allo-HCT from a HLA-mismatched donor including 13 patients who received their graft from a HLA-haploidentical donor. These patients received every alternate day a single volume plasmapheresis followed by IVIg, tacrolimus, and mycophenolate mofetil starting 1–2 weeks before the beginning of transplant conditioning, depending on patient's starting anti-HLA antibodies levels. Reduction of anti-HLA antibodies was seen in 14 of 15 patients, all of these 14 patients engrafted with donor cells [36]. Even though a small number of patients have been treated so far, taken together the experience from these reports suggests that a reduction of anti-HLA antibodies to lower levels is possible and can permit successful engraftment [1].

20.3 Genomic Loss of HLA-Mismatched Human Leukocyte Antigen and Leukemia Immune Escape from Haploidentical Graft-*Versus*-Leukemia Effect

The therapeutic advantage of allo-HCT results not only from the ability to deliver more intensive chemotherapy but also from antineoplastic effects mediated mostly by alloreactive T-cells in the graft. It has become increasingly clear that donor T-cell alloreactivity against host minor HLA antigens as well as tumor specific antigens plays a major role in disease control in a traditional HLA-matched transplants [37]. In the context of haplo-HCT, the large number of alloreactive T-cell targets encoded by the fully HLA-mismatched haplotype can allow a stronger graft-*versus*-tumor (G*v*T) effect and better prevention of disease relapse post-transplant [2]. Disease relapse post-haplo-HCT can still occur via various mechanisms. One of the important mechanisms of relapse recognized after haplo-HCT is loss of heterozygosity (LOH) in the HLA gene region on chromosome 6p of tumor cells. Tumor cells without mismatched HLA expression may be predisposed to selective expansion through *in vivo* escape from immune surveillance by alloreactive T-cells. Even though, this phenomenon is commonly observed in untreated solid tumors, in which the incidence can be up to 70–90% [38, 39], it is rare in leukemia at presentation. However, the LOH has been identified as a mechanism of leukemia immune escape and disease relapse after haplo-HCT [2, 40].

20.3.1 Mechanisms of Loss of Heterozygosity and Leukemia Immune Escape

LOH as a possible mechanism of leukemia immune escape after haplo-HCT has been described by several groups. Vago and coworkers have studied the genomic rearrangements in mutant variants of leukemia by using genomic HLA typing, microsatellite mapping, and single-nucleotide polymorphism (SNP) arrays. They identified the mutant variant of leukemia cells with HLA that differed from the donor's haplotype which had been lost because of acquired uniparental disomy (aUPD) of chromosome 6p in 5 of 17 patients with leukemia relapse after haploidentical transplantation. This mutation resulted in leukemic cell evasion from donor T-cell recognition, whereas the original leukemic cells taken at the time of diagnosis were efficiently recognized and killed. They hypothesized that HLA loss may reflect alloimmune pressure mediated by donor T-cells toward the HLA mismatches [41]. This phenomenon was also confirmed in a report by Villalobos and coworkers, describing two cases of HLA loss by chromosome 6p aUPD resulted in total loss of the mismatched HLA haplotype among three pediatric patients with AML who relapsed after haplo-HCT [42]. The molecular events that form the basis of this type of genomic abnormality remain uncertain, but it has been postulated that aUPD may derive either from mitotic homologous recombination events or from an attempt to correct for the unbalanced loss of chromosomal material by using the remaining alleles as a template resulting in copy number neutral-LOH (CNN-LOH) without a concurrent change in the copy number; therefore, standard cytogenetic methods fail to detect this phenomenon.

Another mechanism of LOH has been described by McCurdy and coworkers in a study of two high-risk AML patients who relapsed after haplo-HCT using PTCy. In this report, the authors demonstrated the absence of mismatched recipient HLA haplotype on the isolated leukemic blasts in both cases. Interestingly, both cases represent distinct mechanisms of HLA loss. SNP array for recipient 1 demonstrated aUPD at 6p, which is the mechanism as previously described [41, 42]. However, the karyotype and SNP array for recipient 2 revealed a deletion of chromosome 6p that encompassed the mismatched HLA locus. The later represents a different, but similar, genomic mechanism and supports that the leukemic cells may lose the mismatched HLA haplotype through multiple means, resulting in evasion of the donor immune system [43].

Another mechanism causing downregulation of mismatched HLA class I antigens was described by Tamaki and colleagues. In this study, this group found a lack of mismatched HLA-A despite a retaining of both HLA haplotypes on the leukemic cell surface of the AML patient who relapsed after haplo-HCT by using flow cytometric analysis. They speculated that this finding might be associated with impaired epigenetic regulation of the gene causing downregulation of HLA class I on unshared alleles, which are preserved on shared alleles [44].

20.3.2 Incidence, Risk Factors, and Clinical Outcomes of Mismatched HLA Loss

While SNP arrays may detect a copy neutral LOH of chromosome 6p, this test is not routinely performed on samples from patients who relapse after allo-HCT, and its sensitivity is limited in early relapse. Moreover, assays to distinguish donor from patient-specific alleles are not clinically available at present. Therefore, the incidence of which HLA loss contributes to relapse is only documented in some small studies with limited number of patients [40–43]. The only large retrospective study to determine the incidence and outcome of HLA loss relapses after allo-HCT and to address the clinical, genetic, and immunologic factors associated with the selection of mutant leukemic variants was done by the investigators from Italy. The investigators retrospectively collected clinical and immunogenetic data from 233 consecutive allo-HCT recipients of partially HLA-incompatible donors (with 162 patients with haplo-HCT). These transplants were performed for myeloid malignancies. At 4 years after HLA-mismatched related transplantation, incidence of HLA loss relapses was 14%, whereas the incidence of the remaining cases of relapse (classical relapses) was 27%. Timing since transplantation was significantly different between the two relapse subtypes: HLA loss relapses mostly occurred late after transplant (median 307 days, range 56–784), whereas "classical" relapses occurred much earlier (median 88 days, range 12–579; $P < 0.0001$). Interestingly, the investigators could not identify cases of HLA loss relapses after HLA-mismatched unrelated (MMUD) transplantation, while incidence of "classical" relapse in HLA-MMUD allo-HCT was 22% at 4 years. In multivariable analysis, active disease at transplant was associated with an increased risk of HLA loss. Conversely, older patient age appeared to significantly decrease the risk. The OS of patients who relapsed in this study was poor and was not different between both types of relapse. The median OS was 94 and 78 days after LOH and classical relapse, respectively [45].

20.3.3 Targeted Therapy of HLA Loss Relapses

It becomes crucial to document the HLA loss in patients who have leukemia relapse after haplo-HCT because it has relevant clinical consequences: not only it demonstrates that the donor-derived T-cells circulating in the patient at the time of relapse become inefficient bystanders but also that any attempt to induce remission by infusion of donor T-lymphocytes is expected to be ineffective against the leukemic cells and potentially harmful to the patient due to the conserved risk of inducing G*v*HD. Based on the net result of the genomic alteration, two possible alternative immunotherapeutic strategies can be considered for these variants of leukemia relapse. The first is a second transplantation from a different HLA-haploidentical donor, selected for being mismatched against the HLA haplotype retained by

leukemic blasts. Theoretically, the advantage of this method is that the donor and leukemia cells would have a full immunologic incompatibility that can help increase G*v*T effect, while an incompatibility between the donor and patient's healthy tissues is only 50%. The second treatment option is an infusion of high-dose purified donor NK cells. It is based on an observation that leukemic cells that undergo genomic loss of one HLA haplotype in several cases also lose the ligands for donor inhibitory KIRs, becoming in principle susceptible to NK cell alloreactivity [46]. Even though, the effectiveness of this strategy is limited in overt leukemia relapse, it might help in preemptive treatment of impending leukemia recurrence, guided by molecular markers of minimal residual disease and early detection of HLA loss relapse.

20.4 Viral Reactivations After Haploidentical Transplants

Although promising survival has been achieved with the establishment of many haplo-HCT protocols, viral reactivation resulting from the impaired immune reconstitution owing to the method of TCD and extensive immunosuppression necessary to overcome HLA disparity remains one of the most important causes of morbidity and mortality.

Graft composition and conditioning may be of great impact on the immune reconstitution after haplo-HCT (see Chaps. 5, 7, and 18). Various approaches have been evaluated to deplete the host and the recipient T-cells in order to prevent graft failure and G*v*HD. However, extensive TCD can cause slow immune reconstitution and leads to various serious infections [3, 47]. Initial studies in TCD haplo-HCT using "megadose" $CD34^+$ allograft have shown that PB counts of NK cells returned to normal within 2–4 weeks after transplantation, while $CD4^+$ T-cell counts were below 100 and 200 cells/mm^3 for as long as 10 and 16 months, respectively, and led to high rate of treatment-related mortality (TRM) (40%) primarily due to serious infections (see Chaps. 1 and 2) [3].

Even though various graft manipulation strategies have been investigated to partially deplete T-cells from the graft with the goal to preserve immunity and G*v*T effects and selectively eliminate the cells mostly responsible for G*v*HD, a high success rate was seen mainly in pediatric patients whose thymic function is still active, while outcomes in adult patients remain poor due to prolonged immune deficiency resulting in high rates of infectious complications [47–51].

Using the new platform for T-cell replete haplo-HCT using PTCy as G*v*HD prevention method showed a low TRM and high feasibility with an acceptable safety profile. This type of haplo-HCT seems to compare favorably with TCD methods, in terms of infectious complications [52]. Ciurea and coworkers have shown the better reconstitution of T-cell subsets including memory and naïve T-cells in patients received unmanipulated haploidentical transplantation with PTCy as compared with TCD haplo-HCT. This group also found a significant lower incidence of viral and fungal infections and a trend for a lower probability of developing any infection in the critical first 6 months post-transplant [53]. In terms of viral infection, Tischer and coworkers compared the incidence of viral infection and outcome of patients

treated with a combined T-cell replete and TCD haplo-HCT and with T-cell replete haplo-HCT PTCy. This group found a significantly lower incidence of herpes virus infection as well as viral infection-related mortality in T-cell replete group suggesting that TCR haplo-HCT using PTCy can better preserve antiviral immunity and allow fast immune recovery of T-cell subset [54]. Nonetheless, it remains unclear how T-cell replete haplo-HCT with PTCy will compare with other *in vivo* and *ex vivo* methods of partial TCD [55].

20.4.1 Common Viral Infections in Recipients of Haploidentical Transplant

Infections from various viral pathogens have been reported in a setting of haplo-HCT. The incidence of viral infection depends on graft type and degree of immune suppression by conditioning and G*v*HD prophylaxis regimens. Tischer and coworkers have reported 139 occurrences of viral infection in 46 out of 55 patients, 68 of them were symptomatic and 20 associated with disease. The most frequently observed viral pathogens in this study were HHV-6; polyomavirus JC/BK, EBV, CMV, and HSV; and adenovirus (ADV) [54].

20.4.2 Cytomegalovirus Reactivation and Infection

Incidence and Risk Factors: Infection from cytomegalovirus (CMV) remains one of the most important complications after haplo-HCT. CMV infection can appear as reactivation, primary infection, or reinfection. It can also cause multiorgan disease including pneumonia, hepatitis, gastroenteritis, retinitis, and encephalitis, and the disease can develop both early and late transplantation period [56]. Donor and/or recipient seropositivity for CMV is a major risk factor for CMV reactivation and CMV disease during and post-transplant [57, 58]. A retrospective study by investigators from MD Anderson Cancer Center showed that of 178 patients treated with unmanipulated haplo-HCT using PTCy for G*v*HD prophylaxis, CMV reactivation was observed in 103 patients (63%) with a median time to reactivation of 39 days. Ten patients (approximately 10%) developed CMV disease (two had pneumonia, three had colitis, two had upper respiratory infections, one had esophagitis, and two had retinitis). The highest incidence was seen when both the patient and donor had CMV IgG seropositive before transplant. Moreover, they found that a low $CD8^+$ T-cell count at day +90 correlated with a higher incidence of CMV reactivation [58]. Same results were found in a recent study of 138 patients treated with T-cell replete haploidentical transplantation and PTCy by Goldsmith and coworkers. In this study, 80 patients (58%) had post-transplant CMV viremia, and 23 patients (29%) progressed to CMV disease. After adjusted for "very-high" disease risk index, CMV viremia was associated with poor OS [59]. CMV reactivation seems to be more common in TCD transplantation than in transplantation using unmanipulated allograft with PTCy [60]. Mulanovich and coworkers found that CMV infection affected most

patients and recurred 1–2 times on average in affected patients. In this study, 30 episodes of CMV infection were reported in 28 TCD haplo-HCT recipients [61].

Prevention and Treatment of CMV Infection: CMV serologic status should be assessed as early as possible when a patient is being considered for haplo-HCT. If a patient is CMV seronegative, CMV-negative blood products should be used during the whole transplant process. Moreover, a CMV serology negative donor is preferable for a CMV serology negative recipient. If only a CMV-seropositive donor is available for a CMV-seronegative patient, the risk of transmission of CMV by the graft to the recipient is approximately 20–30% [62]. The incidence of CMV transmission from TCD haplo-HCT has never been clarified.

Preemptive treatment is preferable over prophylaxis strategy to avoid the unnecessary treatment of patients who will not develop CMV infection or disease. Diagnostic surveillance of patients at risk of acquiring CMV infection is important to guide preemptive therapy. The common tests used include pp65 antigenemia and the CMV DNA PCR [63]. Patients must be screened for CMV viremia at least once a week in the first 100 days post-transplant. However, late CMV viremia and disease can occasionally occur in haplo-HCT setting often in patients on steroids as treatment for G*v*HD and/or due to poor or delayed recovery of CMV-specific T-cells and are associated with poor outcome. In most cases, it occurs between 4 and 12 months post-transplant [56]. Intravenous ganciclovir is most commonly used to treat both CMV viremia and CMV disease followed by foscarnet and cidofovir [64, 65]. The use of ganciclovir can be associated with myelotoxicity and secondary graft failure. Consequently, we recommend the use of foscarnet as first line therapy in patients with normal kidney function who are early post-haploidentical transplant.

20.4.3 Polyomavirus Reactivation

Incidence and Risk Factors: BK virus (BKV) is a human polyomavirus typically acquired in early childhood and becomes latent in urothelial cells of the urinary tract [66]. BKV reactivation after allo-HCT is associated with manifestations ranging from asymptomatic viruria to severe hemorrhagic cystitis (HC), ureteral stenosis, and interstitial nephritis. The incidence of BK viruria is similar in allogeneic (range 46–53%) and autologous (range 39–54%) hematopoietic cell transplantation [67, 68]. A retrospective study by Rorije and coworkers showed that 16% of allo-HCT recipients developed BKV disease (an incidence rate of 0.47/1000 patient-days), while 5.5% had severe disease [69]. Haplo-HCT with PTCy is associated with uroepithelium damage, which may enhance the BKV replication in the bladder. Besides the use of PTCy, impaired immune reconstitution after haplo-HCT may result in an increase in risk for developing a higher BK viral load in urine, enhancing the urothelial mucosal damage and a higher incidence of cystitis [70]. Ruggeri and colleagues reported as high as 62% cumulative incidence of HC at day 180 of T-cell replete haplo-HCT recipients using PTCy, and BKV was positive in blood and urine of 91% of patients at HC onset [71]. Another study by Solomon and coworkers in a series of 20 haploidentical recipients receiving PTCy showed an overall incidence

of 75% of BKV-associated cystitis, with 35% of patients requiring hospitalization [72]. In the MAC setting, using the thiotepa, busulfan, and fludarabine (TBF) regimen and PTCy, Raiola and coworkers reported an incidence of HC of 40%, mainly associated with BKV reactivation [73], while the MD Anderson group reported a incidence of HC of approximately 40% with 25% severe HC requiring hospitalization, bladder irrigation, and occasional nephrostomy tube placement [58]. In haplo-HCT setting, busulfan-based conditioning has also been associated with higher incidence of BKV HC (see Chap. 5).

Diagnosis and Treatment: The diagnosis of BKV-associated HC is considered when gross hematuria or other urinary symptoms occur in the first 90 days post-transplant. Other clinical features include dysuria, frequency, urgency, suprapubic pain, and later on due to complications of urinary tract obstruction and/or renal failure if bleeding and clot formation are severe. The modality of choice for detecting viral DNA in the urine is PCR. However, it does not have high disease specificity because stem cell transplant patients without HC can excrete BKV in urine. Viral culture is not used for detection of BKV replication because growth of the virus in tissue culture can take weeks. Cytologic examination of urine can detect characteristic polyomavirus-infected cells, decoy cells, with enlarged nuclei containing a single large basophilic intranuclear inclusion. However, this feature can also be caused by other viruses, like JC or adenovirus [74]. In general, treatment of BKV-associated HC is supportive including pain and bleeding control. To date, no antiviral drug with proven efficacy against BKV replication has been licensed. Cidofovir, the antiviral drug licensed for the treatment of CMV retinitis in AIDS patients and is a second-line drug for the treatment of ganciclovir-resistant CMV infections, has been used with some success for treatment of BKV-associated HC in allo-HCT recipients [75–77]. Intra-bladder cidofovir has been used with variable success and avoid systemic toxicity. The BKV CTLs are a promising more effective treatment for this common complication.

20.4.4 Adenovirus Infection

Adenovirus (ADV) infection is a well-described complication after allo-HCT, especially in pediatric patients, and is closely associated with delayed immune reconstitution [78]. It can appear as asymptomatic viremia, localized infection, or multiorgan disease. The reported incidence of ADV infection and disease after allo-HCT varies from 8% to 47% and has become increasingly frequent in recent years [79–81]. The progression to a disseminated disease has been suggested in approximately 10–20% of patients and resulting in high mortality rate of up to 80% [82, 83]. Taniguchi and coworkers retrospectively examined the incidence of ADV infection in patients undergoing unmanipulated haplo-HCT. Following 121 transplantations in 110 patients, three had asymptomatic adenovirus viremia, three had localized disease (hemorrhagic cystitis), and seven had disseminated disease. The median time from transplantation to the onset of ADV-associated HC was 15 days (range, 4–39 days), and the median time to the onset of disease was 23 days (range, 7–38 days). The

cumulative incidence of ADV-associated HC was 8.3%, and for ADV disease was 5.8% [84]. Several factors increase the risk of ADV infection, almost always related to a lack of cellular antiviral reactivity that is inherent to the first 100 days after transplantation such as the development of G*v*HD and use of anti-thymocyte globulin (ATG) or alemtuzumab or a TCD allograft [85–89].

Due to a high rate of mortality in patients who have disseminated ADV disease, it is imperative to monitor patients at high risk (i.e., all patients after HLA-mismatch transplants and patients with *in vivo* or *ex vivo* TCD) using sensitive monitoring tools of subclinical ADV infection. Weekly monitoring of the adenoviral load by quantitative ADV PCR in the PB is the most preferable and sensitive method for early detection of ADV disease [90, 91]. Rapidly increasing or sustained adenoviremia can predict the occurrence of severe disease both in children and in adults [91, 92]. Earlier detection of ADV at the infection site such as nasopharyngeal aspiration or stool could be associated with earlier therapeutic intervention and improved outcomes.

Treatment options for ADV infection and disease include antiviral drugs, adoptive immunotherapy, and viral-specific donor lymphocyte infusion (experimental). Ribavirin and cidofovir are commonly used agents in the treatment of ADV, which can be used as prophylaxis, preemptive treatment led by viral load cutoff values, or as therapeutic treatment in case of ADV disease which depends on risk of developing severe disease and institutional guidelines [88, 93].

20.5 Key Points

- Unique complications after haploidentical transplantation consist of viral reactivation and infections, graft rejection related to DSA and relapse related to LOH
- Monitoring, prevention and early treatment of viral infections, as well as detection of DSAs and treatment of allosensitized recipients represent priorities for haploidentical transplant recipients

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Future Prospects: Haploidentical Transplantation

21

Stefan O. Ciurea and Rupert Handgretinger

21.1 Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) for hematologic malignancies continues its expansion worldwide both with autologous and allogeneic grafts [1, 2]. Haploidentical hematopoietic cell transplants (haplo-HCTs) are also increasing in an exponential fashion due to recent improvements in treatment outcomes, primarily by using posttransplantation cyclophosphamide (PTCy), and an enormous need for donors, especially in the developing countries, who usually lack unrelated donor registries or is cost prohibitive to obtain unrelated donor cells, as well as for minority or mixed race individuals in the Western world [3]. In addition, in the developing countries, families are usually larger, and it is easy to identify HLA half-matched relatives for most patients in need; thus haploidentical donors could potentially fit an enormous unmet need for the rest of three quarters of patients without HLA-matched related donors. This could potentially further drive expansion of allo-HCT worldwide for years to come. One can foresee an expansion of haploidentical transplantation worldwide especially in the developing countries where haploidentical donors will become the most commonly used donors for allogeneic stem cell transplantation, while in the Western world, the use of haploidentical donors will continue to expand at a faster pace than any other donor sources.

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21.2 Lessons Learned from the Past

Haploidentical transplants have been performed since the early days of transplantation; however, limitations surfaced immediately, and attempts to overcome these limitations have been presented in other chapters of this book. Early reports with T-cell deplete graft and conventional G*v*HD prophylaxis have shown high incidence of graft rejection, and severe acute G*v*HD led to multi-organ failure [4, 5]. To overcome this limitation, complete depletion of T-cells from the graft has been attempted, which resulted in control of G*v*HD, while improved engraftment was achieved by infusion of higher doses of $CD34^+$ cells to compensate for the lack of donor T-cells in the graft. Unfortunately, the lack of T-cells in the graft has led to a higher infectious complications and prohibitive treatment-related mortality (TRM) [6–8]. After almost 50 years of transplantation using haploidentical donors, it has become clear that using a full graft without effective control of G*v*HD is associated with prohibitive acute G*v*HD and graft rejection [9], while complete depletion of T-cells has been associated with higher graft failure rate, higher incidence of infectious complications, and TRM [7]. Consequently, either a full graft is needed with effective control of alloreactive reactions in graft-*versus*-host or host-*versus*-graft direction or selective or partial depletion of alloreactive T-cells is needed to control G*v*HD, decrease incidence of graft rejection and decrease rate of infectious complications and treatment-related mortality. Several methods have emerged as most effective in accomplishing these goals. An extensive discussion of these methods has been detailed in other chapters of the book and is not the scope of this chapter (see Chaps. 1 and 2). However, we provide a brief sumamry of the main approaches to outline future directions based on how transplants are performed.

21.3 Current Approaches to Haploidentical Transplantation

21.3.1 Posttransplant Cyclophosphamide for Prevention of G*v*HD After T-Cell-Replete Haploidentical Transplantation

PTCy has emerged as the most important method to perform haploidentical transplants and has facilitated expansion of haploidentical transplants including countries with limited resources. High-dose cyclophosphamide applied early posttransplant has proven to be a very effective way to control alloreactive reactions in this setting. Cyclophosphamide administered early after transplant acts in a nonselective way to eliminate rapidly proliferating T-cells susceptible to chemotherapy, generated in the setting of an HLA-mismatched transplant [10]. The low-cost procedure, which is easy to apply in any transplant center performing allogeneic transplants, is the main driver of expansion of haploidentical transplants around the world. Patients treated with PTCy have lower incidence of acute G*v*HD, especially severe grade III–IV acute G*v*HD, and a significantly lower incidence of chronic G*v*HD compared with conventional G*v*HD prophylaxis [11, 12]. This has lowered treatment-related mortality to similar TRM seen with HLA-matched transplants and dramatically improved transplant outcomes. While relapse was initially a concern, multiple studies have now shown that a higher relapse rate compared with

HLA-matched transplants was rather the disease and intensity of conditioning applied, especially for patients with acute leukemia, and not the use of haploidentical donors per se [13, 14]. However, with major improvements in TRM, disease relapse has emerged as the most common cause of treatment failure in haploidentical transplants as seen with HLA-matched donor transplants, and targeting disease relapse has become a priority, not only in haploidentical transplants but also in patients receiving any type of transplant (see Chaps. 7 and 8).

21.3.2 Selective αβ T-Cell Depletion

Depletion of αβ T-cell receptor (TcR)-positive T-cells from the peripheral blood graft has emerged as the main alternative to PTCy approach as it has been shown that the αβ T-cells are a major content of the T-cell population responsible for the occurrence of G*v*HD [15]. As mentioned above, complete depletion of T-cells from the graft was associated with higher TRM due to infectious complications. Selective depletion of αβ T-cells as well as B-cells, while maintaining the γδ T-cells, NK-cells, and other mononuclear cells, requires acquisition of technology and, in order to become the preferred method, would have to show a significant advantage as compared with PTCy method (see Chaps. 3, 4, and 7). A main advantage, which is unclear if it will translate in a survival advantage of patients treated with this method, comparing with those treated with PTCy approach, is the elimination of the need for posttransplant immunosuppression. This, at least in theory, could allow posttransplantation cellular therapy to applied potentially more effectively, although cell therapy has now been applied post-haploidentical transplantation while on immunosuppression with tacrolimus and/or mycophenolate [16, 17].

21.4 Other Approaches to Haploidentical Transplantation

Other approaches to control alloreactivity in haploidentical transplantation discussed extensively elsewhere in this book are photodepletion [18] and administration of regulatory T-cells along with conventional T-cells to prevent G*v*HD in this setting [19]. Comparative studies using these methods with the more common approach using the PTCy method, which is now a new standard of care in haploidentical transplantation, are needed and will likely be performed in the future.

21.5 Future Directions in Haploidentical Transplantation

21.5.1 Comparative Outcomes Between HLA-Haploidentical and HLA-Matched Donor Transplants and Between Different Approaches to Haploidentical Transplantation

One of the most successful and easy to apply methods to control alloreactivity in haploidentical transplantation is using PTCy following a T-cell-replete bone marrow or peripheral blood graft. Using this approach, the posttransplant complications

previously seen with haploidentical transplants have been significantly reduced. These improved outcomes were found to be significantly better compared with complete *ex vivo* T-cell-depleted (TCD) haploidentical transplantation, due to a more rapid immune reconstitution, lower incidence of severe infectious complications, and TRM [20]. Nonetheless, it remains unclear how T-cell-replete haploidentical transplants performed with PTCy-based G*v*HD prophylaxis will compare with other *in vivo* and *ex vivo* methods of partial TCD [21]. To date no retrospective comparison has been performed between modern approaches to haploidentical transplantation, although unpublished data suggests similar outcomes photodepletion and PTCy-based G*v*HD prophylaxis (Denis-Claude Roy, personal communication), and has formed the basis for a prospective multicenter randomized study comparing these two approaches. Another important comparison would be between αβ TCD and PTCy-based G*v*HD prophylaxis; however, αβ TCD has been applied so far primarily in children while PTCy-based approach primarily in adult population.

Owing to the advances and improved outcomes of haploidentical transplantation, multiple retrospective studies are now showing similar outcomes between haploidentical transplants performed with PTCy and HLA-matched transplants, especially with HLA-matched unrelated transplants [22–29]. Two recent larger comparative studies in patients with AML and lymphoma facilitated by CIBMTR showed similar outcomes between these two groups. The first study analyzed 2174 patients with AML treated with either a haploidentical or an 8 of 8 matched-unrelated donor (MUD) who received either myeloablative (MAC) or reduced-intensity (RIC) conditioning showed a similar OS of haploidentical and HLA-MUD transplant in both MAC and RIC subgroups of the patients. However, patients in haploidentical group had significant lower incidence of acute and chronic G*v*HD when compared with the HLA-MUD group likely at least in part related to the use of PTCy-based G*v*HD prophylaxis [22]. Kanate and colleagues also compared transplant outcomes of 917 adult lymphoma patients who received haploidentical transplantation with PTCy ($n = 185$) or 8/8 HLA-MUD transplantation either with ($n = 241$) or without antithymocyte globulin (ATG) ($n = 491$) following RIC regimens. Although, no difference was observed between the three groups in terms of relapse, non-relapse mortality, overall survival (OS), and progression-free survival, multivariate analysis showed that patients in haploidentical group had significant lower rate of acute grade III–IV and chronic G*v*HD compared with HLA-MUD with or without ATG [27]. While survival between haploidentical transplants and HLA-MUD transplants performed without ATG was similar (and worse than MUD transplant with ATG), the incidence of chronic G*v*HD at 1 year posttransplant was much lower in haploidentical transplants (13% *vs.* 51%, $p < 0.0001$) [18].

In addition, a significant proportion of patients who are planned for a HLA-MUD transplant do not end up receiving it, mostly due to disease progression. In a retrospective analysis of all acute leukemia patients who had a MUD search at MD Anderson between 1/2013 and 12/2015, out of 256 MUD searches performed, only 148 had a MUD available in the registry (58%) and 101 had a MUD transplant (68% of MUD identified and 39% of MUD searches performed) after a median time

between MUD search start and donor identified of 92 days (range 42–446 days). Of 47 patients who did not have a MUD transplant, 20 (43%) had either progressed or died before having the transplant (unpublished data). These results suggest that prospective randomized studies with intention to treat comparing haploidentical and HLA-MUD transplants are needed as more patients in the haploidentical transplant group may actually receive the transplant which could translate into better long-term survival.

21.5.2 Extension of Haploidentical Transplantation to Subgroups of Patients with Different Diseases Including Nonmalignant Diseases

Historically, the intense alloreactivity across mismatched HLA was a major concern for applying this treatment modality in patients with nonmalignant diseases. However, the situation has dramatically changed during the past decade since the introduction of various methods of T-cell depletion and the use of PTCy for G*v*HD prophylaxis in T-cell-replete haploidentical transplantation resulted in the encouraging outcomes in adult patients affected by malignant diseases. Consequently, both TCD and T-cell-replete HLA-haploidentical transplants are increasingly used as alternative therapeutic strategies for patients with selected nonmalignant diseases who do not have a HLA-matched sibling or a MUD. The remarkable results of TCD haploidentical transplants in children were seen in various nonmalignant diseases such as severe combined immunodeficiency (SCID) [30–32], sickle cell disease (SCD) [33], and thalassemia [34] as well as with the development of various partial T-cell depletion methods associated with more encouraging immune reconstitution. Based on data of TCR αβ(+)-depleted haploidentical transplants in pediatric patients with hematological malignancies [35], this method has been applied to nonmalignant diseases including SCID and severe aplastic anemia (SAA) [36], while another report showed with no significant difference between haploidentical and HLA-matched transplants. In these reports, the cumulative probability of overall survival was more than 90% with very low TRM of less than 5% [37]. Not only TCD but also T-cell-replete haploidentical transplants have been increasingly and successfully used for patients with nonmalignant diseases. Thanks to the extremely encouraging results with PTCy in hematologic malignancies, in 2012 investigators from Johns Hopkins reported the outcomes of 14 adult patients with SCD who underwent allo-HCT from related haploidentical donors with PTCy-based G*v*HD prophylaxis. Although this cohort had an OS of 100% at almost 2 years posttransplantation, with no documented cases of G*v*HD, graft rejection was a major problem, likely related to the use of NMA conditioning in patients with a strong immune system and heavily transfused before transplant [38]. In contrast, high rates of engraftment were seen in another study in 16 patients with SCD and thalassemia transplanted with T-cell-replete haploidentical donor and PTCy for G*v*HD prophylaxis. In this study, in addition to the fact that no primary graft failure was observed, severe acute G*v*HD was only 12% [39]. Recently, a group from Thailand investigated busulfan-based

conditioning and PTCy-based G*v*HD prophylaxis for haploidentical transplants in 31 children and young adults with severe thalassemia. In this study, 29 patients were engrafted with full donor chimerism, 9 patients developed grade II acute G*v*HD, while only 5 patients developed limited-chronic G*v*HD. The 2-year OS and event-free survival were 95 and 94%, respectively [40]. Taken together, these data suggest that both TCD and TCR haploidentical transplants are suitable options for the definitive treatment of an ever-widening spectrum of nonmalignant disorders suitable for transplantation, and, in the absence of an HLA-identical donor, haploidentical transplants should be performed in particular in those with anticipating the development of life-threatening infections or severe disease-specific organ complications, in which transplantation is urgently needed (see Chap. 12).

21.5.3 Prevention and Treatment of Viral Reactivation: Role of Cytotoxic T-Cells

Although promising survival has been achieved with the establishment of many haploidentical transplantation protocols, viral reactivation resulting from the impaired immune reconstitution owing to extensive immunosuppression and TCD methods necessary to overcome HLA disparity remains one of the most important causes of morbidity and mortality in patients undergoing haploidentical transplantation. Conventional treatment using antiviral agents is expensive, sometimes ineffective and complicated by serious toxicities like secondary graft failure or acute kidney injury. The use of donor lymphocyte infusions (DLIs) derived from seropositive donors is an effective salvage therapy for viral infections in allo-HCT recipients prior to T-cell recovery, but the risk of potentially severe (grades III–IV) G*v*HD is a concern. To restore immune function, prevent, and treat viral reactivation without causing G*v*HD, some investigators have developed adoptive cellular immunotherapy using clones of $CD8^+$ cytotoxic T-cells (CTLs) specific for common viral proteins such as CMV, EBV, and adenovirus and infused these cells to the allo-HCT recipients. Development of viral-specific therapy to broader applicability has been facilitated by several advances in immunobiology such as improvements in *ex vivo* culture methods for the generation of T-cells and antigen-presenting cells (APCs), advances of knowledge of conserved T-cell epitopes for various pathogens, and rapid assays to evaluate the effector function of viral-specific T-cells. This strategy, initially investigated by a group from Seattle, used CMV-specific CTLs clone infused to patients receiving allo-HCT using matched-related graft. There were no adverse effects from the adoptive transfer of these clones. Furthermore, CMV-specific immune responses were reconstituted, and no patients developed CMV disease [41]. Later on, a group from St. Jude Children's Research Hospital successfully used adoptively transferred donor-derived EBV-specific CTLs for prevention and treatment of EBV lymphoproliferative disease in allo-HCT recipients [42]. As a result of these encouraging outcomes, the adoptive cellular therapy approach using T-cells targeting more than one virus was applied in patients receiving haploidentical transplants by the group from Baylor College of Medicine. Results from

this study have proven that bispecific CTLs containing both EBV- and adenovirus-specific T-cells can safely reconstitute an antigen responsive memory population of CTLs after haploidentical transplantation and may provide antiviral activity [43]. Even though alloreactivity is a common concern for using this cellular therapy approach, none of these published studies reported an increased incidence of G*v*HD over what would be expected in the patient population even in mismatched haploidentical transplants. Still, patients who already developed severe G*v*HD and patients who are treated with glucocorticoids were excluded from all of these studies due to a concern about lymphocytic effect of glucocorticoids. In order to make viral-specific CTLs therapy more applicable to all patients in need, some preclinical studies are focusing on gene modification methods to engineer CTLs clones that resistant to corticosteroids as well as calcineurin inhibitors. Future application will likely expand the use of prophylactic CTL infusions especially against CMV and BK virus for patients of high risk of developing reactivation and prevent diseases associated with these viruses (see Chap. 20).

21.5.4 Prevention of Disease Relapse After Transplant

While drug therapy has the potential to decrease relapse rate in certain very specific groups of patients with hematological malignancies, like TKIs in advanced CML and hypomethylating agents for AML and MDS or FLT3 inhibitors for FLT3-mutated AML, conclusive evidence is lacking, and randomized studies are ongoing. The use of cellular therapy posttransplant may enhance the graft-*versus*-tumor effects and have broader application posttransplant (see Chap. 19). Specific approaches using cellular therapy with cells manufactured from the donor mononuclear cells offer the perspective to enhance antitumor effects of the graft and hopefully decrease relapse rate posttransplant. Several methods to prevent disease relapse posttransplant are being explored (see Chaps. 19 and 20).

21.5.4.1 Modified Donor Lymphocyte Infusion with a Safety Switch

Donor lymphocyte infusion (DLI) is one of the most commonly used interventions for treatment of disease relapse post-allo-HCT. However, a higher risk of acute G*v*HD is a major concern of early administration of an unmodified DLI. To control the development of severe acute G*v*HD, infused T-cells can be *ex vivo* genetically modified to express a specific suicide gene which may be turned on to induce cell apoptosis, if G*v*HD occurs. The administration of donor T-cells with a "safety switch" can help prevent relapse when administered earlier after transplant and may accelerate immune reconstitution. The safety and efficacy of this approach have been investigated in several preclinical and early clinical studies [44–47]. In a phase I/II clinical trial by Ciceri and colleagues, donor T-lymphocytes engineered to express herpes simplex thymidine kinase suicide gene (TK cells) were successfully infused to patients with high-risk leukemia who underwent TCD haploidentical transplantation. T-cell apoptosis can be triggered by the use of ganciclovir if the patients develop G*v*HD. The improvement of immune response against CMV and

EBV was seen after TK cell infusions. Ten out of 28 patients developed acute G*v*HD, which can be abrogated by using ganciclovir [48]. However, ganciclovir is a drug commonly used to treat CMV reactivation in allo-HCT; thus, using this drug might not be optimal. The Baylor group developed an alternative approach by using T-cells engineered to express caspase 9 which can be induced by using a dimerizing agent, AP1903. These inducible caspase 9 T-cells provided rapid immune recovery in pediatric patients received allo-HCT with TCD haploidentical cell graft. AP1903 administration could rapidly resolved G*v*HD without a significant effect on antiviral immune reconstitution [47, 49]. Thanks to these encouraging results, ongoing and future studies are investigating the efficacy of this approach to prevent disease relapse after haploidentical transplant in various diseases.

21.5.4.2 Use of NK-Cells for Myeloid Malignancies

NK-cells have shown the ability in eradicating the disease dissociated from G*v*HD [50]. In haploidentical transplantation, HLA mismatches can trigger donor-*versus*-recipient NK-cell alloreactivity without causing G*v*HD as they target hematopoietic cells sparing other body organs. Therefore, donor-derived NK-cells seem to be the ideal candidate for adoptive cellular immunotherapy to prevent disease relapse after allo-HCT in particular with a haploidentical graft, used successfully in children with AML [51]. Owing to these promising outcomes in pediatric patients, many ongoing studies are now focusing on developing various methods of *ex vivo* NK-cell expansion with aim to increase both number of NK-cells to the level that can prove to be effective for adult patients as well as increase cytotoxicity effect of these cells. To date, several methods of NK-cell expansion have been tested in preclinical and early clinical studies [52–56]. Choi and coworkers generated donor NK-cells from the $CD3^+$ cell-depleted portion of the mobilized leukapheresis product and expanded using human IL-15 and IL-21. Expanded doses of NK-cells up to 2×10^8/kg were then infused into 41 patients with hematologic malignancies who underwent haploidentical transplantation using RIC. Even though no significant difference in the cumulative incidences of major transplant outcomes was seen with historical control however, a reduction in leukemia progression was seen in patients who received a high NK-cell dose, and they found that posttransplantation NK-cell infusion was an independent predictor for leukemia relapse prevention [56]. While these results are promising, they are not conclusive of a beneficial effect in reducing relapse or improving survival and further studies are needed. The MD Anderson group is currently investigating a phase 1 dose-escalation study infusing donor-derived NK-cells expanded *ex vivo* with membrane-bound IL21-expressing K562 feeder cells after haploidentical cell transplantation to determine safety of feasibility of this approach as well as maximum tolerated dose (MTD) of NK-cells that can be infused in this setting. So far 13 patients with advanced, high-risk myeloid malignancies were enrolled and treated. All patients are engrafted with donor cells without the development of severe (grade III–IV) acute and chronic G*v*HD. No adverse effects or infusional toxicities occurred after NK-cell infusions, and a very low relapse rate were

observed [17]. Final results of this study will help confirm the efficacy of adoptive NK-cellular therapy for patients with myeloid malignancies.

21.5.4.3 Use of CAR T-Cells for B-Cell Lymphoid Malignancies

CAR T-cells can help reduce risk of relapse by targeting tumor cells and activating cytotoxic T-cells without added risk for the development of G*v*HD. This method of adoptive immunotherapy has been used successfully in tumors that express the $CD19^+$ antigen such as B-cell ALL or B-cell non-Hodgkin's lymphomas [57, 58]. We are exploring the use of haploidentical donor-derived CAR T-cells generated using the *Sleeping Beauty* system and administered early after haplo-HCT to prevent disease relapse, as a part of a multi-arm phase 1 clinical trial [59]. Eight haploidentical transplant recipients with refractory or advanced lymphoid malignancies received CAR T-cells in escalating doses. Overall only three of eight patients relapse, and the rest remain in completed remission at last follow-up. These results are very promising and show that allogeneic CAR T-cell therapy can be safely administered in early post-haploidentical transplantation without significant G*v*HD in the presence of nonsteroid-based immunosuppression. Future studies are needed to determine the efficacy not only of CAR T cells but also other cellular products that target various tumor antigens in different tumor types.

Conclusion

Haplo-HCT has become a feasible alternative form of transplantation and is expanding worldwide. Future studies will explore outcomes for different malignant and nonmalignant diseases, and research will focus on prevention of viral reactivation, one of the most important complication early posttransplant, and attempt to prevent disease relapse, which has become now the most important cause of treatment failure. In addition, comparative studies between different approaches to haploidentical transplantation, as well as prospective randomized studies comparing in an intention-to-treat fashion haploidentical and HLA-matched unrelated donor transplants, are needed.

21.6 Key Points

1. Outcomes after haplo-HCT have improved with better control of alloreactivity and selective depletion of T-cells.
2. Control of viral reactivation early posttransplant and prevention of disease relapse posttransplant have emerged as most important new directions in the arena of haploidentical transplantation.
3. Prospective randomized studies are needed to evaluate different methods of haploidentical transplant and compare outcomes between haploidentical and HLA-matched donor transplants.

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