Stem Cell Biology and Regenerative Medicine

Taner Demirer *Editor*

Haploidentical Stem Cell Transplantation

An Emerging Treatment Modality

Humana Press

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This book is dedicated to the ones we love.....

Preface

This book documents the use of haploidentical stem cell transplantation (SCT) increasingly as an emerging treatment modality in patients with hematologic malignancies. This book covers a wide range of issues such as logic of haploidentical SCT, donor selection and cell dose, graft failure and rejection, graft-versus-host disease (GVHD), infectious complications frequently seen in this type of transplant and graft versus leukemia effect, as well as efficacy of haploidentical SCT in different malignancies and age groups including chapters regarding innovative approaches and future perspectives from the respected authors involved with stem cell tranplantation and research around the world. Clearly, this book is well positioned to provide a comprehensive coverage of haploidentical SCT as an emerging new treatment modality and will be a useful source for practicing hematologists, medical oncologists, and physicians in other disciplines involving with stem cell transplantation.

Because of the unique abilities of stem cells as opposed to a typical somatic cell, they are currently the target of ongoing research. Research on stem cells is advancing knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms. This promising area of science is also leading scientists to investigate the possibility of cell-based therapies beyond haploidentical SCT to treat disease such as diabetes or heart disease, often referred to as regenerative medicine or reparative medicine.

During the last decade, the number of published articles or books investigating the role of stem cells in cell transplantation or regenerative medicine increased remarkably across all sections of the stem cell–related journals. The largest number of stem cell articles was published mainly in the field of neuroscience, followed by the bone, muscle, and cartilage and hepatocytes. Interestingly, in recent years, the number of stem cell articles describing the potential use of stem cell therapy and islet cell transplantation in the diabetes is also slowly increasing, even though this field of endeavor could have one of the greatest clinical and societal impacts.

This book is the main source for clinical and preclinical publications for scientists working toward cell transplantation therapies with the primary goal of replacing diseased cells with donor cells. With the increased number of publications in relation to stem cells and *cell transplantation*, we felt it important to take this

opportunity to share this new treatment modality in the cell transplantation field with our worldwide readers.

Allogeneic hematopoietic stem cell transplantation (HSCT) remains as the sole curative option for many malignant and nonmalignant hematological disorders. The selection of proper donor among the available candidates is a crucial step during the initial work-up. Despite promising results with haploidentical HSCT, there are still several unresolved issues. An area of active research constitutes the selection of the most appropriate haploidentical donors via several predefined criteria including the presence of donor-specific HLA antibodies, donor-recipient HLA mismatch, noninherited maternal antigens, and natural killer (NK) cell alloreactivity. Ongoing efforts to optimize the procedure in order to enhance immune reconstitution and decrease complications, including GvHD, infections, graft failure, and relapse have so far yielded promising results. Historically, the extent of donor-recipient HLA mismatch showed an inverse relationship with HSCT outcomes. The deleterious effects of HLA mismatch have been substantially eliminated after the advent of modern conditioning regimens and GvHD prophylaxis strategies. Recent studies using post-transplantation cyclophosphamide for GvHD prophylaxis have reported similar overall and disease-free survival rates for haploidentical grafts when compared to HLA-matched sibling grafts. Relapse after haploidentical HSCT is still an important problem. The research on several novel approaches including the early use of donor lymphocyte infusions, post-transplant NK cell infusion, and post-transplant consolidation with hypomethylating agents for acute myeloid leukemia and myelodysplastic syndrome have yielded promising results. The results of further research with higher quality features are expected to unveil a more extensive list of indications for haploidentical HSCT in the near future.

Within this context, haploidentical donors offer many advantages, including higher availability, lower operational costs, and a relatively shorter work-up period, when compared to traditional HLA-matched sibling donors (MSDs) and HLA-matched unrelated donors (MUDs).

It is obvious that *Haploidentical Stem Cell Transplantation* is bridging cell transplantation research in a multitude of disease models as methods and technology continue to be refined. Therefore, haploidentical SCT, as a new treatment modality, which has almost a comparable outcome with match sibling transplants, brought hope and Chance of a cure to many patients awaiting for donor. Use of haplo transplants, increasingly, will remarkably decrease the requirement for time-consuming unrelated donor search procedures and cord blood transplants. But it is yet hard to interpret and compare the results of reported studies regarding used approaches and methods, since the current data about haploidentical HSCT mainly come from the results of nonrandomized trials with retrospective comparison. Thus, current recommendations for haploidentical HSCT substantially depend on expert opinions. Future studies should particularly focus onto head-to-head comparisons of other donor sources such as MSD, MUD, and umbilical cord with haploidentical donors, conditioning regimens, and strategies involving graft manipulation. Further research with higher quality features (i.e., randomized, homogenous population and larger sample size) are needed before recommending haploidentical HSCT for a more extended list of indications.

We hope that this book can serve as an important tool and reference guide for all scientists worldwide who work in the field of stem cells and cell transplantation as well as shed light upon some important debatable issues in relation to the use of haploidentical transplants for the treatment of hematologic malignancies.

It will be exciting and interesting for our readers to follow and see the progress which was made with haploidentical SCT, via this book, with a meticulous presentation of our authors in each chapter from basic and clinical aspects of haplo SCT to innovative approaches in order to make this modality more feasible and sophisticated for transplanters all over the world.

I would like to thank to all authors who contributed this book with excellent and up-to-date chapters. I would also like to give a special thanks to Dominic Manoharan, Production Editor, and Aleta Kalkstein, Publishing Editor, and all Springer USA workers for their valuable contribution in order to make this book available.

Sıhhiye, Ankara, Turkey Taner Demirer

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About the Editor

Taner Demirer, MD, FACP graduated from Ankara University Medical School in Turkey and completed his Internal Medicine residency at the Medical College of Wisconsin. He also completed a hematology/oncology and bone marrow transplant fellowship at the University of Washington, Fred Hutchinson Cancer Research Center (FHCRC) in Seattle. He was trained under Professors Don Thomas (Nobel laureate), Dean Buckner, Frederick Appelbaum and Rainer Storb in the clinical division of FHCRC. He is a diplomate of the American Board of Internal Medicine and Board certified in Internal Medicine and Medical Oncology and was given the title of 'Fellow of American College of Physicians' by the American College of Physicians. He has conducted many clinical studies at the FHCRC as principal investigator or co-investigator with the studies mainly related to peripheral blood stem cell (PBSC) mobilization and high-dose chemotherapy (HDC) as well as autologous or allogeneic stem cell transplantations (SCT) in patients with solid tumors and hematologic malignancies. During his career he has published many papers in respected medical journals and written books on PBSC mobilization kinetics, factors influencing the PBSC collection and engraftment and allogeneic as well as autologous SCT in patients with hematologic malignancies and solid tumors. He was chairman of the EBMT Solid Tumors Working Party (STWP) and has conducted, chaired and published studies related to HDC in patients with solid tumors at the EBMT STWP. He was also board member of the Federation of the European Cancer Societies (FECS) on behalf of the EBMT and was the president of the 29th and 41st EBMT Annual Meetings. He has been member of the editorial board of several international journals. He is currently a professor of medicine and hematology/oncology at the Ankara University Medical School in Ankara, Turkey, and a full member of the Turkish Academy of Sciences as well as the European Academy of Sciences and Arts (EASA).

Chapter 1
General Indications and Logic for Haploidentical SCT

Florent Malard and Mohamad Mohty

1.1 Introduction

Recognition of human leucocyte antigen (HLA) incompatibilities by the immune system represents a major barrier to allogeneic stem cell transplantation (allo-SCT). Compatibility between donor and recipient at the HLA-A, -B, -C, -DRB1 and -DQB1 level is therefore an important predictor of success of allo-SCT. Therefore, use of an HLA genotypically identical sibling donor is the gold standard for allo-SCT. However, given the 25% chance that any sibling is fully HLA-matched to the patient and the generally small family sizes in developed country, the probability to identify a fully HLA-matched sibling donor is at best 30%. For others patients, alternative source of donor graft include suitable HLA-matched adult unrelated donors, umbilical cord blood and HLA-haploidentical related donors. The choice of the donor source depends mainly upon the clinical situation and of the practices of each transplant center.

HLA-haploidentical donors, share with the patient a single identical copy of chromosome 6, containing the HLA loci. Therefore, these donor/recipient pairs are mismatched at half of the HLA loci (HLA-A, -B, -C, -DRB1 and -DQB1), leading to an intense bi-directional alloreactivity associated with high incidence of graft rejection and graft-versus-host disease in initial studies of haploidentical allo-SCT [1, 2]. Advances in graft engineering and pharmacologic prophylaxis of GVHD have markedly improved patients' outcome. Therefore haploidentical donors appears as a suitable alternative in patients who lack a matched sibling donor. We will review the most advanced approach to haploidentical allo-SCT: T-cell depletion

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with ‘megadose’ of CD34+ cells; in-vivo T cell depletion with antithymocyte globulin (ATG), granulocyte colony-stimulating factor (G-CSF) primed graft and intensive post-transplant immunosuppression; and high-dose post transplant cyclophosphamide.

1.1.1 T Cell Depletion with Megadose CD34+ Cells

In order to overcome the mains limitation of haploidentical allo-SCT – high incidence of graft rejection and severe GVHD – researchers investigated T-cell depletion of haploidentical graft. In initial studies, ex-vivo T-cell depletion of bone marrow graft was achieved by negative selection by soybean agglutination and erythrocyte resetting and conditioning regimen were myeloablative, consisting of 8 Gy total body irradiation (TBI), 10 mg/Kg thiotepa, 200 mg/m^2 fludarabine and in-vivo T cell depletion with 5 mg/Kg ATG. No additional post-transplant immunosuppressive was administered [3, 4]. However, this strategy was associated with a high incidence of graft rejection and opportunistic infections [5]. Therefore, attempts have been made to improve these results, by using CD34+ selected graft with ‘megadose’ of CD34+ cells and intensified conditioning regimen [6]. While this strategy was associated with improve engraftment rate of 90–95%, low incidence of acute and chronic GVHD and relapse rate comparable to others approaches, this strategy was associated with a high non-relapse mortality (NRM) in the range of 30–50% mainly related to infectious complications [6]. So far high NRM related to infectious complication – mainly viral – remain the main limitation of this strategy, despite several attempts to refine this strategy. New strategies to enhance immune reconstitution after allo-SCT seem indispensable to allow further development of this haploidentical allo-SCT strategy.

1.1.2 The GIAC Protocol: Peking University Experience

Another protocol of haploidentical allo-SCT, called the GIAC protocol has been developed at the Peking University. This protocol, is based on the treatment of donors with G-CSF to induce donor immune tolerance (G), intensified immunosuppressive therapy post-transplant with cyclosporine A, mycophenolate mofetil and methotrexate (I), in-vivo T cell depletion with ATG to prevent GVHD and graft rejection (A) and use of a combination of bone-marrow and peripheral blood stem cell allograft (C). Compare to HLA-matched sibling allo-SCT, they found similar relapse rate, NRM disease-free survival (DFS) and overall survival (OS) [7]. In this study, the conditioning regimen consisted of 2 g/m^2/d intravenous cytarabine on days −10 to −9, 4 mg/kg/d oral busulfan on days −8 to −6; 1.8 g/m^2/d intravenous cyclophosphamide on days −5 to −4 and 250 mg/m^2 oral Me-CCNU on day −3 for HLA-matched sibling allo-SCT. For haploidentical allo-SCT, patients received the

same regimen except for the dose of cytarabine that was increased to 4 g/m^2/d. All patients received 2.5 mg/kg/d ATG on days −5 to −2 and a combination of BM and G-CSF mobilized PBSC as stem cell source. All patients achieved full engraftment with comparable rate of grade II-IV acute and chronic GVHD. Comparing haploidentical to matched-sibling allo-SCT there was no difference in NRM (14% versus 22%, $p = 0.10$), relapse rate (13% versus 18%, $p = 0.40$), DFS (71% versus 64%, $p = 0.27$) and OS (72% versus 72%, $p = 0.72$) [7].

Huang et al. reported a similar protocol that combined G-CSF primed BM and PBSC without in vitro T-cell depletion in 171 patients with hematologic malignancies [8]. Myeloblative conditioning regimen consisted of 4 g/m^2/d intravenous cytarabine on days −10 to −9, 4 mg/kg/d oral busulfan on days −8 to −6; 1.8 g/m^2/d intravenous cyclophosphamide on days −5 to −4 and 250 mg/m^2 intravenous Me-CCNU on day-3. All patients received in-vivo T cell depletion with ATG, either 20 mg/kg/d porcine ATG or 2.5 mg/kg/d rabbit ATG on days −5 to −2. All patients achieved sustained, full donor chimerism. The cumulative incidence of grade III-IV acute GVHD was 23% and chronic GVHD 47%. The 2-year probability of relapse was 12% for standard-risk disease and 39% for high-risk disease. Interestingly, grade III-IV acute GVHD was associated with better DFS ($p = 0.0017$). The 2-year probability of DFS was 68% for standard-risk patients and 42% for high-risk patients ($p = 0.0009$). For standard risk patients, NRM was 9.1% at day 100 and 19.5% at 2 years, while NRM was higher in the high-risk group at 12.7% at day 100 and 31.1% at 2 years [8].

Subsequently Huang et al. reported the outcome of 250 patients with acute leukemia treated with this protocol [9]. All patients achieved sustained full donor chimerism. The incidence of grade II-IV and III-IV acute GVHD were 45.8% and 13.4% respectively. The incidence of chronic GVHD was 53.9%, 22.6% being extensive. For AML patients, DFS was 70.7% and 55.9% for standard risk and high-risk patients respectively, and for ALL 6.0% and 25.9% respectively. For AML patients, NRM at day 100 was 9.1% and 6.8% and 5.9% for standard risk and high-risk patients; and for ALL 6.9% and 25.9%. At 3 years, NRM was 19.4% and 29.4% for the AML group and 21.2% and 50.8% for the ALL group on standard and high-risk status respectively. High-risk ALL were the patients with the highest risk for NRM with RR of 2.422 (95% confidence interval [CI], 1.005–5.835) [9]. Finally, haploidentical allo-SCT using these protocols have ben compared with matched sibling donors allo-SCT, with similar DFS [9–12].

1.1.3 Post-transplant Cyclophosphamide

The John Hopkins group initially evaluate the use of post-transplant cyclophosphamide (PT-Cy) in murine models, showing that administration of Cy, a highly immunosuppressive alkylating agent, on day +3 was able to achieve stable engraftment despite the major histocompatibility mismatched with less lethal and non lethal GVHD [13]. Based on these finding, Luznik et al. evaluate the safety and efficacy

of high-dose PT-Cy to prevent graft rejection and GVHD after non-myeloablative conditioning and T-cell-replete bone marrow transplants from haploidentical donors [14]. The conditioning regimen combine 12.5 mg/Kg/d intravenous Cy on days −6 to −5, 30 mg/m^2/d fludarabine on days −6 to −2, 200 cGy TBI on day −1, followed by bone marrow infusion on day 0. On day +3 or on day +3 or +4, patients received 50 mg/kg of intravenous Cy. Additional pharmacologic GVHD prophylaxis with tacrolimus and mycophenolate mofetil was not initiated until the day following completion of PT-Cy to avoid blocking Cy-induce tolerance. Graft failure occurred in 9 of 66 (13%) evaluable patients and was fatal in one. The median times to neutrophile (>0.5 × 10^9/L) and platelet recovery (>20 × 10^9/L) were 15 and 24 days respectively. The cumulative incidence of grade II–IV and III–IV acute GVHD by day 200 were 34% and 6% respectively. Regarding chronic GVHD, it was lower in the group of patients receiving 2 days of PT-Cy: 5%, versus 25% in the group receiving only 1 day of PT-Cy ($p = 0.05$). It was the only difference between these two groups; in particular there was no difference in the incidence of acute GVHD. The cumulative incidence of NRM and relapse at 1 year were 15% and 51% respectively. Actuarial OS and event-free survival at 2 years were 36% and 26% respectively. Overall, while their results were acceptable regarding graft failure and severe acute and chronic GVHD, use of a nonmyeloablative approach was associated with a very high relapse rate [14].

The John Hopkins group confirms these results in 372 patients with hematologic malignancies who underwent nonmyeloablative conditioning haploidentical allo-SCT with PT-Cy [15]. Probability of NRM at 6 months was low at 8%, as was 6 months probability of grade III-IV acute GVHD: 4% and the 2 years probability of chronic GVHD: 13%. Three-year probability of relapse, DFS and OS were 46%, 40% and 50% respectively. Interestingly stratifying patients according to the disease risk index, show that patient with low-risk disease have a higher DFS: 65% compare to patients with intermediate- and high/very-high-risk disease: 37% and 22% respectively at 3 years ($p < 0.0001$). Similarly, 3 years OS was 71%, 48% and 25% based on low-, intermediate- and high/very-high-risk disease ($p < 0.0001$). While these results confirm that use of PT-Cy is an effective strategy to allow high engraftment rate and low incidence of severe acute and chronic GVHD after haploidentical allo-SCT, the main limit of this approach remain the high relapse rate, particularly in patients with high risk disease, but also intermediate risk disease.

In order to improve disease control, another team recently evaluates the addition of 2 days of busulfan (3.2 mg/Kg/d on days −3 and −2) to the nonmyeloablative John Hopkins regimen in a prospective phase II multicenter trial. 32 patients with high-risk disease (including 61% who were not in remission at time of transplant) have been included in this study [16]. PBSC graft instead of BM was used in this study and PT-Cy was administered on days +3 and +4. The cumulative incidence of grade II-IV and III-IV acute GVHD at day 100 were 23% and 3%, respectively and the cumulative incidence of cGVHD at 1 year was 15%. The cumulative incidence of NRM and relapse were 19% and 19% at 100 days and 23% and 45% respectively at 1 year. OS and DFS at 1 year were 45% and 34% respectively. Overall, despite using increased dose of chemotherapy in the conditioning regimen, this platform

remains associated with a high relapse rate, leading several groups to evaluate myeloablative conditioning regimen in the setting of PT-Cy.

Solomon et al. report a phase II prospective trial that include 20 patients with hematologic malignancies (9 in remission and 11 with relapse/refractory disease) [17]. The first 5 patients received 30 mg/m^2/d intravenous fludarabine on days −7 to −2, 130 mg/m^2/d intravenous busulfan on days −7 to −4 and 14.5 mg/kg/d Cy on days −3 and −2. Fludarabine and busulfan were reduced by 30% and 15% respectively for the 15 subsequent patients due to significant mucositis. All patients reveived PBSC graft and 50 mg/kg/d PT-Cy on days +3 and +4, followed by tacrolimus and mycophenolate mofetil starting from day +5. All patients achieved full donor engraftment on day +30. The cumulative incidence of grade II-IV and III-IV acute GVHD were 30 and 10% respectively, and the cumulative incidence of cGVHD was 35%. This myeloablative regimen was associated with a NRM of 10% at day 100. One year DFS and OS were 50% and 69% respectively. Overall, use of PT-Cy after a myeloablative conditioning regimen appears to be feasible with an acceptable NRM and a higher DFS compare to reduce intensity conditioning.

Finally, Bacigalupo et al. [18] also reported the use of PT-Cy after a myeloablative conditioning regimen consisting of 5 mg/kg/d intravenous thiotepa on days −6 and −5, 3.2 mg/kg/d intravenous busulfan on days −4 to −2 and 50 mg/m^2/d intravenous fludarabine on days −4 to −2 (n = 92) or fractionated TBI (either 9.9 or 12 Gy) and 30 mg/m^2/d intravenous fludarabine on days −5 to −2 (n = 56). The median day to neutrophil engraftment was day +18 (range, 13–32). The cumulative incidence of grade II-IV and III-IV acute GVHD were 24% and 10% respectively. With a median follow-up for the surviving patients of 313 days (100–1162), the cumulative incidence of NRM is 13% and the relapse-related death is 23%. The actuarial 22 months overall survival is 77% for CR1 patients, 49% for CR2 patients and 38% for patients grafted in relapse ($p < 0.0001$). This results confirm the feasibility of haploidentical allo-SCT with PT-Cy after a myeloablative conditioning regimen with a relatively low NRM and an enhance antitumoral effect.

1.2 Conclusion

Haploidentical donors have emerged as an effective alternative in patients who lack a matched sibling donor. Several conditioning regimen have been succesfuly developed by different teams to overcome the HLA mismatch, although so far none of them have emerged as the optimal regimen. Therefore, while use of PT-Cy is effective for to prevent graft failure and GVHD, this platform is often associated with a high incidence of relapse related to the low intensity of the conditioning regimen. So far no prospective study compare patients outcome after haploidentical allo-SCT according to the platform used or the intensity of the conditioning regimen. Overall, despite the advances of haploidentical allo-SCT over the past years, there is still a lot of unanswered questions in this setting. The next years should be productive and provide answer to some of these questions.

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Chapter 2
Donor Selection and Cell Dose in Haploidentical SCT

Riad El Fakih, Mutlu Arat, and Mahmoud Aljurf

2.1 Introduction

Haploidentical stem cell transplantation (haplo-SCT) from a first degree related haplotype-mismatched donor (siblings, children, and parents) has expanded significantly over the past decade. At the early stages, bidirectional alloreactivity was problematic, causing significant acute GVHD and graft failure. Different haplo-SCT platforms were explored to minimize the bidirectional alloreactivity. In the 1980's ex-vivo depletion of T cells, by using CD34-selected grafts, decreased the incidence of acute GVHD at a price of delayed immune reconstitution causing significant increase in the infections, relapses and graft failure. By using megadoses of CD34 cells along with myeloablation, graft failure became less problematic; however the delayed immune reconstitution remained significant, causing high NRM and thus minimizing the benefit from haplo-SCT. During the last decade, major advances to selectively deplete alloreactive T cells from unmanipulated grafts, made haplo-SCT easier, safer and cheaper to perform. Among other benefits, related-haplo-donors are immediately available and motivated; almost any patient, regardless of the race and age, has at least one haplo-donor, there is no need to maintain an unrelated donor registry or to coordinate logistics with distant donor centers. These benefits are very attractive for developing countries; even in developed countries an increasing number of programs are already adopting haplo-SCT as their default option in the absence of a matched sibling donor. This chapter will review the available literature about donor selection and the appropriate cell dose for haplo-SCT recipients.

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2.2 Donor Selection

The presence of multiple potential haplo donors raises the question on how to select the best donor. The impact of such selection on GVHD, relapse, TRM and OS has been reported in several publications [1–12]. Unlike the matched transplant setting, the degree of HLA disparity on the mismatched haplotype does not significantly affect the haplo-SCT outcomes (as long as the donor and recipient share a full haplotype) in both the T-cell replete and T-cell deplete setting [1, 13, 14]. Listed below are variables gaining attention during the process of haplo donor selection, these variables are summarized in Table 2.1:

1. DSA (donor specific antibodies): Allo-antibodies against HLA are frequently encountered in clinical medicine; these are usually due to foreign HLA exposure (transfusion, pregnancy or previous transplant). In transfusion medicine HLA-antibodies cause platelet refractoriness. When these antibodies are specific against the donor HLA, they are called DSA; when they lack specificity against donor HLA these are simply called anti-HLA antibodies. DSAs are a well-known cause of graft rejection in solid organ transplant as well as mismatched hemopoietic cell transplant, especially in the setting of a positive anti-donor lymphocytes cross-match [15, 16]. The correlation between the presence of DSAs and poor graft function or failure, is well established in both the T-cell depleted and T-cell replete (unmanipulated) haplo-SCT setting [4, 17–19]. When a recipient has DSAs against a donor, this donor is usually changed. If there is no alternative then the recipient should receive therapy to minimize the levels of these DSAs (especially when the level of these DSAs is high) before transplant. Several desensitization protocols have been reported with variable degrees of success (plasma exchange, rituximab, bortezomib, intravenous immunoglobulin) [20, 21].
2. CMV: The CMV serostatus in haplo-SCT is no different from other types of transplants. The outcomes are better when the donor and the recipient have the same serostatus.
3. Age: Normal aging is associated with a decline in hematopoiesis and immunity along with an increased risk of clonal hematopoiesis [22–24]; these changes explain in part the worst outcomes when using older donors. In the T-cell depleted haplo-SCT, age did not have impact on transplant outcomes, whereas in the unmanipulated haplo-SCT setting, the use of younger donors was associated with less NRM, a better survival and less GVHD. The graft composition apparently is also different in young donors as compared to older donors [2, 25, 26]. Since most of the haplo-SCT centers use unmanipulated grafts now a day, young haplo donors are preferred.
4. Gender: In the unrelated transplant setting, the use of old multiparous women as donors correlated with more GVHD and lower OS [27, 28]. In the haplo-SCT setting, the use of female donors led to more acute GHVD in some studies, regardless of the platform used (T-cell deplete or replete) [8, 29, 30], other studies using male donors, showed less NRM and a better OS [2].

Table 2.1 Impact of donor-recipient variables on the outcome of haploidentical SCT

Variable	Effect in haplo-SCT
Donor specific antibodies	Associated with poor graft function and graft failure in T-cell replete haplo-SCT. Risk can be minimized by desensitization (depletion of the antibodies)
CMV	Outcomes are better when the donor and the recipient have the same CMV serology
Age	In unmanipulated haplo-SCT young donors use was associated with less NRM, GVHD, and better OS In T-cell depleted haplo-SCT no significant impact of age was observed
Gender	Female donors led to more acute GHVD in some haplo-SCT studies, regardless of graft composition
ABO incompatibility	No or minor ABO mismatch is preferred to avoid graft manipulation
KIR mismatch	KIR mismatched haplo-SCT was associated with better tumor control and outcomes in some studies
Non-inherited maternal HLA antigens (NIMA)	NIMA-mismatched donor use is associated with less acute GVHD
Family relationship	In T-cell replete haplo-SCT, mother donors use was associated with more GVHD, high NRM and lower OS as compared to father haplo-donors Sibling donors were associated with more acute GVHD as compared to children Father donors were better than old sisters

5. ABO: the presence of major ABO incompatibility can cause significant hemolysis, regardless of the transplant type (matched, unmatched, related or unrelated). RBC-depletion from the graft is one way to prevent these reactions, a process that frequently cause loss of cells and thus predispose to graft failure [31, 32]. Minor ABO incompatibility reaction is usually minimized by plasma depletion, a process that does not affect the cell dose in the graft. As such a donor with no or minor ABO mismatch is preferred.
6. NK alloreactivity: NK cells are an integral part of the innate immune system, and have an important role in tumor and viral surveillance. Each NK cell has immunoglobulin-like receptors (KIR). These receptors can activate or inhibit the NK cells. In general self-antigens inhibit NK cells and non-self-antigens activate NK cells, as a result NK cells attack cells that does not have self-antigens [33, 34]. It is speculated that KIR mismatched haplo-SCT leads to better tumor control and less relapse resulting eventually in a better outcome, however data from different groups are not in agreement [35–38].
7. Non-inherited maternal HLA antigens (NIMA): During pregnancy, immune tolerance happens between the mother and the fetus. As a result the fetus tolerates the maternal antigens that he did not inherit from her (NIMA) and the mother tolerates the fetal antigens inherited from the father (inherited paternal antigens or IPA). In the haplo-SCT setting when the recipient and donor share the paternal haplotype, they are called NIMA-mismatched and less alloreactivity develops as

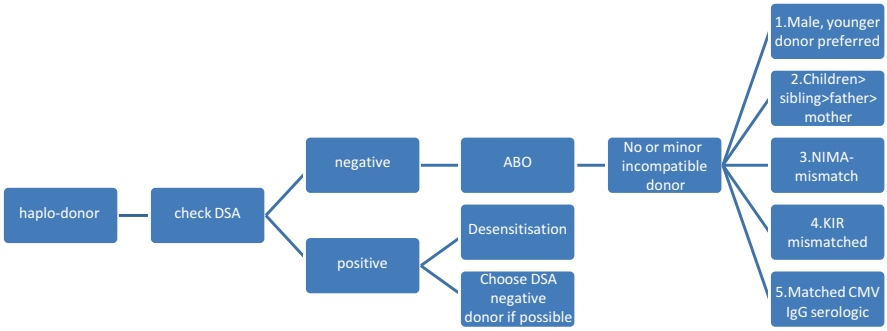

Fig. 2.1 Algorithm for haploidentical donor selection

a result of the immune-tolerance that has happened during pregnancy. When a NIMA-mismatched donor is used, the incidence of acute GVHD is lower as compared to NIPA-mismatched (non-inherited paternal antigens) donor [2, 39–41], therefore a NIMA-mismatched donor is preferred.

8. Family relationship: The effect of recipient-donor relationship on haplo-SCT outcomes has been explored in few trials. In one study of T-cell depleted haplo-SCT, the use of the mother as a haplo donor was associated with improved transplant outcomes and better OS [10]. In T-cell replete haplo-SCT, mother donors were associated with higher NRM, more acute GVHD, and worse OS as compared to father haplo-donors. Grafts from siblings were associated with more acute GVHD as compared to grafts from children. Additionally grafts from fathers were better than grafts from old sisters, and grafts from NIMA mismatched siblings were better than transplants donated by mothers [2]. The use of collateral (second and third degree relatives) haploidentical related donors was associated with more chronic GVHD as compared to immediately related donors [42].

Obviously these variables might have different impacts depending on the transplant strategy and platform used. For example using mother donors showed opposite outcomes when used in the T-cell replete platform as compared to T-cell deplete platform. Conditioning intensity, GVHD prophylaxis strategy, direction of mismatch (GVHD direction vs rejection direction or bidirectional), disease status and patient's condition should be incorporated in the decision-making process when choosing haplo-donors. It is recommended to build an algorithm of donor selection for each institution, depending on the platform used, the available literature and the local experience of the center (Fig. 2.1 is the algorithm used to select donors in unmanipulated haplo-SCT with post-transplant cyclophosphamide).

2.3 Cell Dose

The intense bidirectional alloreactivity in the setting of haplo-SCT, have a relation on the cell dose used to ensure hematologic reconstitution. A low cell dose might cause graft failure or poor graft function and a high cell dose might cause engraftment syndrome, or GVHD. Many factors need to be accounted for when choosing the dose; platform used (T-cell deplete or replete), conditioning (MAC, RIC), disease status and type, GVHD prophylaxis strategy (ATG or post-transplant cyclophosphamide), direction of mismatch (GVHD direction vs rejection direction or bidirectional), degree of HLA disparity, sex match, source of stem cells (blood or marrow), ABO mismatch…etc. There is no existing consensus on the optimal dose to be used; however there is a general agreement about the minimum accepted threshold.

When using a T-cell depleted platform, high CD34 cell count is needed to ensure engraftment. On average a cell dose between 10–20 × 10^6 per kg of recipient (megadose of CD34 cells) was sufficient for hematologic recovery [43–45], CD34 doses less than 8 × 10^6 per kg of recipient were associated with delayed engraftment [46, 47].

Other cell subsets (e.g. CD3+ cells) are also important for engraftment; unmanipulated grafts have good numbers of all cell subsets and therefore a lower number of CD34 cells is needed for engraftment. In T-cell replete haplo-SCT setting, a total nucleated cell dose of 6 × 10^8 per kg of recipient is considered adequate [1, 2], and CD34 cell doses as low as 2.19 × 10^6 per kg of recipient [48] were enough.

The graft source correlates with the composition and the proportions of cell subsets in the graft and therefore affects the dosing. Peripheral blood grafts are different from bone marrow grafts. In the matched transplant settings, peripheral blood source is associated with rapid engraftment, and more chronic GVHD. Unfortunately, in haplotransplant there is no enough and mature data to define cell dosing according to the source of stem cells.

2.4 Conclusion

Breakthrough advances in immunology and better understanding of the cellular subsets responsible for alloreactivity, paved the road to wide clinical application of haplo-SCT in hematology. Over the next few years haplo-grafts will probably be the preferred alternate graft source, and might even replace matched unrelated donor grafts. Almost every patient has multiple haplo-donors immediately available and optimizing donor selection is of utmost importance to improve the haplo-SCT outcomes. Knowledge about the optimal haplo donor is evolving as new data emerge; because of the fundamental differences between haplo-SCT platforms, it is recommended that each institution implement their own donor-selection algorithm and guidelines for cell dosing.

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Chapter 3
Graft Failure and Rejection in Haploidentical Stem Cell Transplantation

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3.1 Introduction

Allogeneic hematopoietic stem cell transplantation (alloSCT) is an established curative procedure for many patients with hematologic malignancies, as well as for those with inherited diseases, such as hemoglobinopathies, bone marrow failure syndromes and as enzyme replacement in metabolic disorders [1–4]. The necessity to find an HLA-matched related donor is a major obstacle that compromises the widespread application of alloSCT. A suitable HLA-identical sibling donor will be available for about 30% of patients. For patients without an HLA identical sibling, the likelihood of identifying an volunteer unrelated donor matched at HLA-A, -B, -C, and -DRB1 is about 75% among caucasian patients but it decreases to 20–40% for patients of other ethnic backgrounds [5, 6]. Furthermore, the search for an HLA-matched-unrelated donor (MUD), can pose an unacceptable delay in performing alloSCT for many patients with aggressive hematological malignancies. In these patients the likelihood of proceeding to transplantation is less than 50%, mainly because of progression of disease during the search process [7]. In addition, the cost of recruiting match unrelated donors and maintaining donor registries might render this approach unaffordable for developing countries [8]. Therefore alternative stem cell sources of hematopoietic stem cells such as unrelated umbilical cord blood and haploidentical family donors are used to enable a rapid transplantation for this substantial number of patients [9, 10].

Unrelated umbilical cord blood (UCB) transplants have been proven to be successful in children and adults and have the advantage of a quick donor search. In addition UCB have shorter time to proceed to transplant when compared with MUD, an important advantage in the context of advanced hematological

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malignancies, where the risk of relapse is high and the goal is to perform a transplant as soon as the patient achieves a state of minimal residual disease after induction therapy. In addition, UCB transplantation allows for a greater degree of HLA mismatch while obtaining relatively low rates of graft-versus-host disease (GvHD), probably due to the lower number of activated alloreactive T lymphocytes present in cord blood [11–13]. However, UCB units have low numbers of hematopoietic progenitor cells and this may lead to delayed engraftment, increased time to hematopoietic recovery, and poor immune reconstitution, increasing the risks of life-threatening infections and graft failure. The possibility of posttransplant immune manipulation, such as donor lymphocyte infusions, for patients who relapse after transplant is also diminished. Although some of these limitations can be overcome by using two or more units of cord blood, this approach is daunted by the high cost of the UCB and maintaining cord blood banks [8, 14, 15].

3.2 Haploidentical Transplants

Haploidentical hematopoietic stem cell transplants (haplo-SCT) refer to an alloSCT from a related donor that has a complete half mismatch with the recipient. HLA-haploidentical donors share with the patient a single identical copy of chromosome 6 containing all the genes in the MHC and the HLA loci. These donors are available for nearly all individuals, and can include any healthy parent or child, approximately half of all siblings and potentially even more distant relatives possessing a shared haplotype. The main advantage of haploidentical transplants is that related donors are readily available and are highly motivated to donate to a family member. Haplo-SCT also has the benefits of speed because relatives are usually easy to contact for stem cell collection, and lower cost than mismatch unrelated donor or umbilical cord products. Another major advantage of haploidentical donors over UCB is the continuous access to the stem cell source that makes posttransplant cellular therapy, such as donor lymphocyte infusions and immune manipulation, available in case of relapse [16–18].

The major disadvantage of haploidentical donors is the HLA disparity. The use of HLA-mismatched allografts is associated with intense bidirectional allo-reactivity, in that the host immune system seeks to eliminate donor cells (graft rejection) and the donor immune system seeks to eliminate the host (GvHD). HLA mismatches, either at the antigen level or at allele level, have been associated with inferior survival after alloSCT, with greater degree of mismatch correlating with worse outcomes [19–21]. Since donor-recipient HLA histocompatibility is the most important independent predictor of outcomes after alloSCT, the host versus graft and graft versus host barriers are more difficult to overcome in haploSCT than in HLA-matched alloSCT. Although the lower risk of relapse due to HLA disparity supports the existence of a “graft versus tumor” (GvT) effect, this positive aspect of haploSCT was offset by markedly increased rates of graft failure, GvHD, and nonrelapse mortality (NRM) that were the cause of failure of initial attempts [10, 20–22].

Over the past two decades, new approaches to haploSCT have effectively controlled this double alloreactivity, resulting in markedly improved outcomes. In this chapter we will address the three most developed strategies to haploSCT: (1) T-cell depletion (TCD) with 'megadose' CD34+ cells [23]; (2) T-cell modulation with granulocyte colony-stimulating factor (GCSF)-primed grafts, antithymocyte globulin (ATG), and intensive post-grafting immunosuppression [24]; and (3) high-dose, post-transplantation cyclophosphamide [25, 26]. Using these approaches, the degree of HLA disparity is no longer a risk factor for GvHD and the results of recent retrospective analyses have demonstrated similar patient survival after haploSCT and HLA-matched-related or HLA-matched-unrelated alloSCT [27–33].

Donor-derived T lymphocytes are key cell effectors in the pathogenesis of GvHD. This was confirmed when GvHD was prevented using initial ex vivo bone marrow TCD techniques without any additional posttransplant GvHD prophylaxis, although high rates of graft rejection, delayed immunoreconstitution and relapses were observed. To improve the results, megadoses of G-CSF mobilized peripheral blood positively selected CD34+ cells have been used as a method of TCD. Despite encouraging long-term results, infectious mortality was 30% due to prolonged immune deficiency, caused by removal of T cells from the stem cell source [23]. The use negative depletion techniques (such as CD3+ and CD19+ negative depletion), results in effective removal of T and B-lymphocytes while maintaining CD34+ cells and other immune cell types such as NK cells, dendritic cells and monocytes. Immune recovery has been improved with the use of alfa-beta T cell and CD19 B cell depletion [34–37].

Wang and co-workers developed the second major strategy for haploSCT also known as the "Beijing protocol" or T-cell replete haplo-grafts. They showed that haploSCT can be performed with intensive in vivo immune depletion with antithymocyte globulin (ATG), cyclosporine A (CsA), methotrexate (MTX), and mycophenolate (MMF), and without ex-vivo T cell depletion [24].

The third strategy is leaded by investigators at Johns Hopkins University that have pioneered a method to selectively deplete alloreactive cells in vivo by administering high doses of cyclophosphamide (Cy) 3–4 days after infusion of mismatched grafts, protecting the recipient from GvHD [25, 26]. The mechanisms of posttransplant Cy (PT/Cy) induced tolerance were delineated in animal models: (1) direct elimination of host T cells responding to donor antigens in the periphery; (2) intrathymic clonal deletion of donor-alloreactive host T cells; and (3) generation of tolerogenic host suppressor T cells [38]. Donor T cells exposed to host antigens on day 0 were largely depleted, whereas non-alloreactive donor T cells, which divided more slowly were relatively spared. However the generation of donor Treg cells was also necessary to prevent lethal GvHD after posttransplant Cy. Hematopoietic progenitor cells and donor Treg cells in both mouse and human models of alloSCT were resistant to posttransplant Cy cytotoxicity owing to increased expression of aldehyde dehydrogenase, the enzyme primarily responsible for in vivo detoxification of Cy, upon allogeneic stimulation in a lymphopenic environment [39–44]. Finally, murine studies have demonstrated that PT/Cy relatively spares pathogen and cancer-specific T cells [42]. Immune reconstitution following haplo-PT/Cy is characterized by a

diverse T cell receptor repertoire and appears dependent on T memory stem cells maturing from naïve T cells that are adoptively transferred in the donor graft and have been shown to survive cyclophosphamide- induced deletion [45, 46].

Several clinical studies have shown that administration of cyclophosphamide on day 3 and 4 posttransplant is a safe and effective method of inducing posttransplant tolerance, dramatically reducing the risk of both GvHD and graft failure in the haploSCT setting, with low transplant related mortality, making this approach affordable to the majority of patients [16–18].

3.3 Graft Failure. Definition and Pathogenesis

Allogeneic SCT is used to rescue patients from the myeloablative (MA) effects of high-dose pre-transplant conditioning therapy and to procure the GvT effects of the graft. Donor hematopoietic cell engraftment is attained when the stem cells of the donor have been taken up by the patient's bone marrow ("have engrafted"), after a conditioning regimen. Engraftment of neutrophils is classically defined as the first of 3 days with neutrophil count $>0.5 \times 10^9$/L. Engraftment of platelets is defined as the first of three consecutive days with platelet count more than 20×10^9/L without transfusions. Hematopoietic reconstitution from donor hematopoietic stem cells is verified by chimerism studies usually performed in T cells and myeloid cells with molecular biology techniques such as VNTR, SNP, or STR-PCR, that demonstrate genetic differences between donor and recipient cells. Full donor chimerism is defined as the presence of >95% of donor-origin hematopoietic cells, whereas mixed chimerism describes the coexistence of recipient and donor hematopoietic cells (ranging from 5–95% of the whole blood cells), following allogeneic SCT [47–49].

Graft failure after allogeneic hematopoietic stem cell transplantation is defined as the inability to achieve donor cell engraftment ("nonengraftment") or as a severe decrease of the donor-derived hematopoiesis after initial engraftment. Graft failure manifests with severe pancytopenia and marrow aplasia and may be classified into primary or secondary. Primary graft failure is defined as no evidence of engraftment or hematological recovery of donor cells, without signs of relapse. In primary graft failure neutrophils never reach $\geq 0.5 \times 10^9$/L after transplant. Secondary graft failure refers to the loss of a previously functioning graft, resulting in cytopenias involving at least two blood cell lineages. In secondary graft failure neutrophils increase to $\geq 0.5 \times 10^9$/L and subsequently decrease to a lower level until additional treatment to obtain engraftment is given. Primary graft failure is usually associated with a more relevant risk of morbidity and mortality in comparison with secondary graft failure. In the latter case, autologous recovery is common; however, marrow aplasia and pancytopenia may also develop [48–51].

Neutrophils can temporarily decrease to lower levels ($<0.5 \times 10^9$/L) due to several causes other than graft failure, such as viral infections, medication or graft versus host disease. In alloSCT with reduced intensity (nonmyeloablative) conditioning, it is possible that the blood cell levels never go below the given limit. It is

also possible, without loss of graft, that some blood cell counts do not reach the given limit for a very long time (particularly platelets). There can be a loss of an allogeneic graft with normal blood cell counts due to autologous reconstitution. All these situations can be confirmed with chimerism studies [47].

Poor graft function can be diagnosed when there are two or three cytopenic lines (Hb <10 g/dL, neutrophil count $<1.0 \times 10^9$/L, platelet count $<30 \times 10^9$/L) for at least 2 consecutive weeks beyond day +28, without transfusion support and with complete or near complete donor chimerism [52].

There are several biological mechanisms that may contribute to graft failure. Graft rejection by the recipient immune cells is a major cause of graft failure. Graft failure may also be caused by infections, mainly of viral origin, such as those caused by cytomegalovirus, human herpes virus type 6 and parvovirus especially when associated with macrophage activation. Use of drugs producing myelotoxicity such as ganciclovir can also induce graft failure. Increased risk of graft failure has been reported in HLA-mismatched, and major ABO-mismatched transplants, as well as with low cell dose in the graft, T-cell depletion, reduced intensity conditioning regimens and cord blood transplantation [12, 48–51, 53–61]. The increasing use of reduced intensity conditioning (RIC), and wider application of HLA-mismatched donors in recent years have turned graft failure into an increasing problem [49, 50, 62].

Recipient T-cells are regarded as the main contributors to immunological rejection of the donor hematopoietic stem cells; although natural killer cell mediated rejection has also been demonstrated. Patient natural killer cells surviving the preparative regimen can also kill donor hematopoietic stem cells through the mechanism of 'missing-self recognition', provided that the patient: (1) expresses a killer immunoglobulin receptor (KIR)-ligand missing in the donor HLA genotype; and (2) expresses the specific KIR, leading to a KIR/KIR-ligand mismatch in the host-versus-graft direction [20, 34, 49, 63, 64]. Although immune-mediated graft failure is typically mediated by recipients either T or NK cells directed against major or minor non-shared donor histocompatibility antigens, it has been shown that the presence of donor-reactive antibodies in allosensitized recipients may also be involved in the pathophysiology of this complication. Allosensitization towards major HLA antigens or, less frequently, minor histocompatibility antigens that develop as a consequence of previous blood products transfusions, can contribute to the increased rejection rate observed in nonmalignant diseases. Immunologically mediated rejection can be caused by sensitization of the recipient to nonshared HLA antigen. Therefore the risk of graft failure is higher in transplant recipients who have donor-specific anti-HLA antibodies [65–69].

3.4 Incidence of Graft Failure

Graft failure can be mediated by cellular or humoral immunity or it may reflect insufficient or damaged stem cells. A recent series of 967 consecutive patients who underwent alloSCT in a single institution revealed that the overall rate of graft failure was

5.6%; primary graft failure was 0.6% and secondary graft failure 5% [50]. This retrospective study included first alloSCT performed in recent years (1995–2010) mainly in patients with leukaemia but also in patients with other malignancies and non-malignant diseases. Most donors were HLA-identical siblings (38%), match unrelated donors (MUD, 47%), and mismatch donors (MMD, 13%). Peripheral blood stem cells were more frequently used than bone marrow or UCB as a graft source. Conditioning regimens included TBI and non-TBI myeloablative regimens as well as nonmyeloablative (RIC) fludarabine-based regimens. Acute GvHD (aGvHD) prophylaxis was mainly based on the Cyclosporine-methotrexate combination although varied according to the type of transplant, including anti-thymocyte globulin for MUD or MMD, and TCD grafts for some mismatch transplant recipients. Univariate analysis confirmed that type of disease, HLA match, ABO match, cell dose; intensity of conditioning, and type of GvHD prophylaxis influenced the risk of graft failure. Multivariate analysis revealed that graft failure was significantly higher in patients with non-malignant diseases, MUD, nonmyeloablative (NMA) conditioning regimens, low total nucleated cells (TNC) grafts and TCD transplants [50].

It is interesting to note that time to engraftment with match related donors (MRD), match unrelated donors (MUD), and haploidentical donors are similar when comparable cellular products and conditioning regimens are used [16, 70–72]. In this setting UCB products have 10-fold fewer stem cells compared with adult donor stem cell products, with a median TNCs and $CD34^+$ cells infused between 1.0 and 3.3×10^7 cells per kilogram and 0.74 and 1.2×10^5 cells per kilogram, respectively that results in slower count recovery than in MRD, MUD and haploidentical transplants [12–14, 16, 65, 73]. In addition, in UCB transplantation, there are few passively transferred T cells from the donor to protect against graft rejection. Therefore, graft failure ranges between 15% in RIC UCB transplantation and 20% in myeloablative UCB transplantation [12, 74, 75]. Engraftment failure after UCB transplant is a serious complication and is usually treated with a second UCB transplant or an haploidentical donor transplant [66].

In the setting of alloSCT from haploidentical family donors, the infusion of unmanipulated transplants allows reaching an optimal rate of engraftment, since residual recipient T lymphocytes are eliminated or inactivated by donor T cells. On the contrary, extensively T-cell-depleted haploidentical transplants are associated with high rate of graft rejection as a consequence of the paucity of immune-competent donor T cells [49, 76].

3.4.1 Graft Failure in TCD-haploSCT

Results of TCD-alloSCT from donors other than HLA-matched siblings showed that graft failure was a persistent problem, affecting more than 20% of patients. It is well established that T-cell depletion both increases the risk of graft failure and leukemic relapse because of reduced cellular alloreactivity of the graft and emergence of conditioning-resistant, anti-donor T cells in the host [76–78]. Graft failure

in animal models of TCD alloSCT was reduced depleting the residual host T cells by using monoclonal antibodies or increasing the doses of radiotherapy or chemotherapy in conditioning regimens [79]. Induction of tolerance was also improved increasing the doses of CD34 stem cells by means of a "veto" effect [17, 80, 81].

These concepts were integrated by the Perugia group into a transplant model that intensified the conditioning regimen incorporating thiotepa, fludarabine, TBI and ATG, and increased the stem cell dose (so-called "mega-dose") with a CD34+-selected PBSC graft. The median CD34+ cell dose was 13.8×10^6 cells per kilogram (range, $5.1–29.7 \times 10^6$ cells per kilogram). No post-grafting immunosuppression was given. With this approach the rate of graft failure was 5–9% [23, 82]. Acute and chronic GvHD were less than 10% and relapse rates between 25% and 30%. NK cell recovery was rapid, and those patients receiving grafts from NK cell alloreactive donors seemed to have a lower risk of relapse. These data were confirmed in a recent report of the European experience of TCD-haploSCT in 266 patients with acute leukemias in which the rate of graft failure was 9%. Acute GvHD grade III-IV and chronic GvHD occurred in 6% and 14% respectively and 2-year non relapse mortality (NRM) ranged from 36% to 66% depending on the disease type and stage at haploSCT [83]. Studies with this transplant model in children have given similar results. However, despite encouraging long-term results, mortality due to infections remained high, about 30%, as a consequence of prolonged immune deficiency, caused by removal of T cells [84, 85].

Immune recovery has become more rapid with the use of negative depletion of lymphocytes during graft manipulation. In contrast with positive CD34+ selection in which almost no cells other than the CD34+ stem cells are transplanted, the double negative depletion of CD3+ and CD19+ cells produce a graft containing dendritic cells, NK cells, monocytes, and other myeloid cells in addition to the CD34+ progenitors, which might enable better immune recovery without leading to GvHD. However the number of alloreactive lymphocytes "contaminating" the donor graft is approximately ten times higher in CD3+/CD19+ depletion, thus making necessary the use of pharmacological GvHD prophylaxis after these type of transplants [36]. Successful engraftment was obtained in all but one of 29 patients transplanted with this approach in combination with a RIC regimen [34]. In another study after myeloablative conditioning in a pediatric population the rate of graft failure was 13%. However acute GvHD grade III-IV and chronic GVHD was 7% and 20% respectively, and 63% of patients relapsed with a NRM of 20% at 5 years [35]. More recently, the depletion of T-cell receptor + T lymphocytes and CD19+ B cell depletion has been used. This method retains + T lymphocytes in the graft. + T lymphocytes are non-alloreactive and hence do not contribute to GvHD, but exhibit potent antitumor and anti-infectious properties. This procedure has been tested in 23 children with nonmalignant disorders. Four patients had graft failure, skin acute GvHD was observed in three and chronic GvHD in none for a NRM of 9.3% and EFS of 91.1% [86]. In 41 children -36 with malignant disease- transplanted by the German group the incidence of graft failure was 12% (5 patients) aGvHD grades II and III-IV occurred in 10% and 15% respectively. Extensive chronic GvHD (cGvHD) occurred in 9% of the patients whereas limited and transient cGvHD occurred in 18%. Three children died of NRM and 17 of relapse, although the

EFS was 100% for the patients transplanted in complete remission [87]. No graft failure was observed in a group of 42 children treated in South Korea. Grade II-IV and III-IV acute GvHD were 31% and 12% respectively, and chronic GvHD was 15%. NRM was 2.6%. Sixteen patients relapsed and 11 died of disease [88]. No graft failure was observed in 33 acute myeloid leukemia pediatric patients treated in Russia. Acute GvHD grade II-III incidence was 39% and chronic GvHD 30%. NRM was 10%, EFS 60% and OS 67% at 2 years [89]. However the same group has published a 27% incidence of graft failure in children with primary immunodeficiencies [37].

3.4.2 Graft Failure in T-Cell Replete (TCR) haploSCT

The GIAC protocol (G-CSF stimulation of the donor, Intensified immunosuppression with CsA-MTX-MMF/basiliximab, Antithymocyte globulin use, Combination of PBSC and bone marrow) was developed at the Air Force General Hospital [90, 91] and the Peking University [92] both in Beijing, China. Although both groups used similar strategies, the Air force General Hospital group used bone marrow alone and added basiliximab to immunosuppression achieving full donor chimerism in all transplanted patients with an incidence of II-IV aGvHD of 11% [91]. Similarly, the group of the Peking University, using combined PBSC and bone marrow reported 100% sustained engraftment in 171 patients that underwent T-cell replete haploSCT family donor. The cumulative incidence of grade III-IV acute GvHD was 23% and that of extensive chronic GvHD, 47% [92]. This group showed analogous outcomes in a pediatric series of 42 patients less than 14 years with no graft failures and cumulative incidences of grade III-IV acute GvHD and extensive chronic GvHD of 13.8% and 29.5% respectively [93]. An update of the results in 250 consecutive patients with acute leukemia showed that all but one patient achieved full donor chimerism [94]. Modifications of this protocol using RIC regimens and other variations trying to decrease GvHD increased the rate of graft failure to 7–8% [95, 96].

Two comparative retrospective studies have shown that haploSCT using the GIAC protocol provides similar results to those achieved with MRD [27], and MUD allo-SCT [33]. All patients achieved full engraftment although the rates of GvHD were lower in the MRD group. These data have been confirmed in a recent prospective multicenter study in adult patients with acute leukemia comparing 231 patients that underwent haploBMT versus 219 patients that underwent a MRD alloBMT using biological randomization based on donor availability [97].

3.4.3 Graft Failure in the Posttransplant Cyclophosphamide (PT/Cy) Protocols

The use of high-dose PT/Cy to induce tolerance in the haploSCT setting was pioneered at the Johns Hopkins Hospital in a phase I study that was published in 2002 [25]. The patients were conditioned with a RIC regimen including fludarabine,

cyclophosphamide and 200 cGy TBI, and then received bone marrow TCR haploidentical graft and on day 3 posttransplant received 50 mg/kg Cy as well as MMF and Tacrolimus for additional GvHD prophylaxis 24 h after the Cy. Among the 10 patients evaluated eight had successful engraftment, and six developed grade II–IV acute GVHD [25]. This was followed by a phase I/II study in which some patients received two doses of 50 mg/kg Cy on days 3 and 4 posttransplant and Tacrolimus was continued until day 180. The rates of graft failure, grade III-IV aGvHD and NRM were 13%, 6% and 15% respectively [26]. Posterior studies with large number of patients and longer follow up using the PT/Cy platform have confirmed low rates of graft failure (2–10%), NRM (8%) and severe acute GvHD (4%), with a 3 year probability of relapse, progression free survival and overall survival estimates of 46%, 40% and 50% respectively [18, 28, 98, 99].

Modulations of this platform including myeloablative-conditioning (MAC) regimens have been performed in an attempt to further improve engraftment and reduce relapses. A study using PT/Cy and MAC regimens has shown a low incidence of graft failure (<5%), a low incidence of acute grades II–IV GvHD (18%), a very low rate of severe grades III-IV acute GvHD (3%) and relapse rate of 26%. In this study CsA and MMF were started before PT-Cy and the PT CY was given in day +3 and +5 [100]. Another study that used a TBI-based myeloablative conditioning with PBSCs for haploSCT, showed a 100% of engraftment, and 78% survival rate, with low NRM (3%) and relapse (24%) after 2 years of follow up, although with higher rates of acute (23% grade III–IV) and chronic (22% moderate or severe) GVHD [101].

Several centers have elected to use exclusively peripheral blood (PBSC) as a stem cell source instead of bone marrow (BM) after the original Baltimore NMA conditioning regimen based on donor preference, inability to secure operating room hours, and outcome considerations. This strategy was explored in a multicenter study using T-replete PBSC haploSCT with RIC conditioning and PT/Cy in 55 patients with high risk hematologic disorders [9]. Patients received a mean of 6.4×10^6/kg CD34+ cells and 2×10^8 /kg CD3+ cells. The median times to neutrophil and platelet recovery were 17 and 21 days respectively. All but two of the patients achieved full engraftment. The cumulative incidence of grade III-IV acute GvHD and chronic GvHD was 8% and 18% respectively. Overall survival and event-free survival at 2 years were 48% and 51%, respectively. The 2-year cumulative incidences of NRM and relapse were 23% and 28%, respectively. The authors concluded that PBSC could be substituted safely and effectively for BM as the graft source for haploidentical transplantation after RIC. In a recent paper, unmanipulated BM and PBSC have been compared in the nonmyeloablative setting: incidence of acute and chronic GvHD, non-relapse mortality, relapse, and survival were quite comparable. The cumulative incidences of ANC and platelet engraftment were 87% and 95% after BM and PBSC infusion, respectively. The median time to obtain full donor chimerism was 60 days (range, 15–108), which was similar between the BM and PBSC groups [102]. However results were a little different when unmanipulated PBSC was given after myeloablative regimens, such as full dose TBI: in the Atlanta program with TBI 12Gy, all patients

engrafted with a median time to neutrophil and platelet recovery of 16 and 25 days, respectively. All evaluable patients achieved sustained complete donor T cell and myeloid chimerism by day +30. Acute GvHD, grades III-IV was seen in 23% and severe chronic GvHD in 10% and NRM at 2 years was 3% [101]. The same group has also reported a busulfan based regimen (BU 110–130 mg/m^2 on each of the 4 days) + FLU and CY, with a 100% engraftment and donor chimerism from day +30, NRM of 10%, and a disease-free survival of 60% [103]. In the Atlanta experience with these protocols there were very few or no episodes of viral infections, invasive mold infection or infectious death in the first 100 days after transplant. The reduced risk of infectious complications following MA haplo-PT/Cy translated into low NRM, approximately 10% in the first year after transplant. This experience compares favorably to the results reported with T cell-depleted MA haploSCT where NRM of approximately 40% have been seen, with much of this attributable to infectious mortality [99, 104].

One group has devised a so-called two-step approach: patients receive a conventional dose of total body irradiation (12Gy) over 4 days and then a high dose of donor lymphocytes (2×10^8 /kg), followed after 72 h by CY 50 mg/kg × 2, followed by Tacrolimus + Mycophenolate on day −1. Finally, on day zero, patients receive CD34 selected cells, from G-mobilized peripheral blood. In the first published report, 23 out of 25 patients had full donor engraftment. Neutrophil recovery occurred at a median of 12 days (range, 9–15) and platelet recovery occurred at a median of 20.5 days (range, 15–46). Cumulative incidence of engraftment for neutrophils and platelets was 85.2% and 74.1%, respectively. Two multiparous females with multiple HLA-antibodies rejected grafts from their daughter [105]. A confirmatory study with 28 additional patients in complete remission of their disease showed no graft failures. All patients engrafted neutrophils and platelets at a median of 11 and 17 days after haploSCT respectively. The 2-year NRM was 3.6% and disease-free survival of 74% [106].

Retrospective studies comparing the PT/Cy haploBMT approach with alloBMT MRD or alloBMT MUD gave similar outcomes [28, 30–32, 98]. While haplo patients were more likely to have received bone marrow (BM) grafts, which have been associated with engraftment delays, neutrophil recovery was similar after haploBMT with PT/Cy and HLA-matched BMT. There were low rates of graft failure and time to neutrophil engraftment was similar (18 days in both) [30] or slightly delayed (18 compared with 13 days [31] or 16 compared with 14 [32] after haploBMT with PT/Cy and HLA-matched BMT. In one study, neutrophil recovery was no different after RIC MUD and RIC haploBMT; however, day 30 neutrophil recovery was 97% after MAC MUD compared with 90% after MAC haploBMT, respectively (0.02) [107]. Bashey et al. compared neutrophil and platelet engraftment among haploSCT patients who received either PBSC grafts or BM grafts and found no difference in time to recovery by graft source (16 days to neutrophil engraftment and 26 days to platelet engraftment in both groups) [32].

Immune reconstitution was different at early time points after HLA-matched and haploBMT, with a decrease in CD3+ and natural killer (NK) cell counts at day 30 [31] and CD4+ counts at day 50 in the haplo cohort [30]. However, there were no

differences in CD4+, CD3+, or NK cell counts after these early time points. CD20+ cell counts were similar across transplantation techniques at all time points examined [31]. Engraftment and immune reconstitution of CD3+, CD4+, and NK cells were similar in haplo and MRD BMT after the early posttransplant time period. While the slight delay in neutrophil engraftment and reduction in T-cell counts before day 50 may be associated with either the haplo graft or the PT/Cy, it is possible that the use of BM as a stem cell source, which has been associated with engraftment delay [108] and was used preferentially in the haplo cohort, may also have contributed. However, the study that compared neutrophil engraftment after haplo PBSC and haplo BM allografting found no difference in time to neutrophil or platelet recovery [32].

In another retrospective study the PT/Cy haploBMT was compared with TCD haploBMT. All patients received a preparative regimen consisting of melphalan, fludarabine, and thiotepa. The T cell replete group received post transplantation treatment with Cy, Tacrolimus, and Mycophenolate. Patients with TCD received ATG followed by infusion of CD34+ selected cells without posttransplant immunosuppression. Engraftment was achieved in 94% of PT/Cy patients versus 81% of TCD patients (P = NS). The rates of NRM, chronic GvHD, progression-free survival and overall survival were significantly better after PT/Cy [104].

Two parallel prospective BMT Clinical Trials Network (CTN) studies assessed RIC with PT/Cy haploBMT or double umbilical cord blood transplantation (dUCBT). For both trials, the transplantation conditioning regimen incorporated Cy, fludarabine, and 200 cGy of total body irradiation. The day +56 cumulative incidence of neutrophil recovery was 94% after dUCB and 96% after PT/Cy haploBMT. The incidence of grade II-IV acute GvHD was 40% after dUCB and 32% after PT/Cy haploBMT. The 1-year cumulative incidences of nonrelapse mortality and relapse after dUCB transplantation were 24% and 31%, respectively, with corresponding results of 7% and 45%, respectively, after PT/Cy BMT [75]. Findings of another retrospective study, which compared UCB and PT/Cy haploBMT, showed markedly faster platelet engraftment, lower rates of acute and chronic GVHD, a lower relapse rate and better progression free survival for patients who received PT/Cy haploBMT [109].

The ease of application, the reduced cost, and the ready availability of haplo donors have led to the widespread adoption of haploSCT with PT/Cy as an alternative donor approach [16, 17, 26, 98, 110]. Table 3.1 summarizes the incidence of graft failure in the different studies analyzed in this chapter [17]. Despite these excellent results, graft rejection remains a potential complication of haploSCT with any approach, and is usually related to donor HLA-specific antibodies (DSAs) being present pre transplantation in the recipient [68, 69]. In patients with detectable DSAs to all potential HLA-haploidentical donors, desensitization procedures can reduce DSAs titers such that haploBMT can be successfully performed [69, 111, 112].

Table 3.1 Graft failure in haploidentical SCT

Reference	Patient number	GvHD prophylaxis	Graft failure (%)	Acute GvHD II-IV (%)	Chronic GvHD (%)	Non-Relapse Mortality NRM (%)
Aversa F et al. [82]	43	TCD[a]/megadose CD34+	5	0	0	40
Lang P et al. [140]	63	TCD/CD34+/CD133+	17	7	13	29
Aversa F et al. [23]	104	TCD/megadose CD34+	9	8	7	37
Ciceri F et al. [83]	266	TCD/megadose CD34+	9	5–18	10–19	52–48
Klingebiel T et al. [85]	102	TCD/megadose CD34+	13	22	17	37
Bethge WA et al. [34]	29	CD3/CD19 depletion	3	48	10	28
Lang P et al. [35]	46	CD3/Cd19 depletion	13	27	21	20
Balashov D et al. [37]	37	TCR and CD19 depletion	27	22	3	3
Bertaina A et al. [86]	23	TCR and CD19 depletion	17	13	0	9
Lang P et al. [87]	41	TCR and CD19 depletion	12	15	9	7
Im HJ et al. [88]	42	TCR and CD19 depletion	0	31	15	3
Maschan M et al. [89]	33	TCR and CD19 depletion	0	39	30	10
Kaynar L et al. [141]	34	TCR and CD19 depletion	9	30	6	12
Ji SQ et al. [90]	15	GIAC protocol	0	33	100	33
Ji SQ et al. [91]	38	GIAC + basiliximab	0	11	89	32
Huang XJ et al. [92]	171	GIAC protocol	0	55	74	23
Liu D et al. [93]	42	GIAC protocol	0	57	57	20
Chen XH et al. [27]	56	GIAC protocol	4	27	23	13
Huang XJ et al. [94]	250	GIAC protocol	0	46	54	26
Lee KH et al. [95]	83	ATG[b]		20	34	18

(continued)

Table 3.1 (continued)

Reference	Patient number	GvHD prophylaxis	Graft failure (%)	Acute GvHD II-IV (%)	Chronic GvHD (%)	Non-Relapse Mortality NRM (%)
Di Bartolomeo P et al. [96]	80	GIAC protocol	7	24	17	36
Luo Y et al. [33]	99	ATG[c]	0	42	41	30
Wang Y et al. [97]	231	GIAC protocol	0	36	42	13
McCurdy SR et al. [98]	372	PT/Cy (RIC)	8	32	13	14
Bashey A et al. [28]	53	PT/Cy (MAC-PBSC)[d]	2	30	38	7
Raiola AM et al. [30]	92	PT/Cy (MAC)	NR	14	15	18
Brunstein CG et al. [75]	50	PT/Cy (RIC)	2	32	13	7
Ciurea SO et al. [104]	32	PT/Cy (RIC)	6	20	7	16
Castagna L et al. [110]	49	PT/Cy (RIC)	4	26	5	16
Raj K et al. [9]	55	PT/Cy (RIC)	4	53	18	23
Grosso D et al. [106]	28	"Two step approach" PT/Cy + CD34 selection	0	39	22	4
Solomon SR et al. [101]	30	PT/Cy (MAC-PBSC)[d]	0	43	56	3

[a]*TCD* T cell depletion
[b]*RIC* reduced intensity conditioning
[c]*MAC* myeloablative conditioning
[d]*PBSC* peripheral blood stem cells

3.5 Treatment of Graft Failure

3.5.1 Graft Failure

Prevention is the best treatment of graft failure. As it has been shown in the previous section, transplant strategies have been modified through the time to avoid this major complication of haploidentical transplants. However, there still are up to 10% of patients that experience this condition. There is no good quality evidence regarding the best way to approach graft failure, especially in haploidentical transplants. The preferred treatment in graft failure is performing a second transplant

[113–115]. In cases of non-malignant diseases other than aplastic anemia, an autologous SCT can be considered when cryopreserved cells are available [116, 117]. In the rare cases of patients with malignant diseases in complete remission that have stored autologous umbilical cord blood, this has been successfully used with no recurrence of the disease [118]. For the majority of the patients, and considering both that haploSCT patients usually lack a matched related or unrelated donor and that the second transplant needs to be done with haste to avoid the complications related to a prolonged pancytopenia, a second haploSCT or a UCB transplant are the only options.

The largest series of UCB transplants for graft failure treatment is the one reported by Waki et al. [119]. It included 80 UCB transplants performed with RIC between January 2000 and April 2006. The incidence of graft failure was 41%, and the overall survival at 1 year 33%. UCB transplant care has improved through the time. In a more recent study, seven patients transplanted between June 2009 and June 2015 with RIC and 2 cord blood units, all patients engrafted in a median of 27 days. Two died of TRM, one of relapse and four were alive after a median of 29 months from the transplant [120].

When a second haploSCT is considered, the first question is whether to change the donor or not. Although in an analysis performed by the CIBMTR in HLA identical siblings no significant differences were found [121], in some series changing the donor has been beneficial [122]. Also, changing the donor avoids the possibility of graft rejection by residual host T cells sensitized against the first donor [116]. In any case, the presence of DSAs has to be ruled out in the donor selection procedure as it considerably increases the possibility of graft failure [68, 123].

Taking into consideration the proximity of the first transplant in primary graft failures, immunosuppression should be the main goal of the conditioning regimen. Lympholytic agents, such as fludarabine, cyclophosphamide, ATG, and/or Campath1, and total body irradiation (200 cGy) are commonly used. A myeloablative regimen, if possible, can be useful in secondary graft failures where the recipient hematopoiesis is active [49].

The Société Française de Greffe de Moelle published in 2000 the results of 82 s alloSCT, including 19 other than HLA-matched siblings initially transplanted with T-cell depletion. Primary graft failure was observed in 31 patients, and secondary in six additional ones. The use of PBPCs for second transplant, an inter-transplant time interval $\geq$ 80 days and a positive recipient cytomegalovirus serology were significant predictors of day 40 neutrophil recovery. The estimated 100 days transplant related mortality (TRM) was 53 $\pm$ 6%, due to bacterial and fungal infections, conditioning-related multi-organ failures and veno-occlusive disease [124]. These results may not be transferable to the present days due to the improvements in peritransplant care and conditioning strategies. Unfortunately, there are not recent large series assessing the efficacy of post-haploSCT graft failure treatment protocols. When graft failure treatment is mentioned, usually the rules above-mentioned are followed although maintaining the type of haploSCT. The majority of the patients are successfully rescued.

Seeking to reduce conditioning toxicity, the Tübingen group published in 2008 two works testing a total lymphoid irradiation (TLI)-based reconditioning regimen [84, 125]. They treated 18 pediatric patients and seven adults. The first transplant was a haploSCT in nine of them. PBSC were T-depleted by either CD34+ selection, or CD3/CD19 depletion. Four adults received unmanipulated grafts. All pediatric patients engrafted, no grade III-IV acute GvHD was observed and two had extended chronic GvHD with a 1 year overall survival in the range of 70%. The adult patients fared considerably worse with 6 TRM deaths and 1 relapse. This protocol was further explored in a more homogeneous cohort of 19 children, 16 of them with previous haploSCT [116]. Positive selection of CD34+ cells was used in 1 patient, CD3/CD19 depletion in 15, and αβTCR/CD19 depletion in 3. Median CD34+ cells dose exceeded 16×10^6/kg, and median CD3+ cells dose was 4 times higher in the CD3/CD19 depletions. The conditioning regimens also included OKT3 and/or ATG. One patient died in day +3, engraftment was achieved in the remaining 18 patients. Two patients developed grade II aGvHD and two grade III aGvHD. One patient experienced limited chronic GvHD and one patient developed extensive chronic GvHD. TRM was 11%, OS and EFS estimates at 3 years were 68% and 63%. Park et al. [126] also used ex-vivo CD3 or CD3/CD19 depletion for retransplantation after graft failure in 14 haploSCT patients with fludarabine-ATG based regimens with or without TBI. All patients engrafted. Three patients developed grade II acute GvHD, and two patients who received a relatively large amount of CD3+ cells (2.5 and 1.25×10^6/kg) had grade III acute GvHD. Three patients developed limited skin chronic GVHD. No patient developed extensive chronic GVHD. TRM was 10% and the 2-year probability of OS and EFS was 88%.

Yoshihara et al. [66] used unmanipulated grafts in eight patients with graft failure after haploSCT or UCB transplant. Conditioning regimen was fludarabine, thiotepa, rabbit antithymocyte globulin and low-dose TBI. All patients engrafted at a median of 10 days. Two patients developed grade II GvHD, whereas two patients developed grade III. TRM was 37% (3 patients) one relapsed. Overall survival and disease-free survival at 5 years was 75 and 56%. Kanda et al. [127] published a 1-day RIC conditioning protocol including fludarabine, cyclophosphamide, alemtuzumab and TBI. The graft was not manipulated and included a median of 12.1 (range, 8.0–20.0) $\times 10^6$/kg CD34+ cells. Eleven adult patients were treated at a median of 35 days from the initial transplantation. One patient experienced graft failure. Viral infections/reactivations were frequent. No patient developed grade III or IV acute GvHD. Three patients developed limited chronic GvHD and one extensive. TRM was 27% and overall survival at 1 year 73%.

Epperla et al. [123] used the PT-Cy platform for haploSCT graft failure rescue in five patients. One graft failure was observed in a patient in whom low levels of DSAs had been detected. One patient experienced acute GvHD (skin stage 3, grade 2) and subsequently developed moderate chronic GvHD. One patient died from cerebral toxoplasmosis and two from veno-occlusive disease. The two surviving patients were alive and disease-free at days +412 and +585.

3.5.2 Poor Graft Function

Donor cells chimerism needs to be closely monitored in haploSCT patients. When autologous T cells persist or increase, a rapid withdrawal of immunosuppression is advisable. If no response is observed, it can be followed by donor lymphocyte infusions (DLI). The starting recommended dose of T cells is 25×10^3/kg in the ex-vivo T cell depletion protocols [23, 35, 89]. In the non-manipulated grafts protocols, 1×10^6 CD3+ cells/kg has been described a safe cell dose when PT-Cy is used [128]. When it is not used, 100×10^6 mononuclear cells containing 19–74.9 $\times 10^6$ CD3 + cells/kg have been infused, although the addition of GvHD prophylaxis is required [129].

When poor graft function is observed with full donor chimerism, 3 days of G-CSF can indentify long term responders [130]. Thrombopoietin analogues can also be used [131]. Infusing a boost of hematopoietic stem cells with no previous conditioning regimen has also been proposed. Larocca et al. [132] demonstrated that the infusion of positively selected CD34+ cells was more effective than infusing the complete marrow of peripheral blood stem cells (75% vs 36% trilineage recovery), and reduced the likelihood of GvHD (36% vs 10%). Several groups have confirmed the efficacy of this strategy with complete recoveries ranging from 72 to 92%, with acute GvHD grades II-IV from 5 to 22%, grades III–IV from 0 to 13%, and a 3 year overall survival from 40 to 63% [133].

Mesenchymal stromal cells (MSC) have been shown to promote engraftment [134] and also have immunomodulatory capacities including alloreactive T-cells [135]. Meuleman et al. [136] infused a single dose of 2×10^6 /kg MSC. Two out of six patients showed hematopoietic recovery. Other authors have used repeated MSC infusions obtaining recovery in 63–100% of the patients [137–139].

3.6 Conclusions

Graft failure is an important complication in haploSCT particularly in the T-cell depleted haplo-SCT. New strategies have allowed decreasing its incidence to levels similar to those of conventional alloSCT. Although large studies on the treatment of graft failure and poor graft function after haploSCT are lacking, some general recommendations can be offered that permit engraftment in most of the patients, however their care and prognosis remains challenging.

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Chapter 4
Toxicity of Conditioning Regimens in Haploidentical SCT

Meltem Kurt Yüksel and Taner Demirer

4.1 Introduction

Conditioning regimens were used to eradicate the tumor and create a space to facilitate engraftment. At first total body irradiation (TBI) and cyclophosphomide (CY) were the major components of the regimens. Later, chemotherapy without TBI gained popularity and today combination chemotherapies containing fludarabine, cyclophosphamide and busulfan became the most popular ones in allogeneic transplantation [1]. The concept of using high dose chemotherapy to eradicate the tumor and to create a space for the donor stem cells has replaced by establishing a new immune system by the help of conditioning regimens. Hence, a must to have a HLA full match donor to overcome the desperate outcome of leukemia, has replaced by having a haploidentical stem cell donor, in recent years. The toxicity of conditioning regimens used in this new era, haploidentical hematopoietic stem cell transplantation is the subject of this chapter.

4.2 Conditioning Regimens

4.2.1 Definition of Conditioning Regimens

Hematopoietic Stem Cell Transplantation (HSCT) involves the treatment of recipients with irradiation and/ or chemotherapy followed by infusion of cells obtained from bone marrow, cytokine-mobilized peripheral blood or umblical cord blood.

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The cellular content of the donor graft has the major impact on the outcome of HSCT. Graft versus tumor (GVT) and graft versus host disease (GVHD) are important issues determining the success of allogeneic transplantation. The two important purposes of the conditioning regimen are: first to provide adequate immunosuppression to prevent rejection of the transplant grafted and second to treat the disease for which the transplant is being performed. Conditioning regimens should be applied in effort to balance interactions between the competing issues such as maximizing the GVT effect, minimizing acute and chronic GVHD, minimizing graft rejection, minimizing opportunistic infections through rapid immune reconstitution [2]. To date three main categories of the conditioning regimens were defined: 1-A myeloablative conditioning regimen (MAC), which will cause irreversible (or close to irreversible) pancytopenia. Stem cell support is required to rescue marrow function, and prevent aplasia related death 2-A non-myeloablative conditioning regimen (NMA), which will produce minimal cytopenia, and there is no need for stem cell support 3-Reduced intensity conditioning regimen (RIC) is defined as a conditioning regimen which does not fulfill MA or NMA [3, 4].

The terminology for the categorization of conditioning regimens reflects the early regimen related toxicity towards host marrow cells, and not the biologic effect of the transplant. The latter component is complex, involving engraftment of donor lympho-hematopoetic cells, followed by displacement of host lympho-hematopoietic cells, through an immune mediated myeloablation [3, 5]. The design of HSCT conditioning regimen is complicated by the multipl pharmacological properties required to achieve successful engraftment and the potential toxicities associated with the agents used. The primary consideration in designing an effective conditioning regimen must always include patient specific characteristics. Up to date the largest retrospective registry based study comparing outcomes after Tcell repleted (TCR) haplo SCT with post transplant cyclophosphamide (PT-CY) prophylaxis for patients with AML and ALL after RIC and MAC has been reported from the acute leukemia working party of the EBMT [6]. The majority of patients in the RIC group received PBSC (65%) compared to a similar distribution of PB and BM as stem cell source in the MAC cohort (52.5% and 47.5%, respectively; $p = 0.001$). The percentage of patients receiving in vivo T cell depletion, mainly performed with Thymoglobulin, was not significantly different between groups ($p = 0.26$). Apart from T cell depletion, GVHD prophylaxis consisted of different combinations depending on the choice of institution. A combination of PT-CY with one calcineurin inhibitor and mycophenolate-mofetil was used in 66 (25%) patients in RIC and 125 (32%) in MAC groups. There was no impact of conditioning regimen intensity in OS in AML but a trend was seen for worse OS with RIC in ALL. The main factor impacting outcomes was disease status at transplantation. Although the patients were not stratified according to disease risk index (DRI), the main factor impacting outcomes was disease status at transplantation. GVHD prophylaxis with PT-CY based regimen was independently associated with reduced NRM without impact on relapse incidence. Therefore, Rubio et al. concluded that TCR haplo – HSCT with both RIC and MAC, in particular associated with PT-CY are valid options in first line treatment of high risk AML and ALL.The outcomes of transplantation is shown in the Fig. 4.1.

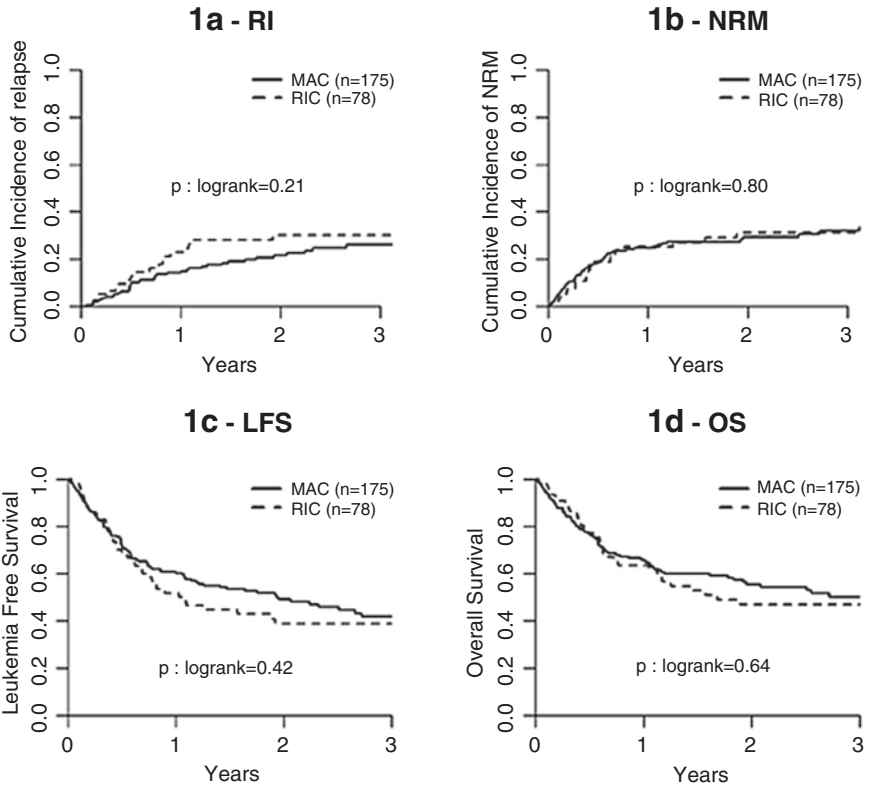

Fig. 4.1 Probability of (**a**) relapse incidence (RI); (**b**) non-relapse mortality (NRM); (**c**) leukemia-free survival; and (**d**) overall survival (OS) after MAC or RIC haplo-SCT for AL in CR1 (Ref. [6])

4.2.2 Drug Interactions and the Additive Toxicity of Conditioning Regimens

The total number of HSCT have steadily increased in recent years. The introduction of less toxic conditioning regimens allowed to expand HSCT to even more fragile elderly patients. However, the drug interactions and its complications should always be kept in mind.

Cardiac toxicity of CY was shown to be fatal in patients receiving CY in combination with carmustine, cytarabine and thioguanine. Besides, previous anthracycline therapy and concurrent chemo- radio therapy may dispose a patient to CY induced cardiac toxicities [7]. Dose escalation of Busulfan 8–20 mg/kg in the presence of CY 50 mg/kg × 4 does not alter the maximum tolerated dose of Busulfan. Combination therapy with Busulfan and CY increased the incidence of both veno-occlusive disease (VOD) and hemorrhagic cystitis (HC) in the Busulfan treated patients compared to the CY and TBI group [8]. However, there are few data about the PT-CY for GVHD prophylaxis and correlation between the increased toxicities such as VOD and HC.

TBI is the oldest therapy for transplantation. Several rat studies have suggested that TBI may alter the enzymatic metabolism of drugs, with reduced rates of oxidative demethylation and hydroxylation, but with no effect on the hepatic CYP450 enzyme system [9]. High dose CY, often given immediately after TBI, is dependent on the CYP450 iso-enzyme system for degradation to its active metabolite. However, TBI might not affect the metabolism of CY itself, the effects of hepatic sinusoidal damage might be potentiated by the interaction between these two modalities. When used alone neither high dose CY nor TBI at 12–15 Gy causes significant liver injury, however, when used together, the glutathione reduction caused by CY renders the hepatocyte vulnerable to radiation damage [8, 9]. The conversion of CY to the active metabolites may be reduced in patients with hepatic impairment, resulting in reduced efficacy. It is recommended that if the level of serum bilirubin is 3.1–5 mg/dl or transaminases >3 times of upper limit of normal value then 75% of the total dose should be administered. But if the serum bilirubin is >5 mg/dl CY usage should be avoided [10]. Between 5% and 25% of CY is excreted unchanged in the urine. Cyclosporine (CSA) and tacrolimus are metabolized by the CYP3A4 isoenzyme, changes in CSA and tacrolimus levels will occur fairly rapidly when the two drugs are used concurrently. Although there is retrospective study about interaction of CY and CSA, there is not any retrospective or prospective study about interaction of CY and tacrolimus. However, tacrolimus levels should be closely followed as in CSA. The antiemetic aprepitant is also a CYP3A4 inhibitor. In a randomized, double blind placebo controlled study, patients undergoing HSCT did not show any significant changes in CY pharmacokinetics and chemotherapy induced nauseas and vomiting were well tolerated.

To minimize the conditioning regimen related toxicities in special groups such as obese patients American Society for Blood and Marrow Transplantation (ASBMT) practice guideline committee position statement on chemotherapy dosing is as follows: If the total amount of CY is 200 mg/kg then the lesser ideal body weight (IBW) or actual body weight (ABW) should be used. However if the total dose of CY is 120 mg/kg then either IBW or ABW for adult patients ≤120% IBW, and AB25 for the pediatric patients >120% IBW should be used [11].

4.3 Toxicity of Conditioning Regimens and the Impact of PT-CY for GVHD Prophylaxis

Decision making about identifying the patients which should be treated with high dose conditioning regimens, which are best suited for RIC regimens and which patients should not be offered allogeneic transplantation is challenging. Conditioning toxicity can be prevented by the help of risk assessment before conditioning. The choice of conditioning regimen heavily influences the effectiveness and outcome of transplantation. Patient specific characteristics, the nature of the disease, the source of the stem cells, the GVHD prophylaxis, supportive care methods employed are

some of the factors which have been addressed. There are some risk assessment scores to objectively stratify the patients according to performance status and health conditions such as, Karnofsky, Charleson, Sorror and adjusted HSCT-CI [12, 13]. Sorror et al. developed a new tool for capturing pretransplant comorbidities that could be used in predicting outcomes and stratifying patients for HCT [12]. The same risk assessment tools are used for both haplo and match or mismatch related and unrelated donors and cord blood transplantations. On the other hand the outcome of allogeneic transplantation is not only influenced by the comorbidity but also influenced by the donor features. There are also risk scores to identify the best donor [14]. Hence, not only the health status of patient and the donor features but also the nature of the disease, status at transplantation are important factors for the transplant outcome. In one study, it was shown that the EBMT risk score can predict the outcome of leukemia after unmanipulated haploidentical blood and marrow transplantation [15]. Although there are some reports evaluating patient and donor related risk factors for haploidentical transplantation, there is still gap in this issue. The rate of utilization of this treatment modality is unremitting. However, this comes at a price. In order to standardize the indications in the leadership of EBMT and ASBMT many countries have their own transplantation indications standardized for reimbursement from the social security agencies [16, 17]. Although haploidentical transplantation seems more cost effective than others, especially than MUD, it is not a standardized procedure for refractory patients in most of the countries. The disease risk index (DRI) is an other tool which has been found to stratify risk factors for heterogeneous adult patient cohorts regardless of conditioning intensity and graft source. In 2014, refined and validated disease risk index (DRI) is published which is a composite of disease risk (diagnosis) and pre-transplantation disease status called stage risk. DRI scoring is classified into three groups low, intermediate, and high/very high risk disease (Table 4.1) [18]. In a study, by Mc Curdy et al., it has been reported that, the refined DRI effectively risk stratified a diverse group of patients who received the NMA haplo BMT with PTCY. The outcome of the patients in terms of 3-year PFS and OS are similar, in the DRI groups, when taking the graft source into the consideration. HLA matched related-donor or MUD BMT had 3-year PFS probabilities of 66% (65% in haplo-RIC), 31% (39% in haplo-RIC) and 15% (25% in in haplo-RIC) for low-risk, intermediate-risk, and high very/high risk groups, respectively, with corresponding 3-year OS probabilities of 70% (73% in haplo-RIC), 47% (49% in haplo-RIC) and 25%(37% in haplo-RIC), respectively. The data suggest that the index is helpful regardless of HLA mismatching and the type of post grafting immunosuppression [19].

4.3.1 Organ Toxicities

Total body irradiation TBI has been the mainstay of preperative regimens since the inception of HSCT. The major limitations of fractionated TBI include mucositis, lung toxicity, infertility. Long term complications following TBI used as part of a

Table 4.1 Disease risk index

Disease and stage	DRI subgroup	2yOS (95CI)	DRI group	2yOS (95CI)
Hodgkin lymphoma, indolent B-NHL, MCL or CLL, **any CR**	Low-1	74% (69–78)	Low	66% (63–68)
Indolent B-NHL or CLL, **PR**	Low-2	62% (59–65)		
AML favorable cyto,**any CR**				
CML, **chronic phase**				
T-NHL, **any CR**	Int-1	52% (51–54)	Int	51% (50–52)
ALL, **1st CR**				
AML intermediate cyto, **any CR**				
Myeloproliferative neoplasms, **any stage**				
Low-risk MDS, any cyto, **early stage**[a]				
Multiple myeloma, **CR/VGPR/PR**				
Aggressive B-NHL, **any CR**				
Hodgkin lymphoma or MCL, **PR**				
Aggressive B-NHL or T-NHL, **PR**	Int-2	46% (43–49)		
Low-risk MDS Int cyto,**Advanced stage** or High-risk MDS Int cyto, **early stage**				
CML, **advanced phase**				
Indolent B-NHL or CLL, **advanced stage**[a]				
Aggressive NHL, **PR**				
High-risk MDS Int cyto, **advanced stage**[a]	High-1	39% (36–43)	High	33% (31–35)
AML favorable cyto, **advanced stage**[a]				
Burkitt lymphoma, **CR**				
AML adverse cyto, **CR**				
ALL, **2nd CR**				
High-risk MDS Adv cyto, **any stage** or Low-risk MDS Adv cyto **advanced stage**[a]	High-2	31% (28–33)		
Hodgkin lymphoma, MCL or T-cell NHL, **advanced stage**[a]				
ALL, **3rd or higher CR**				
Multiple myeloma, **advanced stage**[a]				
AML intermediate cyto, **advanced stage**[a]				
CML, **blast phase**	Very high	23% (20–27)	Very high	23% (20–27)
ALL, **advanced stage**[a]				
Aggressive NHL, **advanced stage**[a]				
AML Adv cyto, **advanced stage**[a]				
Burkitt lymphoma, **PR or advanced stage**[a]				

Adapted from Armand et al. [18]

MCL mantle cell lymphoma, *cyto* cytogenetics (classified as in original DRI except that complex karyotype was defined as >3 abnormalities for both MDS and AML and *t*(8;21) was favorable for AML), *int* intermediate, *adv* adverse, *pts* patients, *OS* overall survival

[a]Advanced stage is induction failure or active relapse, including stable or progressive disease for lymphoma and CLL; for MDS, early stage is untreated, CR, or improvement with therapy without CR

HSCT preparative regimen are common. In a study, adults surviving at least 1 year following TBI and HSCT who were followed for a median time of 4 years, the most common complications include, asymptomatic alterations in pulmonary function, cataracts, sicca syndrome and thyroiditis [9, 20, 21]. It is not known that whether administration of PT-CY increase the toxicity of conditioning regimen in combination with TBI or not.

Many centers are transitioning away from the use of radiation based regimens in preference for chemotherapy based regimens. The primary advantage of regimens that lack TBI is reduced toxicity. In addition, the cost is lower, the regimen is easier to administer and schedule. Drug combinations have been selected based upon the biologic activity of the particular drug, the ability to escalate the dose of the drug, and non -overlapping toxicities when delivering the drugs at maximally tolerated dosages [22].

GVT effect requires the engraftment of donor type immune competent cells which does not necessarily require a high dose MAC regimen. As a result, the possibility of achieving donor specific engraftment using NMA or RIC regimens has been extensively explored [23]. All of the MAC regimens have side effects that can be life threatening. In addition to myelotoxicity, other common toxicities include, mucositis, nausea and vomiting, alopecia, diarrhea, rash, peripheral neuropathies. Pulmonary and hepatic toxicity are also relatively common and infertility, which can be devastating for young patients is almost universal when using MAC regimens.

4.3.2 Myelotoxicity

Patient who underwent haploidentical HSCT experienced lower rates of neutrophil recovery after 30 days whether they receive myeloablative or RIC compared with MUD HSCT [24].

4.3.3 Kidney Injury

Acute Kidney Injury (AKI) is highly prevelant in both myeloablative and non-myeloablative regimens. Risk factors leading to AKI differ between two conditioning methods. If AKI develops in a patient receiving HSCT then it is associated with increased short and long term mortality and also higher rate of progression to chronic kidney disease (CKD) [25, 26]. There are different grading systems to diagnose and classify AKI. Risk, injury, failure, loss of kidney function, end-stage kidney disease (RIFLE) is one of them [25]. According to RIFLE AKI can be divided into three categories, risk, injury and failure. Risk denotes serum creatinin 1.5 times higher than the normal level or 25% reduction in GFR and urine output <0.5 ml/kg/h for more than 6 h. Injury denotes serum creatinin 2 times higher than the

normal level or 50% reduction in GFR and urine output <0.5 ml/kg/h for more than12 h. Failure denotes serum creatinin three times higher than the normal level or 75% reduction in GFR or serum creatinin ≥4 mg/dl (≥353.6 μmol/L and urine output <0.5 ml/kg/h for more than 24 h or anuria more than 12 h. However, although most of the drugs are common in match related, unrelated and haploidentical transplantations, it is not exactly known which transplantation type has the greatest toxicity [27, 28].

4.3.4 Cardiac Toxicity

Cardiac toxicity is an uncommon but a serious complication of high dose chemotherapy. High dose CY containing regimens have been most commonly associated with cardiac toxicity [29]. Fatal cases of diffuse hemorrhagic myocardial necrosis and acute myopericarditis have been reported in patients receiving >180 mg/kg CY. Cardiac damage may occur between 2 and 3 weeks after the start of therapy. The exact dosing of CY on a m2 basis rather than on a weight basis has reduced the incidence of cardiac toxicity [30]. Risk factors for high dose chemotherapy – associated cardiac toxicity are the dosage, schedule of administration, concomitant admistration of other agents, history of radiation therapy to the mediastinum or left chest wall, history of anthracycline exposure, older age, obesity and left ventricular function less than 50% [29]. Nowadays, no data has been reported in terms cardiac toxicity due to PTCY after RIC or MAC haplo HSCT.

4.3.5 Neurological Complications

Neurologic complications among HSCT recipients are variable both in incidence and clinical severity. The incidence varies from 3% to 44% and severity, ranging from mild transient disorder to serious clinic illness [31]. Drug-related toxicities and metabolic alterations are the most common neurologic complications during preengraftmant and engraftment period. Early and late infectious complications including bacterial, fungal, viral and protozoan pathogens may represent as neurologic complication. Moreover, post-transplant lymphoproliferative disorder, disease relapse and transplantation associated thrombotic microangiopathy should also be considered in the differential diagnosis. Recently described CNS manifestations of chronic GVHD and immune-mediated disorders, including neuromuscular and peripheral nerve diseases are infrequent but difficult to manage [32]. Up to date, there is no data about the effect of PTCY for GVHD prophylaxis and haploidentical HSCT on the incidence or outcome of neurological complications. However, there is a report about the severe cytokine-release syndrome after the administration of TCR peripheral blood haplo HSCT. Administration of anti-IL-6 is safe and well tolerated in this life threating situation [33].

4.4 Early and Late Transplant Related Complications in Haploidentical Transplantation in the Presence of PTCY as GVHD Prophylaxis

4.4.1 Early Transplant Related Complications

Apart from the most common early effects such as nausea, vomiting and pain, there are some other early complications that, albeit infrequent, are important cause of morbidity and mortality. Haemorrhagic cystitis, idiopathic pneumonia syndrome (IPS) and there are some best defined syndromes, caused by the injury to the vascular endothelium such as: Venoocclusive disease of the liver (VOD), Capillary leakage syndrome, Engraftment syndrome, Diffuse alveolar haemorrhage and HSCT associated thrombotic microangiopathy [34]. But there is no clear-cut data about the frequency of these above mentioned complications in the stting of haploidentical transplantation. With the accumulation of data regarding early complication of haploidentical transplantations we believe in that literature data will shape out in near future. Therefore we will shortly mention HC and infections in this chapter.

4.4.1.1 Hemorrhagic Cystitis (HC)

The incidence of post HSCT polyoma virus associated (PV-HC) ranges between 4% and 75% [35]. Several risk factors have been described for the development of HC: intensity of the conditioning regimen, unrelated donor, cord blood transplantation, HLA mismatch, sources of PBSCs, older age, high pre HSCT BK virus Ig G antibody titer, CMV infection and acute GVHD. The reported incidence of PV-HC after haplo HCT ranges between 0% and 75% [36]. Rimondo et al. reported HC in a particular patient group, all patients received T cell-replete haplo HSCT with PT-CY and the type of the CNI was either CSA or tacrolimus. The onset of PV-HC was defined as the first day of urinary symptoms associated with hemorrhagic signs, and remission was defined as the last day of bleeding. They first reported the possible link between the use of tacrolimus as part of GVHD prophylaxis and increase in the incidence of PV-HC in the HSCT setting. The cumulative incidence of PV-HC was higher in tacrolimus group than CSA group. They also reported that compared to thiotepa- busulfan containing regimens with truly NMA conditioning regimens, the incidence of HC was statistically lower in the NMA group [37]. In an another study, conditioning regimen containing ATG had 62% PV-HC at 180 days and the factors associated with HC were second transplant and CMV reactivation [38]. Solomon et al. found 75% rate of PV-HC in the presence of tacrolimus and after MA conditioning, which is probably linked to a greater cytotoxicity on the urothelium [39].

4.4.1.2 Infections

Infections still remain a main cause of morbidity and mortality in patients undergoing HSCT. Infections after HSCT can be divided into three phases chronologically as follows: phase I preengraftment,(early), phase II neutropenic and phase III late phase (days 100 > 365) [34]. Although the advances in graft processing and pharmacologic prophylaxis of GVHD have reduced the risks of bidirectional allo reactivity causing engraftment failure and GVHD in haploidentical HSCT, infections are still mostly responsible for toxicity and NRM due to prolonged immunosuppression related or not, to GVHD. Infection related issues in haplo HSCT will be mentioned in a different chapter of this book (Chap. 6).

4.4.2 Late Complications

Advances in HSCT technology and supportive care techniques have led to improvements in long term survival after HSCT. These survivors are at risk for developing late complications secondary to pre-, peri- and post-transplant exposures and risk factors. Infections, ocular complications, general sicca syndrome, oral complications, respiratory complications including idiopathic pneumonia, bronchiolitis obliterans syndrome (BOS), cryptogenic organizing pneumonia (COP), cardiac and vascular complications, liver complications, renal and genitourinary complications, muscle and connective tissue complications, and nervous system complications represent the most common ones [40]. However, there is no data about the effect of PT-CY and haplo HSCT on the incidence and outcome of complications.

4.5 The Impact of PT-CY on the Risk of Relapse and Mortality

HLA- haploidentical HSCT was originally developed as a therapeutic alternative for hematologic malignancy patients who were referred for transplantation but who lacked full match donor. With the favorable toxicity profile, this option has been extended to older adults with hematologic malignancies. Patients up to age 75 have been eligible if they meet eligibility criteria for organ function, including a left ventricular ejection fraction ≥35%, forced expiratory volume in 1 s and forced vital capacity >40% of predicted, bilirubin ≤ 3.0 mg/dl, and alanine and aspartate transaminases less than five times the upper limit of normal [41]. Fuchs et al. has shown that NRM after reduced intensity haploidentical HSCT with PT-CY according to patient age was not higher among patients 70 years of age, compared with patients in their 50s and 60s. PFS also did not differ significantly between those over 70 versus younger patients.

The analysis of 2174 adult patients with AML, from the Center for International Blood and Marrow Transplant Research, who underwent haploidentical (n = 192) or 8/8 HLA-matched unrelated donor (n = 1982) HSCT shows that both in the myeloablative and reduced intensity setting 3-month acute grade 2 and grade 4 GVHD and 3-year chronic GVHD rates were lower in haploidentical group compared with MUD group. The OS probabilities were similar in both MAC and RIC conditioning in two different donor transplantation types. The comparison of OS, PFS rates in different transplant types are shown in the Table 4.2.

In another study, multivariate analysis has shown that active disease at transplant was associated with a higher risk of NRM (Fig. 4.2). On other hand, the use of PT-CY, CSA/Tacro and MMF was associated with decreased NRM as compared to other GVHD prophylaxis including CSA/ Tacro + MMF or MTX; CSA + MMF + MTX + Basiliximab; and Sirolimus + MMF [6]. The main causes of NRM were infectious complications in 53 (32%) vs 75(33%) patients and GVHD in

Table 4.2 Causes of death according to donor HLA matching (Ref. [42])

	HLA 10/10	HLA 9/10	HLA 8/10
Relapse	552 (47.5%)	173 (43.4%)	21 (36.2%)
Infection	249 (21.3%)	100 (25.1%)	21 (36.2%)
GVHD	202 (17.3%)	80 (20.1%)	10 (17.2%)
Graft failure/rejection	11 (0.9%)	1 (0.3%)	0
Cardiac toxicity	8 (0.7%)	4 (1%)	1 (1.7%)
Haemorhage	14 (1.2%)	5 (1.3%)	1 (1.7%)
VOD	12 (1%)	8 (2%)	0
Idiopathic pneumonia	25 (2.1%)	9 (2.3%)	0
Second malignancy	24 (2.1%)	5 (1.3%)	0
Other SCT-related	70 (6%)	14 (3.5%)	4 (6.9%)

GVHD graft-versus-host disease, *SCT* stem cell transplantation, *VOD* veno-occlusive disease

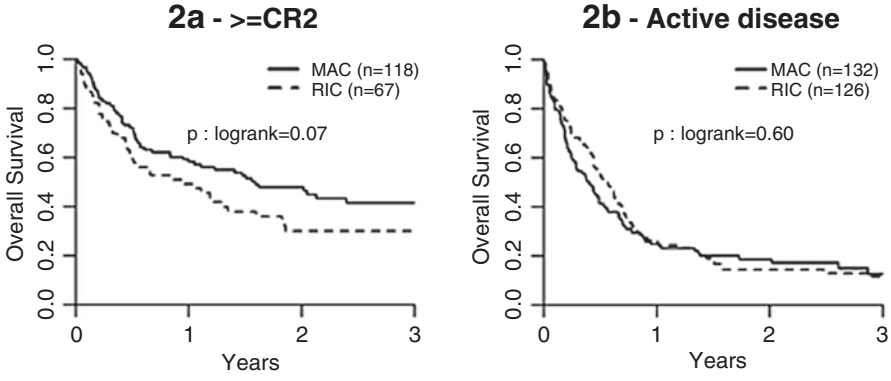

Fig. 4.2 Probability of overall survival (OS) after MAC or RIC haplo-SCT for AL in (**a**) ≥ CR2 and (**b**) with active disease at transplant (Ref. [6])

23 (14%) vs 36(16%) of patients in RIC vs MAC groups respectively. Death from organ toxicity was very low in both groups. In particular sinusoidal obstructive syndrome (SOS) was reported in 2 (1.2%) and 5 (2.2%) of patients in RIC and MAC groups, respectively [6].

4.6 Conclusion

Referring patients to allogeneic HSCT is a challenging task both for physicians and patients since this therapy can lead to a significant transplant related mortality. A randomized trial is the gold standard for comparing outcomes between donor types. However, in recent years it has been shown that, haploidentical HSCT is as effective as HLA match related or unrelated HSCT. Although there are different kinds of methods to overcome GVHD hurdle, currently PT-CY is the cheapest and the most preferred method. The CY proved its immunosuppressive potency without causing any increase in the conditioning regimen related toxicity and transplant related mortality. Haploidentical conditioning regimens are as effective as other match sibling or MUD regimens without increasing drug interactions, sacrificing therapeutic efficacy or resulting in excessive toxicity.

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Chapter 5
An Overview of the Prophylaxis and Treatment of GvHD in Haploidentical SCT

Fabio Ciceri

The great interest in transplantation from haploidentical donors arises from the immediate availability of a suitable one-haplotype mismatched donor for virtually all patients in the appropriate timing. In the absence of a HLA full matched donor, alternative family haploidentical donors have been intensively investigated in the past decade [1, 2].

Primary prevention and treatment of Graft-versus-Host Disease (GvHD) has been a major challenge in this peculiar HLA mismatched setting of hematopoietic stem cell transplantation (SCT). Two main clinical platforms have been developed: ex-vivo T-cell depletion and more recently unmanipulated grafts transplantation.

5.1 Ex-Vivo T-Cell Depletion

The physical removal of donor T-cells from the graft has been pioneered by the group of Perugia in the late 90' [1]. The original concept was to prevent GvHD through a graft with a T cell content not exceeding a total T-cell graft dose of 1×10^4/kg of recipient body weight. The most experienced ex-vivo manipulation has been the positive selection of CD34+ cells realized by CliniMACS® CD34 System Milltenyi, providing a T-cell depleted grafts with high cell dose of CD34+ cells starting from G-CSF mobilized peripheral blood stem cell graft of family haploidentical donors [1–3]. This profound T-cell depleted graft required the development of conditioning regimens aimed at a maximal host immune suppression through the use of anti-thymocyte globulins (ATG), full dose total-body irradiation (TBI) and the combination of intensive immunesuppressive agents fludarabine and thiotepa. Despite the application of intensive immunoablative regimens, the rate of graft rejection has been 10–15% requiring a salvage subsequent 2nd HSC transplantation

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providing an overall engraftment rate >95%. According to the primary objective, ex-vivo T cell depletion by CD34+ selection allows a stable engraftment with a GvHD rate <10% in the absence of any additional post- transplant immunesuppressive treatment. Unfortunately, this intense graft T-cell depletion translated into a slow post-transplant immune recovery with a prolonged and profound T-cell lymphopenia [3, 4]. Unfortunately, in this clinical platform transplant-related deaths have been observed in a significant proportion of recipients [2]. Leading causes of deaths reported were opportunistic infections occurring even as late as 1 year post transplant in the absence of GvHD and any immunesuppressive treatment. The improvement of post-transplant immune reconstitution while controlling Graft-versus-Host Disease (GvHD) prompted the concurrent development of several additional strategies of cell therapy [4–6]. Donor T-cells genetically modified to express HSV-thymidine kinase suicide gene (Zalmoxis®) have been recently registered by European Medicine Agency as adjunctive therapeutic tool post haploidentical SCT.

A partial T-cell depletion less profound than CD34+ selection can be provided by alternative selections, such as CD3/CD19 negative selection. The CliniMACS CD3/CD19 Product Line was developed for the simultaneous depletion of unwanted T and B cells in combination with the CliniMACS System. This approach keeps stem and progenitor cells untouched and leaves immune effector cells, such as NK cells and dendritic cells, in the cellular product [7–9]. Starting form G-CSF mobilized PBSC in adults, grafts contained a median of 7.0×10^6 CD34+ cells/kg, 4.2×10^4 CD3+ T cells/kg and 2.7×10^7 CD56+ cells/kg; incidence of grade II–IV acute graft-versus-host-disease and chronic graft-versus-host-disease was 46% and 18%, respectively, requiring the post-transplant use of a calcineurin inhibitor as additional GvHD prophylaxis in adults patients.

More recently, Miltenyi developed CliniMACS TCRα/β and CD19 depleted stem cell grafts from haploidentical donors for hematopoietic progenitor cell transplantation in children and adults. The ex-vivo protocol has been designed to selectively remove donor T cells with TCRα/β that are recognized to mediate GvHD. Preliminary clinical experience in children showed a very low rate of skin GvHD and no visceral acute or chronic GVHD [10, 11].

Overall, ex-vivo T cell depletion is a platform clinically useful to provide hematopietic engraftment with low GvHD in haploidentical setting. Furthermore, the different cell population selection in the graft provide a unique clinical setting to dissect the biology of different immune cells as NK, TCRα/β and TCRγ/δ T-cells in the clinical post-transplant immune reconstitution, anti-tumour and immune protective in vivo effects [12–15].

5.2 Unmanipulated Haploidentical Graft

Ex-vivo T cell depleted platforms require a specific graft laboratory expertise and, despite improved results and long-term relevant outcome free of GvHD [2], have been associated with a definite complex procedure to spread into a standard clinical

practice. This prompted the development of alternative platforms for haploidentical transplantation based of unmanipulated donor graft.

In the past 15 years, four main different protocols of unmanipulated graft have been promoted and translated into a significant increase in the clinical application of haploidentical SCT [16]: the Chinese protocol, post-transplant cyclophosphamide, rapamycin-based PBSC and alemtuzumab-based protocols.

5.2.1 The "Chinese" Concept of Haploidentical SCT

Huang et al. experienced in large series a GVHD modulation mediated by G-CSF priming of T cells in the bone marrow (BM) and peripheral blood (PB), antithymocyte globulins (ATG) in the conditioning and a powerful posttransplantation GVHD prophylaxis [17–20]. In a prospective comparative trial, unmanipulated haploidentical HSCT achieved outcomes similar to those of HLA-identical sibling HSCT for acute myeloid leukemia patients in early phase [21]. An Italian cooperative group further developed a GVHD prophylaxis of a combination of five drugs with different points of attack: antithymocyte globulin, cyclosporine (CsA), methotrexate (MTX), mycophenolate mofetil (MMF) and basiliximab, an anti-CD25 mAb targeting activated CD25+ T-cells [22, 23]. In this protocol based on a complex post-transplant immune suppression, the incidence of II–IV, III–IV acute and chronic GVHD were 24%, 5% and 6%, respectively. In addition to the intense immunesuppression, the rationale of this transplantation strategy is based on the use of unmanipulated BM cells harvested from donors primed with low-dose G-CSF. Relevant quantitative and qualitative modifications in the BM cell composition and function are induced by G-CSF priming: the number of BM $CD34^+$ cells increases 1.4–1.7-fold, the number of colony-forming cells 3-fold and the number of long-term culture-initiating cells 50–90-fold. Furthermore, G-CSF exerts an intense immune regulatory effect on BM T cells by down-regulating the expression of adhesion and CD28/B7 molecules and by increasing the absolute number of DC2 APCs favouring a T-cell shift from Th1- to Th2-type cells and inducing an higher production of IL-4 and IL-10 anti-inflammatory cytokines [22, 23].

5.2.2 Post-transplant Cyclophosphamide (PTCy)

Luznik and colleagues in Baltimore first developed clinical trials based on post bone marrow transplantation cyclophosphamide [24]. The rationale for this study was based on preclinical evidences that alloreactive donor T lymphocytes are activated after the infusion into the recipient, enter a proliferative phase, and are thus sensitive to the cytotoxic effect of cyclophosphamide 72 h later. On the other hand, non-alloreactive, non-proliferating T cells are spared the purging effect of PT-CY and may provide protection against infections in the short term and allow for a more

robust immune reconstitution. Furthermore, PT-CY does not affect engraftment because of the enzymatic resistance of hemopoietic stem cells to cyclophosphamide. Initial series of haploidentical SCT based on PTCy with BM source were impressively translating into a high rate of hematopoietic engraftment with a very low rate of GvHD [25, 26]. In the past 10 years the use of PTCy platform has been increasingly used globally, accounting for an overall significant increase of haploSCT [16, 27–33]. Registry data from both CIBMTR and EBMT document substantial non-inferiority outcome results of unmanipulated PTCy haploSCT when compared to HLA-identical sibling, Cord Blood and Volunteer Unrelated donor transplantation [34–36]. However, in-vivo alloreactive T-cell depletion provided by PTCy is associated with a significant rate of viral infections and delayed immune reconstitution [37, 38], requiring extensive and systematic use of post-transplant donor lymphocyte infusions as a complementary tool to unmanipulated graft [39]. Furthermore, the use of PBSC graft in the PTCy platform is associated with an increased risk of chronic GvHD as compared to the classical use of BM [40].

5.2.3 Rapamycin-Based Protocols

Peccatori J et al. developed a calcineurin inhibitor-free GvHD prophylaxis based on rapamycin, mycophenolate mofetil (MMF) and anti-T lymphocytes globulin (ATG-Fresenius), in the attempt to promote a fast post-transplant immune recovery with a preferential accumulation of regulatory T cells (Tregs) [41]. Rapamycin is an immunosuppressive drug that, in contrast to calcineurin inhibitors, promotes the expansion of natural Tregs. With this calcineurin-inhibitor free GvHD prophylaxis and after PBSC grafting, incidence of acute GvHD grade II–IV was 35% and chronic GvHD 47%. Interestingly, occurrence and severity of acute GvHD negatively correlated with in vivo Tregs frequency in patients. Sirolimus-based GVHD prophylaxis was then explored with PTCy in PBSC unmanipulated haploSCT (Sir-PTCy) [42]: post-HSCT recovery of lymphocyte subsets was broad and fast, with a median time to CD4 > 200/μL of 41 days. Cumulative incidences of grade II– IV and III–IV acute GVHD were 15% and 7.5%, respectively, and were associated with a significant early increase in circulating regulatory T cells at day 15 after HSCT, with values <5% being predictive of subsequent GVHD occurrence. The 1-year cumulative incidence of chronic GVHD was 20%.

5.2.4 Alemtuzumab-Based Protocols

Alemtuzumab is a humanized monoclonal antibody directed against human CD52 that is expressed on many T and B cells and some dendritic and NK cells. Alemtuzumab is extensively used to facilitate engraftment and reduce incidence of graft-versus-host disease (GVHD) in allogeneic SCT from HLA-matched sibling

donor or unrelated donor [43–46]. Alemtuzumab prevented severe GvHD while enabling haploidentical engraftment both after myeloablative and reduced intensity conditioning. However, CD4+ and CD8+ T-cells and thymus T-cells output remained low within 1 year after HSCT, suggesting that the use of alemtuzumab of a lower than standard dose should be explored in future studies to accelerate immune recovery after HSCT [47].

5.3 Conclusions

Many different HSC transplantation platforms have been developed with the primary objective of a stable haploidentical hematopoietic engraftment at a low GvHD rate. Both ex-vivo T-cell depletion and unmanipulated grafts transplantation are feasible and effective in GvHD prevention despite major HLA mismatches. However, those platforms are realized within specific transplant packages including conditioning and GvHD drug treatment requirements.

Haploidentical transplants should be performed in centers with major experience with HSCT procedures and preferentially performed within the framework of a local clinical protocol designed specifically to address the prevention of GvHD.

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Chapter 6
Prevention and Treatment of Infectious Complications in Haploidentical SCT

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6.1 Infectious Complications in Haplo-HSCT

Infectious complications remain a significant cause of morbidity and mortality in patients undergoing hematopoietic stem cell transplantation (HSCT). The increased risk for infections which may be caused by bacteria, viruses, fungi or parasites is due to the complex immune suppression as a consequence of (myeloablative) chemotherapy, immunosuppression induced by conditioning, and immunosuppressive therapy for the prophylaxis or the management of acute and chronic graft-versus-host disease (GvHD). The immunosuppression may affect different arms of the immune system including skin, mucosa, phagocytes, various lymphocyte subsets, cytokines, and interferons [1, 2]. Notably, haplo-HSCT is associated with a higher risk of severe GvHD and graft failure which require a more profound immunosuppression than conventional HSCT. Hence, infectious complications are particular frequent and potentially severe in HSCT with haploidentical donor (haplo-HSCT).

6.2 Comparison of Immune Recovery Between Haploidentical and Other Transplantation Modalities

HLA-disparity is a predominant feature of haplo-HSCT, making a profound and prolonged immunosuppression necessary to prevent acute and chronic GvHD and graft failure in these patients. Strategies to prevent GvHD include the

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transplantation of T-cell depleted grafts, which results in a slow reconstitution of the T-cell compartment. In this regard, it was shown that haplo-HSCT recipients have lower counts of T-cells and dendritic cells during the first 90 days after HSCT [3] compared to HLA-matched HSCT recipients, and explains both the high frequency and the specific types of infectious complications, which are still responsible for most cases of non-relapse mortality in haplo-HSCT recipients. In contrast, regimens which result in faster $CD4^+$ T-cell reconstitution are associated with significantly lower rates of infectious complications [4], which further illustrates the inverse correlation between immune recovery and infection related deaths. It therefore remains a significant task to balance the risk of GvHD and infectious complications in haplo-HSCT regimens using T-cell depleted vs. non-depleted grafts. A recent study compared a strategy of T-cell depletion with ATG and $CD34^+$ selected haplo-HSCT grafts without posttransplant immunosuppression versus T-cell replete grafts followed by immunosuppressive therapy with cyclophosphamide, tacrolimus and mycophenolate-mofetil (MMF). The results demonstrated that immune reconstitution was superior in the group of T-cell replete grafts, and these patients had lower rates of infections and non-relapse mortality [5].

6.2.1 Phases of Immune Recovery and Infectious Complications After Haplo-HSCT

As in conventional HSCT, the differential kinetics of recovery of different immune cells after haplo-HSCT results in defined phases of risk for infectious complications due to specific pathogens (Fig. 6.1, Table 6.1). The first phase is the pre-engraftment phase, which is characterized by profound neutropenia. This phase lasts up to 20 days, depending on the type of graft modality. Importantly, during this time, immunological barriers are also disrupted in most patients: severe mucositis is associated with higher gut permeability, and the skin barrier is impaired by the insertion of central venous lines. Major pathogens causing infectious complications in this phase are Gram-positive and Gram-negative bacteria as well as *Candida* or *Aspergillus* spp. [6]. Since neutropenia occurs universally after various modes of HSCT, recipients of haplo- and non-haplo HSCT are at a comparable high risk of bacterial infections during the pre-engraftment phase [7]. After neutrophil recovery, the risk of infections with common bacteria decreases, whereas patients remain at high risk of viral and fungal infections during this post-engraftment phase, which lasts until day 100 after HSCT. This post-engraftment phase is characterized by both impaired T- and B-cell immunity, by a high risk of acute GvHD (aGVDH), and by the frequent need of immunosuppressive therapy to prevent or to treat aGvHD. In this period after haplo-HSCT, patients with aGvHD suffer particularly frequent from viral and fungal infections [7]. Frequent pathogens causing infectious complications are summarized in Table 6.1.

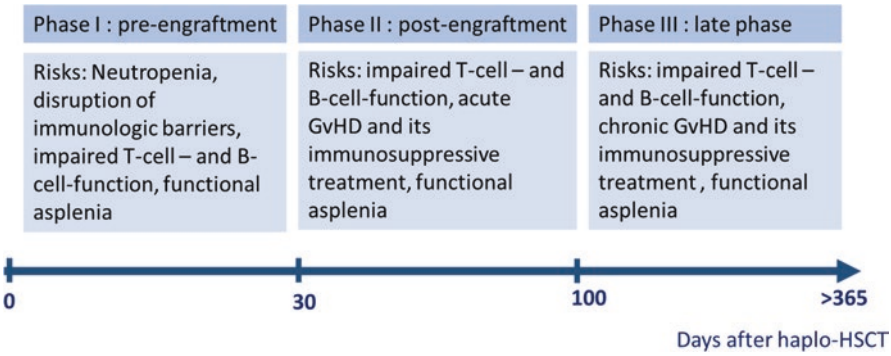


Fig. 6.1 Phases of immunosuppression after haploidentical hematopoietic stem cell transplantation (HSCT) [8]

Table 6.1 Predominant infections according to phase of haploidentical hematopoietic stem cell transplantation (HSCT) [8]

	Phase I pre-engraftment	Phase II post-engraftment	Phase III late phase
Bacteria	Gram negative bacteria Gram positive bacteria	Gram negative bacteria	Encapsulated bacteria
Fungi	*Aspergillus* spp. *Candida* spp.	*Aspergillus* spp.	*Aspergillus* spp. Pneumocystis jiroveci
Viruses	HSV	HSV CMV EBV, PTLD Adenovirus	HSV CMV EBV, PTLD Adenovirus

HSV Herpes-simplex virus, *CMV* Cytomegalovirus, *EBV* Epstein Barr Virus, *PTLD* post-transplant lymphoproliferative disease

The late post-transplantation phase starts at day 100 after haplo-HSCT and is characterized by potentially severe chronic GvHD (cGVHD). cGvHD results in impaired cellular and humoral immune responses which render the patient at a particular high risk for fungal and viral infections, and – due to functional asplenia and hypogammaglobulinemia – infections with encapsulated bacteria such as Haemophilus or Pneumococcus spp. [7]. During this phase of post-transplantation, $CD4^+$ T-cell counts correlate with the risk and the type of infectious complications, which is comparable to patients suffering from HIV.

6.3 Bacterial Infections

6.3.1 Common Bacterial Infections

The management of common bacterial infections does not generally differ between haplo-HSCT and other modalities of HSCT or chemotherapeutic regimens. During the neutropenic phase, bacterial infections remain an important and life-threatening

complication of HSCT including haplo-HSCT, and have to be considered as an emergency situation requiring the immediate institution of empirical broad-spectrum antibiotics which also cover *Pseudomonas* spp. In the post-engraftment and late phase, the risk of infection with common bacteria is still present, but substantially lower than in the neutropenic phase. The risk for bacterial infection may vary according to preparative conditioning regimens, underlying disease, severity and duration of neutropenia, presence of mucositis, the use of central venous or urinary tract catheters, patient's age and comorbidities [9]. Strategies which aimed to replace myeloablative conditioning by a reduced intensity conditioning regimen were shown to significantly reduce the severity of mucositis as well as the duration and extend of neutropenia, which ultimately resulted in a reduced risk of bacterial infections [10].

One study reported that at least one bacterial infection occurred in 64% of 70 patients receiving haplo-HSCT. Of these patients, 14% had infections with Gram-positive bacteria, 30% with Gram-negative bacteria, and 20% with both Gram-positive and Gram-negative bacteria. Most frequently, *Escherichia coli* (28%), coagulase negative staphylococci (20%), *Staphylococcus aureus* (9%), and *Clostridium difficile* (8%) were identified [11].

The main source for Gram-negative bacterial infection in the neutropenic phase is being thought to result from bacterial translocation from the gastrointestinal flora, and most Gram-positive infections result from invasion of skin-resident bacteria e.g. via vascular devices or of oral bacteria in cases of mucositis [8]. Beside the common pathogens, bacteria which rarely cause systemic complications in immunocompetent patients (e.g. Salmonella enterica or *Listeria* spp.) may cause severe septic complications in the neutropenic phase of haplo-HSCT.

The vast majority of bacterial infections were observed between day 0 and 30 after haplo-HSCT, whereas only few bacterial infections occurred in the late phase after HSCT, in particular in patients who had no GvHD and were not receiving immunosuppressive agents [11].

The most common manifestation of bacterial infections after haplo-HSCT are bloodstream infections without specific organ involvement, followed by pneumonia, gastrointestinal infections including pseudomembranous colitis (caused by *C. difficile*), urinary tract infections, and skin infections [9]. However, it is important to keep in mind that specific situations such as abscess formation, which may proceed without relevant clinical symptoms in severely immunosuppressed patients, or infections with limited permeability for some antibiotics such as meningitis or osteomyelitis may occur.

6.3.2 Preventive Measures for Bacterial Infection

Due to the universal risk of bacterial infection, prophylactic strategies to prevent bacterial infections in haplo-HSCT patients are usually applied. For example, antibacterial prophylaxis in the neutropenic phase after haplo-HSCT is usually

recommended, and, according to the guidelines by the European Conference on Infections in Leukaemia (ECIL), suitable agents include fluorochinolones or co-trimoxazole, but local resistance profiles have always to be considered. In this regard, resistance to co-trimoxazole is an emerging issue in numerous countries [12]. Whereas the use of systemic prophylactic antibacterial agents such as fluoroquinolones is commonly accepted, the use of metronidazole monotherapy or of selective gut decontamination with non-absorbable antibiotics to prevent bacterial infections is controversial.

Strict hygiene measures, like hand washing, are also an important cornerstone of prophylaxis of bacterial infections after haplo-HSCT with proven efficacy and should be systematically applied in all cases. In contrast, the value of low-bacterial diets ("neutropenic diet") which is based on a theoretical rationale has never been proven and is increasingly questioned. For example, no reduction of bacterial infections has been reported in a large analysis of pediatric patients with AML in whom low-bacterial diet restrictions were recommended compared to patients who did not receive these recommendations. Similarly, another study has failed to show a survival benefit in 153 patients after HSCT who strictly ingested cooked food compared to patients who were allowed to consume raw food as well [13, 14].

6.3.3 Antibacterial Therapy

Antibiotic therapy of neutropenic fever and of manifest sepsis has to be initiated promptly under the consideration of comorbidity, previous prophylactic therapy, local resistance profiles and the severity of sepsis. Suitable agents for the empirical treatment of neutropenic fever without specific focus include beta-lactam antibiotics with broad activity against Gram-positive and Gram-negative bacteria including *Pseudomonas spp*. such as piperacillin/tazobactam, third-generation cephalosporines such as cefepim or ceftazidime, or carbapenems [15]. Carbapenems are recommended as initial therapy if the local incidence of Gram-negative bacteria producing extended-spectrum beta-lactamases is high. In patients with severe mucositis, presumed catheter-associated infection, skin or soft tissue infection, or severe sepsis, initial therapy should include a glycopeptide to cover resistant Gram-positive bacteria including MRSA [16]. According to the ECIL guidelines, an additional aminoglycoside (preferably amikacin) might be added in patients with suspected severe Gram-negative sepsis or pneumonia, to cover resistant *Pseudomonas* spp. or other resistant Gram-negative bacteria [12]. Persistent fever in an otherwise stable patient does not necessarily require escalation of antibiotic therapy, but diagnostic efforts to identify the cause of fever should be continued, including repeated cultures of blood and potential foci. In addition, invasive fungal infection and viral infection should be excluded [16].

The world-wide increase of multidrug-resistant (MDR) bacteria, especially Gram-negative rods with extended-spectrum spectrum beta-lactamases (ESBLs), methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*

spp. is worrisome and results in an increased number of haplo-HSCT patients requiring complex antibacterial treatment regimens. It has to be kept in mind that prophylactic antibiotic therapy may not cover MDR bacteria or even result in the selection of MDR bacteria [17]. Antibiotics which may be required to treat Gram-negative MDR infections include colistin/polymyxin B, fosfomycin or tigecyclin, whereas Gram-positive MDR resistant to vancomycin may be treated with teicoplanin, tigecyclin, linezolid, or daptomycin [16, 18]. However, some of these drugs are burdened with significant side effects (e.g. renal toxicity of colistin/polymyxcin B), and drug-drug interactions have to be considered.

6.3.4 Infections with Encapsulated Bacteria

Infections with encapsulated bacteria are a typical complication in the late phase (>day +100) after haplo-HSCT. The risk for this infection is increased due to functional asplenia, hypogammaglobulinemia, and chronic GvHD which requires immunosuppressive therapy. The most important pathogens causing the infectious complication are encapsulated bacteria such as *Streptococcus pneumonia*, *Haemophilus* spp., and *Neisseria meningitidis*. Importantly, patients with an infection with encapsulated bacteria may present with fulminant sepsis including Waterhouse-Friderichsen syndrome. Prophylactic measures include vaccination at 6–12 months after HSCT (if GvHD is controlled) [19], prophylactic administration of penicillin G or a macrolide during immunosuppressive therapy as well as immunoglobulin replacement in cases of severe hypogammaglobulinemia [8]. However, one has to recognize that increasing rates of resistance are reported [20]. It has to be noted that beside infections due to encapsulated bacteria, rare bacterial infections such as infections due to *Mycobacterium tuberculosis*, *Nocardia* spp., *Listeria* or *Legionella* spp. may occur in the late phase after haplo-HSCT [8].

6.3.5 M. tuberculosis

In contrast to solid organ transplantation, tuberculosis (TB) is less common among HSCT recipients (approximately 10 time less frequent). The main risk factor for TB is undergoing transplantation in a country with a high endemic rate of TB. Similarly, the risk of TB is increased among patients who have a migration history from an area with high TB prevalence. Depending on the risk of TB infection, assessment of *M. tuberculosis* infection prior to haplo-HSCT should be performed by medical history, routine chest X ray studies, tuberculin skin tests (TSTs) or interferon-gamma release assays (IGRA). Importantly, the sensitivity of TST and IGRA can be reduced in immunosuppressed patients [21]. Impaired T-cell immunity associated with haplo-HSCT bears a relevant risk of reactivation of controlled or latent *M. tuberculosis* infection. Hence, in patients with test results suggestive for previous (latent)

M. tuberculosis infection, prophylactic therapy is recommended and may be performed preferentially with isoniazide (INH) for at least 9 months and until immunosuppression dosages can be substantially reduced. Manifest disease has to be identified early and treated rigorously according to general practice [8].

6.4 Viral Infections

Viral infections are caused by de novo infection or by re-activation of a pre-existing silent infection, and are a frequent and potentially severe complication in patients undergoing haplo HSCT. In these patients, in particular infections due to viruses of the herpes virus family, of the adenovirus family, and of the polyomavirus family as well as due to hepatitis viruses may cause specific syndromes associated with immune suppression. In addition, common viral infections such as influenza virus infection or respiratory virus infection may present with altered clinical features such as delayed or extremely severe clinical course during immune suppression.

6.4.1 Cytomegalovirus (CMV)

CMV is a double-stranded DNA virus of the herpesvirus family which usually persists in multiple tissues life-long after initial infection. Of note, hematopoetic stem cells and macrophages are a specific cellular reservoir of CMV persistence. Hence, CMV infection after haplo-HSCT can be a consequence of infection with CMV by the graft in CMV seronegative recipients, of de novo infection of the recipient after HSCT, or of re-activation of CMV infection in CMV seropositive recipients [22].

Patients undergoing haplo HSCT are at a high risk for CMV infection and CMV-associated death not only during the early posttransplantation period (<day 100 after HSCT), but also later (>day 100 after HSCT) in the posttransplantation course (Fig. 6.1). The serostatus of the donor as well as the pre-transplant serostatus of the HSCT recipient have an important impact on the risk for CMV infection and CMV-associated morbidity in the setting of HSCT. In this regard, seropositive recipients of a HSCT are at particularly high risk of CMV reactivation after HSCT, in particular if the donor is CMV negative, because protective immune responses are abolished by the HSCT procedure. This is in contrast to solid organ recipients, who are at highest risk of severe CMV infection if they are seronegative for CMV and receive a transplant of a CMV positive donor. However, there is a considerable risk of CMV infection in CMV negative HSCT recipients as well, in particular if a bone marrow of a CMV positive donor is transplanted. The risk of CMV infection in this scenario is approximately 20–30% and warrants similar prophylactic strategies than in CMV positive donors [22–24]. Prophylactic as well as preemptive antiviral therapy can significantly reduce the risk for manifest CMV infection (see below).

Due to the broad cellular tropism of CMV, a large variety of tissues can be infected by CMV. The most common sites of clinical apparent CMV infection are the lungs (causing pneumonia), the intestines (causing ulcerative mucositis from the esophagus to the colon), the kidneys (causing renal failure), the liver (causing hepatitis), the bone marrow (causing cytopenia) and, in particular after long-lasting severe immunosuppression, the brain and retina causing encephalitis and retinitis, respectively [22, 24, 25]. Mortality in untreated patients is high, and therefore, a high suspicion of CMV infection is warranted and should prompt immediate diagnostics.

The best diagnostic test of CMV infection is real-time PCR-based detection of viral DNA, which should be performed in blood samples. However, blood testing may not detect organ manifestations such as CMV colitis, and therefore, if clinically suspected, tissue biopsies should be performed for PCR testing for CMV DNA and immunohistochemistry. As an alternative, the pp65 antigen test may be done, which is, however, less reliable to detect active CMV infection [22, 25]. Of note, serologic testing for CMV is generally not helpful to discriminate latent and active CMV infection and may not be sufficient to indicate de novo infection with CMV in the setting of post-transplant immune suppression [22].

6.4.1.1 Prevention and Treatment of Clinically Manifest CMV Infection

As the serostatus of the donor has a major impact on the risk for CMV infection, seronegative donors should be selected for seronegative HSCT recipients if possible. In addition, blood transfusions for HSCT recipients should be derived from CMV seronegative donors. In patients who are at risk of manifest CMV infection (i.e. seropositive recipients or seronegative recipients of a seropositive graft), strategies to prevent clinically relevant CMV infection should be applied and consist of either prophylactic antiviral therapy from the time of HSCT or preemptive antiviral therapy if CMV DNA is detected during scheduled surveillance testing (Table 6.2) [22, 24]. As available antiviral agents to combat CMV are burdened with relevant side effects, the preemptive antiviral strategy has a wider acceptance. However, preemptive antiviral therapy requires thorough monitoring by quantitative PCR of blood samples (at least once weekly during the first year of HSCT) and immediate initiation of antiviral therapy if CMV DNA is detected [26]. There are a number of antiviral agents which are used in immunocompromised patients with CMV infection. Intravenously administered ganciclovir is the most effective agent for preemptive antiviral therapy and for the treatment of clinically manifest CMV infection. However, ganciclovir has a relevant potential of bone marrow toxicity which may limit its application in HSCT recipients [27, 28]. Valganciclovir, an orally bioavailable prodrug of ganciclovir, may be used to prevent CMV infection in haplo HSCT recipients, but its role for preemptive therapy is under study. Foscarnet is an alternative agent, but the most common side effect of this drug is renal toxicity [28]. Cidofovir may be used as a second-line agent for the treatment of CMV infection. However, cidofovir is also nephrotoxic and patients with viral breakthrough during

Table 6.2 Management to prevent CMV-Infection after haplo HSCT [22]

Indication	Strategy
Seropositive recipient	PCR – or antigenemia – guided early ganciclovir treatment or Ganciclovir prophylaxis until day +100 (recommended if neither PCR no antigenemia testing is available)
Seronegative recipient/seropositive donor	PCR – or antigenemia – guided early ganciclovir treatment and Seronegative or leucocyte-reduced blood products
Seronegative recipient/seronegative donor	Seronegative or leucocyte-reduced blood products

cidofovir therapy have been observed [29, 30]. As aciclovir has a moderate antiviral activity against CMV, patients receiving aciclovir for the prevention of herpes simplex virus infection have a lower risk of CMV infection compared to patients not receiving antiviral therapy [31]. There is an increasing body of evidence that CMV specific T-cell therapy may be an effective approach to target CMV infection [32], whereas the application of CMV-specific antisera has failed to show a benefit in the prevention of CMV infection in HSCT patients [33].

6.4.2 Epstein Barr Virus (EBV)

In the setting of haplo-HSCT, EBV is another human herpes virus of high clinical relevance. Approximately 90% of all humans are chronically infected with EBV. Similar to CMV, EBV persists lifelong in a latent state, with B-cells serving as a reservoir for EBV persistence [34]. In the immunocompetent host, clinically relevant reactivations are uncommon. Whereas in the setting of haplo-HSCT-related immunosuppression, EBV reactivation can cause organ infection such as hepatitis. More importantly, EBV reactivation can present as post-transplant-lymphoproliferative disease (PTLD), which may be a potentially life-threatening syndrome ranging from benign polyclonal B-cell-proliferation to malignant B-cell-lymphoma. This complication develops as a consequence of uncontrolled proliferation of EBV-infected lymphoid or plasma cells, and is usually accompanied by EBV DNAemia. As EBV DNAemia precedes in most patients clinically manifest EBV-related disease, EBV DNA monitoring in blood should regularly be performed in asymptomatic haplo HSCT recipients, starting from the first month after haplo HSCT for at least four months [35]. As the risk of EBV-related PTLD is predominantly determined by the degree of T-cell impairment, as T-cells are required to control persistent EBV infection, additional risk factors are the constellation of a seropositive haplo HSCT donor for an EBV negative recipient or pediatric HSCT. The mortality of PTLD is high [35]. The risk of EBV-infection seems to be reduced if B-cells are sufficiently reduced together with the T-cell depletion as this is the case in $CD34^+$ selection or in CD3/CD19 negative selection.

Clinical symptoms of PTLD vary and may include fever, lymphadenopathy, the rapid development of progressive lymphoma, or signs of organ infiltration and subsequent organ failure.

If EBV-related PTLD is suspected, EBV DNA measurement by PCR should be performed in blood samples and, if applicable, in suspicious tissue fluids such as pleural effusion. Notably, the definite diagnosis of PTLD depends on histological analysis of enlarged lymph nodes or infiltrated organs, which also helps to distinguish "benign" from neoplastic PTLD. PCR-based EBV measurement in lymph node specimens is not recommended due to a high sensitivity but poor specificity, whereas in situ hybridization of EBV-encoded RNA is recommended to confirm the relevance of EBV in PTLDs. Staging of PTLD can be performed in line with the Ann Arbor algorithms [35].

Unfortunately, no antiviral agents with sufficient activity against EBV are available. Therefore, prevention and therapy of EBV-related PTLD relies on strategies to booster EBV-directed immune responses and to target EBV-infected proliferation B-cell clones, respectively. Therefore, whenever possible, immunosuppressive therapies should be reduced in the setting of haplo HSCT-related EBV reactivation. Rituximab, a monoclonal anti-CD 20 antibody directed against B cells is widely used to treat both benign and neoplastic PTLD [36]. If no sufficient response of PTLD to rituximab monotherapy is achieved, conventional chemotherapy with or without rituximab may be given [37, 38]. Rituximab can also be used for preemptive EBV therapy in case of EBV DNAemia detected in asymptomatic patients during routing monitoring. Typically, 1-4 doses of once weekly rituximab are sufficient in this setting. However, the threshold of EBV DNAemia to initiate preemptive therapy with rituximab is controversial and may be considered as 1000–40,000 EBV copies/ml [35].

Another therapeutic approach is the administration of donor or third party EBV-specific cytotoxic T-cells, which are highly effective in preemptive therapy of EBV disease and should also be considered for the management of manifest PTLD [39, 40]. Unselected donor lymphocyte infusions might be applied as second line therapy of PTLD in order to restore broad T-cell reactivity including EBV-specific responses, but this approach bears a relevant risk of severe GvHD [41].

6.4.3 Herpes Simplex Virus (HSV)

Approximately 95% and 10–30% of all individuals world-wide are chronically infected with HSV type I and II, respectively. The virus persists in neural ganglia, which are a reservoir for clinically manifest reactivations in both immunocompetent and immunocompromised patients. In the context of haplo-HSCT, HSV reactivation can be observed frequently and may cause severe infection of the skin and of the mucosa, as well as severe organ infections such as pneumonitis, hepatitis or encephalitis. Diagnosis of HSV-related disease relies on PCR-based detection of HSV DNA in blood or fluid samples, bronchoalveolar lavage or biopsy specimens. Due to the potentially high mortality of organ infection, prompt initiation of high-dose

antiviral therapy with aciclovir is warranted in patients in whom HSV organ infection is suspected without waiting for positive test results. Since aciclovir is highly effective to prevent HSV associated disease, most centers perform prophylactic therapy of aciclovir from the onset of conditioning until the time of resolution of mucositis or even longer [42].

6.4.4 Infections with Other Viruses of the Herpes Family

Varizella Zoster Virus (VZV) can cause severe skin and organ infections in the setting of haplo-HSCT, either by de novo infection or reinfection of persistent VZV infection. Clinically relevant are VZV infections of the central nervous system including meningitis and encephalitis, of the lung and liver causing pneumonitis, and hepatitis, respectively. VZV infection can be diagnosed by PCR-based VZV DNA detection. Aciclovir, administered at high doses, is a potent antiviral agent for the treatment of VZV infection, and VZV-specific immunoglobulins should be rapidly administered to prevent VZV infection after contact to individuals with manifest VZV infection (<96 h). There is also an active immunization with a VZV life-vaccine, which should not be given prior to 2 years after haplo-HSCT in presumed immunocompetent patients [42]. Unfortunately, a dead vaccine which has been evaluated in solid organ transplant recipients is not available to date for HSCT recipients.

Human herpes virus 6 (HHV-6) reactivations are relatively frequent (50–70%) after HSCT and may cause severe encephalitis causing limbic and hippocampus-derived symptoms. HHV-6 disease can be treated with foscarnet or ganciclovir [43].

6.4.5 Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), and Hepatitis E Virus (HEV)

HBV reactivation after haplo-HSCT is a serious concern in any patient who had been previously infected with HBV, including individuals who have successfully generated protective immunity against HBV (i.e. anti-HBs-antibodies) [44]. Therefore, anti-HBc as a marker of active or immune-controlled HBV infection as well as HBs-antigen should be tested in any patient prior to HSCT. Prophylactic antiviral therapy with HBV polymerase inhibitors such as entecavir, tenofovir or lamivudine is required in any haplo-HSCT recipient who is positive for anti-HBc or HBs-antigen, independently whether protective anti-HBs-antibodies are present prior to conditioning or not [45, 46]. Duration of prophylactic antiviral therapy should continue for at least 1 year after immune reconstitution.

Severe hepatitis C reactivation has been observed as early complication after HSCT, and chronic hepatitis C progresses faster in immunocompromised than in immunocompetent patients [47, 48]. The possibility to eradicate HCV infection with 8–12 weeks of therapy with modern direct-acting antiviral agents should lead to a low threshold of the initiation of antiviral therapy in HSCT patients with HCV infection [46, 47, 49].

HEV infection is acquired orally via ingestion of contaminated food such as insufficiently cooked pork meet or wild boar. In immunocompetent patients, acute HEV infection is universally cleared spontaneously, whereas, in organ transplant recipients, chronic HEV infection has been described causing chronic hepatitis [50]. Diagnosis of chronic HEV relies on HEV RNA measurement by real-time PCR, and treatment consists of the reduction of immunosuppression or ribavirin [46, 51].

6.4.6 Adenovirus

Infections due to human adenovirus (HAdV) are an important cause of morbidity and mortality in immuncomprimised patients including haplo-HSCT recipients. HAdV may persist in epithelial cells and lymphoid tissues in healthy individuals, which may be a source of endogenous reactivation [52], in particular during the period of severely suppressed T-cell function. There is a high genetic variability of HAdV with seven genotypes und multiple subtypes identified. Children appear to be more frequently affected by HAdV after haplo-HSCT than adults [53]. Clinical manifestations of HAdV infection include upper respiratory disease, gastroenteritis, (kerato-) conjunctivitis, and other, often severe organ manifestations such as hemorrhagic cystitis, nephritis, hepatitis potentially resulting in liver failure, encephalitis, myocarditis, and pneumonia. PCR-based assays are suitable diagnostic tools for rapid, specific, quantitative, and highly sensitive detection of HAdV infection. Monitoring with quantitative PCR of HAdV load in peripheral blood is recommended for patients with haplo-HSCT. Prevention of HAdV relies on strict isolation and hygiene measures, as HAdV is highly resistant even in dry environments and remains infectious at room temperature for up to 3 weeks. No protective effect of intravenous administered immunoglobulins has been proven. Only a moderate antiviral activity of ganciclovir, ribavirin and cidofovir has been shown in vitro against HAdV, and the latter is often used as preemptive therapy in patients with HAdV viremia. Currently, brincidofovir is under clinical evaluation in both pediatric and adult HSCT recipients with HAdV viremia [54]. A promising option to treat HAdV-infections are specific donor-derived T-cells [53, 55].

6.4.7 BK Polyomavirus

BK-virus is a member of the Polyomaviridae family, which can cause relevant morbidity in haplo HSCT recipients. The virus persists in immunocompetent patients in kidney cells, and poor immunoreconstitution after HSCT is associated with symptomatic infection [56]. The typical clinical manifestation of BK virus infection in the setting of haplo-HSCT is significant hemorrhagic cystitis, which is extremely painful for the patients. Less often, interstitial nephritis is seen, which may result in renal failure. Reliable PCR-based assays to diagnose BK virus infection have been

established, but positive test results in blood or urine have to be interpreted in context of clinical symptoms of BK virus infection. Cidofovir has been reported to be effective for treatment of BK virus-related diseases [57, 58].

6.5 Invasive Fungal Infection

Invasive fungal infections are a frequent and severe infectious complication in haplo-HSCT recipients. Risk factors for invasive fungal infections include prolonged and severe neutropenia and functional defects of phagocytes such as during the administration of high-dose steroids, delayed reconstitution of the T cell compartment, and severe mucositis. Since these risk factors are frequently present in patients receiving a haplo-HSCT, these patients are at particular high risk for invasive fungal disease [59]. *Aspergillus* spp. and *Candida* spp. are the most common causes of invasive fungal disease in haplo-HSCT patients, but other fungi such as mucormycetes or *Fusarium* spp. are also seen in these patients [60].

Clinical signs of invasive fungal disease are frequently unspecific, and may range from neutropenic fever to severe organ manifestation such as encephalitis or ophthalmitis. Hence, the threshold to perform diagnostic tests for invasive fungal disease should be low (Table 6.3).

6.5.1 Common Pathogens Causing Invasive Fungal Disease in Haplo-HSCT Patients

6.5.1.1 *Candida* spp

Established risk factors for invasive *Candida* infections are prolonged neutropenia, treatment with corticosteroids, mucositis and other mucosal lesions, central venous lines and urinary catheters, parenteral nutrition, and the use of broad spectrum antibiotics. Invasive Candida infection frequently presents as disseminated disease originating from infected catheters, may involve virtually all organs including the liver, spleen, brain and the eye, and may proceed to severe sepsis in up to 30% of patients. *Candida albicans*, as well as Candida non-albicans *C. glabrata, C. parapsilosis,* and *C. tropicalis* are most frequently isolated species causing invasive Candida infection [62]. Importantly, *C. krusei* and *C. glabrata* demonstrate resistance and reduced sensitivity to fluconazole.

6.5.1.2 *Aspergillus* spp

In contrast to *Candida* spp., which are part of the commensal flora, the ubiquitous *Aspergillus* spp. typically invades the organism *via* inhaled contaminated aerosols. Therefore, the predominant site of invasive aspergillosis is the lung, where large

Table 6.3 Potential clinical signs of invasive fungal disease

Organ/system	Features	Possible pathogen
Skin	Scattered lesions, exanthema	Acute disseminated candidiasis, disseminated aspergillosis, *Fusarium* infection
Sinus and palate	Facial pain, bloody nasal discharge, nasal eschars, ulcerations	Invasive aspergillosis or mucormycosis
Chest	Unspecific symptoms such as cough, pleural pain or back pain	Invasive pulmonary aspergillosis, PcP, other fungal pneumonia
Central nervous system (CNS)	Headache, neck stiffness, altered mental status, seizures, other focal neurologic signs	CNS aspergillosis or mucormycosis, cryptococcal or *Candida* meningitis
Eyes	Visual disturbances up to sudden blindness	Acute disseminated candidosis
Liver	Right upper quadrant pain hepatosplenomegaly	Chronic disseminated candidiasis

Adapted from Lehrnbecher et al. [61]

solid or multiple disseminated lesions may occur. Infection of second sites, in particular of the CNS, occurs in approximately 30% of patients, whereas cutaneous disease or abdominal aspergillosis affecting liver and spleen are less commonly seen [62, 63]. Invasive aspergillosis is associated with high mortality rates of up to 80% in HSCT patients, but the prognosis depends on the recovery of the immune system. The risk of invasive aspergillosis is especially high during the neutropenic phase prior to engraftment, but a significant percentage of invasive aspergillosis occurs after neutrophil engraftment during T-cell impairment, which underlines the importance of specific anti-fungal T-cells [63, 64].

6.5.1.3 Other Molds

Non-Aspergillus molds which may cause invasive fungal disease in haplo-HSCT patients include *Fusarium* Spp., *Scedosporium* spp., mucormycosis. The clinical presentation of these infections may be similar to that of invasive aspergillosis, though mucormycosis often presents as progressive, locally destructive mass lesions involving sinuses and orbita. The management of these infections is extremely difficult, and the prognosis is usually poor [62].

6.5.2 Diagnosis of Invasive Fungal Disease

Diagnosis of invasive fungal disease can be challenging and relies on imaging, serologic and PCR-based diagnostic tests, histopathology, and microbiology. Tissue specimens and body fluids suspected to be infected with fungi should be subjected to microscopy for fungal structures, for culture, and, if possible, for further analysis

including fungal DNA analysis. Isolation of fungi and culture is considered as gold standard for the definite diagnosis of invasive fungal infection. Fungal culture also allows testing for antifungal resistance, which is extremely important not only in infections with rare fungal species, but also because of the increasing incidence of azole-resistance in *Aspergillus* spp. [65]. In case of a negative culture, PCR based techniques and immunohistochemistry may be helpful in specifying genus and species of a fungus. In addition, genotyping of the fungus may reveal mutations in the fungal genome associated with reduced susceptibility or even resistance to specific antifungal agents [66–68]. Unfortunately, culture-based diagnosis of invasive fungal disease cannot be obtained in the majority of cases with clinical evidence of invasive fungal disease. In these scenarios, the detection of fungal antigens may assist the clinician to establish at least the probable diagnosis of invasive fungal disease.

Galactomannan (GM) is a polysaccharide cell-wall component that is released by most *Aspergillus* spp. during its hyphal growth. GM can be detected by an FDA-approved enzyme immunoassay that uses EB-A2 rat monoclonal antibodies (Platelia™ Aspergillus Enzyme Immunoassay, Bio-Rad). GM positivity in serum, bronchoalveolar lavage (BAL) fluid and cerebrospinal fluid are included as a mycological criterion in the revised definitions of invasive fungal disease from the EORTC/MSG consensus group [69]. GM testing can achieve a 90%–100% specificity and 80%–100% sensitivity of invasive aspergillosis in granulocytopenic adult patients [70–74]. In addition, its negative predictive value for excluding invasive aspergillosis is very high (>90%). As circulation of GM in serum is transient, testing should be carried out at least twice a week when GM is being used for screening [75]. In this setting, the GM assay may be positive before clinical suspicion of an infection and may also be useful for further monitoring of the therapeutic response [75]. It is important to note that causes for false-positivity of the GM test have to be considered, such as some batches of the ß-lactam antibiotics piperacillin/tazobactam and ampicillin, cross-reactivity with fungal species other than *Aspergillus* spp. such as *Penicillium marneffei* or *Histoplasma capsulatum*, cross-reactivity with transfused blood or antiglobulin sera and cyclophosphamide [76]. On the other hand, GM testing may be false-negative in patients receiving anti-mold active prophylaxis, and again, GM is not able to indicate an infection due to non-Aspergillus molds [77]. Although some authors stated the GM has a higher false-positivity in children [78], a recent meta-analysis demonstrated that the usefulness of GM is comparable in children and adults [79].

1 → 3-ß-D-Glucan (BG) is a cell wall polysaccharide component of many pathogenic fungi such as *Aspergillus* spp., *Candida* spp., *Fusarium* spp., *Trichosporum* spp., *Saccharomyces* spp. or *Pneumocystis* jiroveci, and therefore, BG is able to indicate infections due to a broad range of fungi. Similar to GM, BG is included as mycological criterion in the revised definitions of invasive fungal disease by the EORTC/MSG consensus group [69]. A recent meta-analysis in adult patients reported that for the cut-off recommended by the manufacturer, two consecutive positive test results increase the diagnostic performance of the BG assay in proven or probable IFD, and sensitivity and specificity were 50% and 99%, respectively,

and estimated positive and negative predictive values for an IFD prevalence of 10% were 84% and 95%, respectively [80]. As in GM, there are a number of causes of false-positive results, and the occurrence of BG is not limited to fungi, but found also in bacteria, algae and higher plants, which can result in false-positive results. As in children there is a paucity of data on BD and a need to validate a pediatric specific cut-off, current guidelines recommend that BG should not be used to guide pediatric clinical decision making [15].

The lack of standardization and absence of validated commercial systems of PCR-based methods for the detection of fungal pathogens explains the wide range of sensitivity and specificity across the reports and resulted in the exclusion of PCR testing in the revised 2008 EORTC/MSG diagnostic criteria [69]. Current data indicate that when testing blood samples, sensitivity of Aspergillus PCR using plasma seems to be superior to that of serum [81]. However, compared to blood samples, testing BAL samples seems to achieve higher sensitivity and specificity rates [75].

Recent studies indicate that the combined use of different biomarkers may increase their usefulness [82].

Imaging techniques are of great value in the diagnosis of invasive fungal disease. Whereas imaging by conventional X-ray is insufficient for early diagnosis of invasive mold infection, chest computed tomography (CT) plays an important role in this setting, in particular in invasive aspergillosis, as most of those infections are localized in the lungs. Characteristic CT findings for invasive aspergillosis include particular nodules with halo sign, air crescent sign and cavitation, which are all included as clinical criterion in the revised EORTC/MSG definitions [69, 83]. However, the occurrence of these findings are not restricted to aspergillosis and can also be seen in infections caused by other molds. The so-called reversed halo sign has been described as an early sign of invasive mucormycosis and may assist differentiation between those two groups of molds exhibiting different antifungal susceptibilities [84]. Notably, in children, typical CT signs as described above are often not observed and radiographic findings are more unspecific lit [85, 86].

It is important to note that in patients with suspected pulmonary aspergillosis, magnetic resonance imaging (MRI) of the brain is recommended even in the absence of neurological signs and symptoms [87, 88]. Imaging studies are also crucial in the diagnosis of hepatosplenic candidiasis (= chronic disseminated candidiasis, CDC), which is a distinct phenotype of deep seated candidiasis localized mainly in the spleen and liver. In this setting, ultrasound and MRI are the recommended imaging modalities [89].

Early diagnosis of invasive fungal disease is often difficult, in particular in patients suffering from invasive mold infection. However, early institution of antifungal therapy is associated with an improved outcome. In the clinical setting, different antifungal strategies may be applied. For example, in patients at high-risk for invasive fungal infection, such as haplo-HSCT recipients, who are neutropenic, afebrile and do not have symptoms suggesting invasive fungal infection, screening with non-culture based methods is a potential strategy (Fig. 6.2). When these patients develop fever which does not respond to broad-spectrum antibiotics, there are two potential strategies: in the empirical antifungal therapeutic approach, anti-

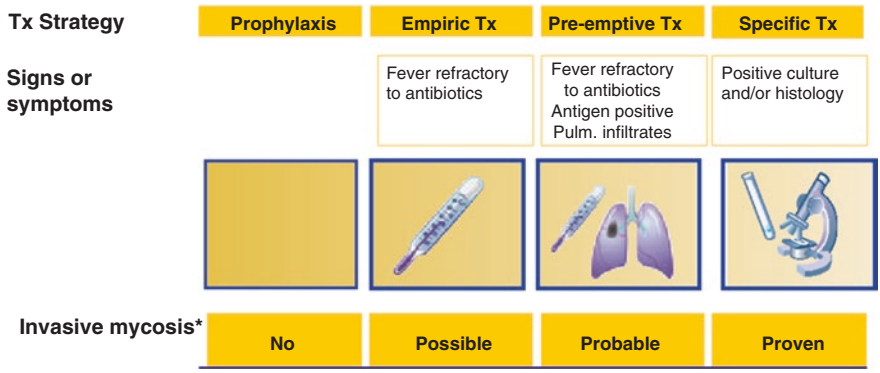
fungal therapy is started in all persistently febrile neutropenic patients, whereas in the pre-emptive strategy, antifungal therapy is only instituted when imaging and/or non-culture based diagnostics indicate invasive fungal infection. A diagnostic work-up should be performed which might help to modify and guide antifungal therapy.

6.5.3 Prophylaxis of Invasive Fungal Infections

Non-pharmacologic anti-infective measures to prevent invasive fungal disease in haplo-HSCT patients include HEPA-filtered rooms, and instructing patients to avoid construction areas. Although conflicting results have been reported, most experts agree that these measures may reduce the risk of air-borne infections such as invasive pulmonary aspergillosis [90].

Recent guidelines recommend primary antifungal chemoprophylaxis for patients undergoing haplo HSCT during the time of neutropenia and during phases of pronounced immunosuppressive therapy for severe GvHD [12, 62]. Depending on factors such as local epidemiology, patient's age, and co-medication, systemic antifungal prophylaxis may consist of a lipid formulation of amphotericin B (e.g., lipososmal amphotericin B, amphotericin B lipid complex), an echinocandin (e.g., micafungin) or an azole (e.g., fluconazole, itraconazole or one of the broad-spectrum triazoles voriconazole or posaconazole) [91]. Whereas most azoles can be administered either orally or intravenously, only intravenous formulations are available for amphotericin B and the echinocandins. It is important to note that the choice of antifungal prophylaxis is controversial and different guideline groups have used different approaches regarding evidence synthesis and recommendation generation [62, 92, 93].

Fluconazole might be used during the neutropenic phase after haplo-HSCT to prevent fungal infections, especially in the setting of HEPA-filtered rooms when patients are at a high-risk for *Candida* infections. Fluconazole is not active against molds and it is important to note that the agent has no or only reduced activity against some important *Candida* non-albicans spp. Itraconazole prophylaxis has also been applied in the setting of HSCT, but it is burdened with relatively high toxicity and with inefficacy against some relevant fungi such as *C. glabrata* or *Fusarium* spp. [94]. Prophylactic therapy with the newer triazole voriconazole has been shown to reduce the frequency of invasive aspergillosis compared to fluconazole or itraconazole, but a survival benefit has not been shown [95]. Notably, for both itraconazole and voriconazole, therapeutic drug monitoring is strongly recommended [62, 96]. In contrast, prophylactic therapy with posaconazole, which is active against mucormycetes, has been shown to improve survival in adult HSCT patients with GvHD compared to patients receiving fluconazole [97]. Unfortunately, posaconazole is not approved for the use in pediatric patients younger than 12 years in the US and younger than 18 years in the EU. The administration of azoles is limited by multiple drug-drug interactions, and in patients with contraindications of azole administration, echinocandins may be an option for antifungal prophylaxis. In



* in clinical practice, not EORTC/MSG criteria!

Fig. 6.2 Potential antifungal strategies (Modified according to Lehrnbecher et al. [61])

this regard, a recent study showed comparable efficacy of micafungin compared to fluconazole [98].

6.5.4 Empirical and Pre-emptive Antifungal Therapy

Empirical antifungal therapy is a widely accepted strategy to institute antifungal agents in haplo-HSCT patients after 3–4 days of unexplained fever not responding to broad-spectrum antibacterial therapy as these patients are at high risk of invasive fungal disease and fever refractory to antibiotics may be a sign of invasive fungal infection (Fig. 6.2). Echinocandins, liposomal amphotericin B, and, possibly, voriconazole are suitable agents for empirical antifungal therapy [15]. In patients receiving mold-active antifungal prophylaxis, switching to another class of antifungal agents appears reasonable [12, 62]. However, experts strongly recommend further diagnostic procedures in this setting although patients are already receiving antifungal therapy (Fig. 6.3).

An alternative approach to empirical therapy is pre-emptive antifungal therapy, which is initiated on the basis of suspect imaging findings or other indicators of possible fungal disease (such as a positive GM test) in order to reduce the overall exposure to potentially unnecessary antifungal agents [15, 93] (Fig. 6.2). If cerebral fungal infection is suspected, the choice of the antifungal drug should be restricted to agents which sufficiently pass the blood-brain-barrier, such as lipid formulations of amphotericin B (e.g., amphotericin B lipid complex, and liposomal amphotericin B), or the broad-spectrum triazole voriconazole. In contrast, the echinocandins do not seem to achieve therapeutic levels in the CNS [99–101]. Although limited data are available, a study suggests that the approach of pre-emptive therapy reduces the

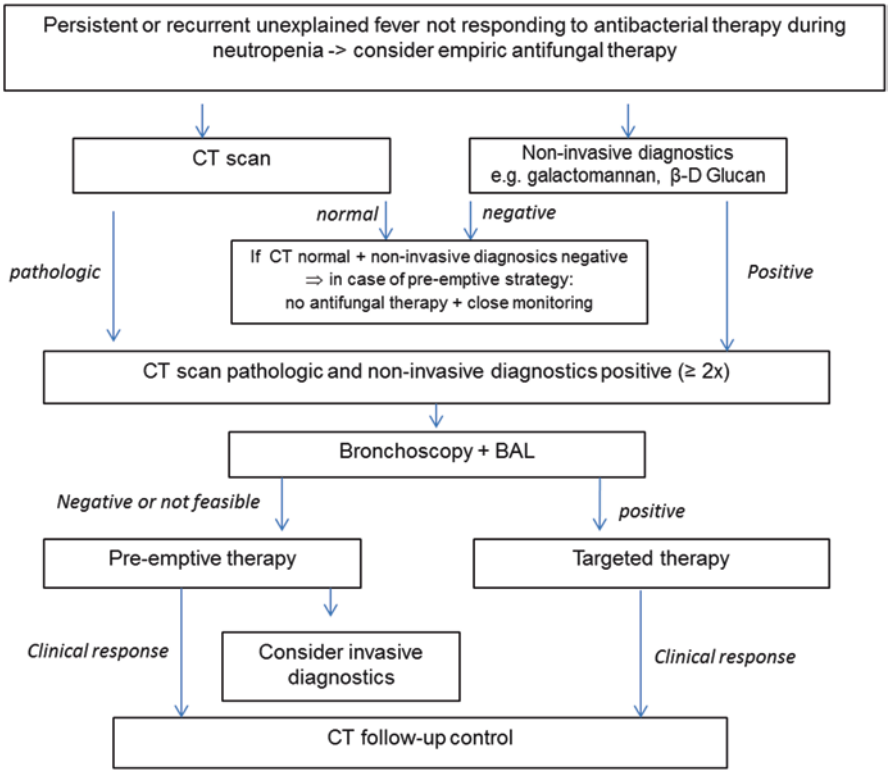


Fig. 6.3 Diagnostic workflow in neutropenic patients with persistent fever (Adapted from Lehrnbecher et al. [15])

use of antifungals without increasing mortality [102]. Importantly, this strategy cannot be recommended in children as no data are available [15].

6.5.5 Species-Directed Antifungal Therapy

6.5.5.1 *Candida* spp

Invasive Candida infection requires prompt initiation of antifungal therapy, and surgical intervention. The exchange of intravenous and urinary catheters have to be considered. Echinocandins are the preferred agents to treat invasive Candida infections in immunocompromised patients, unless CNS or intraocular manifestations are present. Liposomal amphotericin B is also effective to treat invasive Candida infection, but the toxicity profile of this antifungal agent is less favorable. Susceptibility testing should be included in the management of invasive Candida infections, as for example *C. parapsilosis* demonstrates in vitro higher

susceptibtibility to fluconazole compared to echinocandins, although clinical experience may not support this finding [93, 103].

6.5.5.2 *Aspergillus* spp

Voriconazole is the recommended first-line agent for the treatment of invasive aspergillosis, in particular in patients with CNS infection [12, 62]. Liposomal amphotericin B is an alternative to voriconazole, in particular in patients with prior antifungal prophylaxis with broad-spectrum azoles, whereas caspofungin may be considered as a second-line therapy [62, 103]. Therapeutic drug monitoring is recommended for patients receiving voriconazole [96]. As the recurrence of invasive aspergillosis is high, secondary antifungal prophylaxis with a mold-active agent is recommended in patients undergoing haplo-HSCT [104, 105].

6.5.5.3 Other Fungi

Successful treatment of mucormycosis crucially depends on rapid initiation of antifungal therapy with amphotericin B and surgical debridement. Second-line options include posaconazole and lipid amphotericin B plus caspofungin, whereas itraconazole is not effective to treat this infection [62]. Voriconazole is recommended as treatment of invasive fusariosis and scedosporoiosis, which should be monitored by TDM [62].

6.5.5.4 Additional Interventions to Support Antifungal Therapy

In addition to the administration of antifungal agents, current guidelines recommend to improve host immunity, e.g. by tapering immunosuppressive therapy in allogeneic HSCT recipients with GvHD, the use of colony-stimulating factors such as G-CSF or granulocyte transfusions in the neutropenic host, although for the latter, a significant benefit has not been proven to date [62]. Given the impaired antifungal T-cell responses in patients after haplo-HSCT, administration of antifungal T-cells or dendritic cells may be a promising approach to combat invasive fungal disease, although these strategies have to be evaluated in future studies [106, 107].

6.5.6 Pneumocystis jiroveci

Patients undergoing haplo-HSCT have a relevant risk of Pneumocystis jirovecii pneumonia (PCP). This infection, however, is almost completely preventable by adequate prophylaxis therapy with trimethoprim-sulfamethoxazole (TMP-SMX). This agent does not only reliable protects against Pneumocystis jirovecii, but also to

a certain extent against other pathogens such as *Toxoplasma* spp., Nocardia, spp., *Plasmodium* species, and some respiratory pathogens. Prophylaxis with TMP-SMX is highly effective but may lead to side effects such as myelosuppression, hypersensitivity or nephritis. Alternative drugs to prevent and treat PCP are pentamidine, dapsone, or atovaquone. Since TMP-SMX can delay engraftment, prophylactic therapy with TMP-SMX is usually initiated in the post-engraftment phase and given for at least 6 months after haplo-HSCT [8, 108–110].

6.6 Parasitic Infections

There are a number of parasites such as Cryptosporidium, Giardia lamblia, Entamboeba histolytica, or Toxoplasma spp. which can cause infections in patients undergoing haplo-HSCT. High incidence rates of up to 50% of toxoplasmosis in haplo-HSCT recipients have been reported in endemic areas [10]. The infection can be a result of reactivation or of de novo infection and may present with uncharacteristic signs and symptoms such as fever, lymphadenopathy, hepatosplenomegaly, meningitis, brain abscess, chorioretinitis, pneumonitis, myocarditis, or disseminated disease. Toxoplasmosis frequently occurs within 3 months after haplo-HSCT, but later presentations are possible, especially after termination of PCP prophylaxis with TMP-SMX. Diagnosis of toxoplasmosis relies on typical imaging results (especially of the CNS), serologic testing, and PCR-based detection of the pathogen [10].

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Chapter 7
Graft Versus Leukemia (GvL), Graft Versus Lymphoma Effect in Haploidentic SCT

Jakob R. Passweg, Michael Medinger, and Joerg P. Halter

7.1 How Do We Assess Graft Versus Leukemia Effects

Since graft versus leukemia or graft versus lymphoma effects are difficult to assess clinically, relapse rate reduction or response to immunologic interventions in post-transplant persisting disease or relapse has been accepted as a surrogate of GvL strength since many years. Indeed, it was the seminal paper published in BLOOD in 1990 [1] describing both the role of T cells as important effector cells for graft versus leukemia (GvL) effects as well as the close link between GvL and GvHD in patients with acute and chronic leukemia receiving allogeneic bone marrow transplantation (hematopoietic stem cell transplantation, SCT). This study showed decreasing relapse rates from syngeneic and T-cell depleted allogeneic [2] to T-cell repleted allogeneic SCT without GvHD to allogeneic SCT with patients having acute and chronic GvHD having the lowest relapse rates (Fig. 7.1). Similarly, more than 20 years later an analysis comparing the strength of the GvL effects in patients with acute myeloid leukemia receiving consolidation by allogeneic SCT versus other types of consolidation used reduction of relapse risks to demonstrate the comparable power of GvL in different cytogenetic risk categories [3]. This is shown in Fig. 7.2. Today there is no better way to measure GvL effects than to compare relapse reduction in patients undergoing allogeneic SCT versus other types of treatment. This is not different with other types of immunotherapy, e.g. in patients with melanoma treated by checkpoint inhibitors lower relapse rates are used to define the effects of cancer immunotherapy. GvL effects may be operative in different disease entities, mainly hematologic malignancies of myeloid or lymphoid origin. Whereas CML is well known for exquisite sensitivity to GvL effects other disease entities, e.g. T-cell lymphomas may exhibit such sensitivity as well. It is quite difficult to

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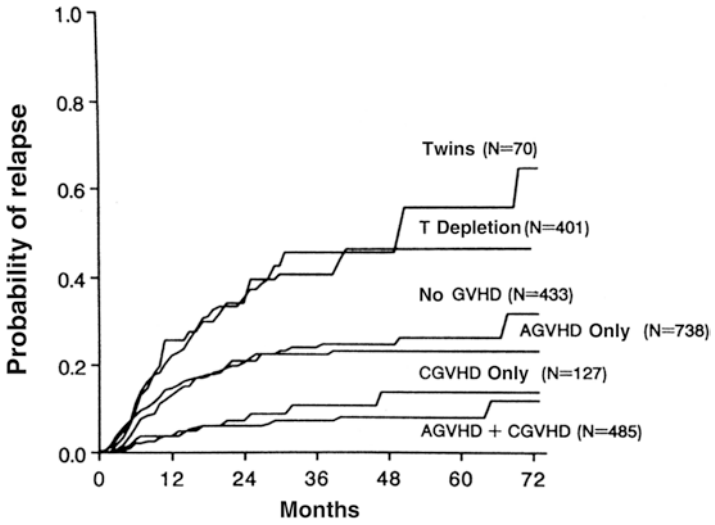

Fig. 7.1 Figure depicting relapse after allogeneic bone marrow transplantation (From Ref. [1] as described in the text)

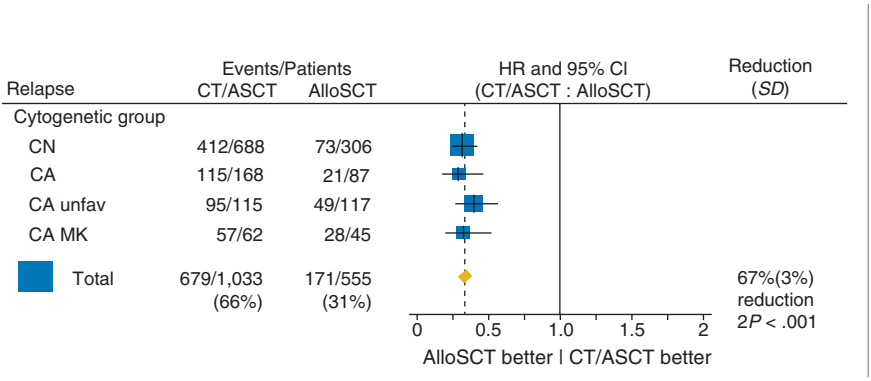

Fig. 7.2 Figure from Ref. [2] showing relapse risk reduction as a forest plot in patients with AML in CR1 in different cytogenetic risk groups by allogeneic SCT as compared to other types of consolidation. The strength of GvL is measured as relapse risk reduction and shows similar risk reduction across different cytogenetic risk groups

categorize disease entities by GvL sensitivity as this is very much dependent on disease stage, disease aggressiveness, disease growth dynamics and escape mechanisms. In this chapter GvL is used as the term to define immunotherapeutic effects of alloreactive cells irrespective of graft versus leukemia or graft versus lymphoma activity.

7.2 HLA Mismatch Associated Alloreactivity

With haploidentical SCT GvL effects are postulated to be particularly strong as the mismatched haplotype allows for additional allorecognition and thus elimination of malignant cells. The strength of this allorecognition is exemplified in a study by Vago et al. where particularly late relapse (after a median of 307 days) after haploidentical SCT was associated with loss of the mismatched haplotype in leukemic blasts probably through uniparental disomy [4]. This was observed in a particular context of non T-cell depleted haploidentical SCT in myeloid diseases where relapsing leukemic blasts had to overcome the mismatched haplotype induced alloreactivity in order to cause frank relapse. Other studies have not found a stronger GvL effect in haploidentical SCT as compared to matched sibling donor SCT [5]. However in this quoted study only a minority of the haploidentical SCT used post-transplant cyclophosphamide as a GvHD prophylaxis. Furthermore, many studies include myeloid and lymphoid neoplasms, which may depend on different pathways for alloreactivity and relapse.

7.3 Competing Risks with Graft Versus Host Disease

There is a complex competition between GvHD and GvL, as severe GvHD not only reduces risks of relapse and is thus seen as evidence of measurable GvL but GvHD is also a major driver of non relapse mortality and removes patients from being at risk of relapse. Appropriate statistical models are required as exemplified in the analysis by Ringden [5] where time dependent covariate models of relapse are used entering GvHD at the time of occurrence to compare, in this example, relapse risk in patients receiving identical sibling transplants to patients receiving haploidentical SCT. In this particular example haploidentical SCT was not associated with a reduced relapse risk as compared to identical sibling transplants, therefore not arguing in favor of stronger GvL effects of haploidentical to sibling transplants in the analyzed patient cohort.

7.4 Contribution of Cellular Subsets e.g. NK Cells, Gamma/Delta T-Cells Towards Graft Versus Leukemia Effects

In early studies GvL was thought to be exclusively mediated by T-lymphocytes, particularly as T-cell depletion protocols abrogated GvL effectively [2]. It is now generally accepted that other cellular subsets, e.g. NK-cells may mediate GvL effects, whereas the relative contributions of donor T- and NK-cells to the GvL effect may depend on the transplant protocol. Protocols using extensive T-cell depletion for haploidentical SCT showed that NK cell reconstitution was rapid and

that NK alloreactivity using the missing ligand model could predict for lower relapse rates [6, 7]. Rapid NK cell reconstitution is possibly the consequence of a lack of competition for cytokines by T-cells being absent. Hence, observing a strong NK alloreactive effect is probably dependent on having few T-lymphocytes in the system. Next to NK alloreactivity defined by missing ligands the number of activating KIR genes appears also to play a role in alloreactivity and thus in exerting GvL effects [7] although the role of activating KIR gene content is far from being clear. Furthermore gamma/delta T-cells have been described in some but not all models as being beneficial by exerting GvL without GvHD [8]. This has led to GvHD prophylaxis regimens using alpha/beta and CD19 depletion, eliminating alpha/beta T-cells and B-cells while preserving NK-cells and gamma/delta T-cells.

7.5 Conditioning Intensity and GvL

Impact of conditioning intensity on outcome of allogeneic SCT in particular on relapse incidence is well documented [9]. Several comparative studies have shown lower relapse rates with myeloablative conditioning as compared to reduced intensity conditioning. These studies have also uniformly shown higher toxicity of myeloablative conditioning and often the benefit of lower relapse incidence was offset by higher non relapse mortality resulting in low or no overall benefit. Whether there is a strong interaction between conditioning intensity and GvL effects is difficult to determine. The strength of GvL effect is mainly measured as a reduction in relapse incidence, which is influenced by conditioning intensity as well. Even if haploidentical SCT using post-transplant cyclophosphamide as GvHD prophylaxis has been described after reduced intensity conditioning and myeloablative regimens [10–12], there are too few comparative studies to allow to estimate the relative contribution of intensity of conditioning versus GvL effect on transplant outcome.

7.6 Donor Choice and GvL

Optimal donor choice for haploidentical SCT to maximize GvL effects is not well defined. Whereas under some conditions like intensive T-cell depletion maternal donors with NK alloreactivity were found to be particularly well suited [6] other studies have identified male donors [13] or donors with higher BMI [14] to be providing better transplant outcomes. The role of activating KIR gene content is not clarified but some results look promising. Most centers will choose haploidentical donors according to some algorithm that includes age and gender of the donor, NK alloreactivity based on a ligand mismatched model, they will more readily accept a haploidentical donor mismatched for fewer than 5/10 alleles. Clearly the preference of stem cell source, the likelihood to collect enough cells for transplantation as well as the presence of donor specific anti-HLA antibodies in the recipient also impacts on donor choice.

7.7 Stem Cell Source and GvL

In a study comparing haploidentcial peripheral blood versus bone marrow transplants after non myeloablative conditioning relapse rates were higher with marrow as a stem cell source, however there was no difference in overall survival. This can be interpreted as being a sign of stronger GvL effects with peripheral blood than with marrow [15]. Stronger GvL with peripheral blood as compared to marrow may be readily explained by higher T-cell content of the peripheral blood product. The EBMT activity survey report [16] shows that in Europe peripheral blood is already used more frequently than marrow in haploidentical SCT in spite of the majority of the studies reported using ptCy as GvHD prophylaxis having used marrow instead of peripheral blood as a stem cell source.

7.8 GvHD Prophylaxis

GvHD prophylaxis is used to avoid severe acute and chronic GvHD and includes immunosuppressive drugs, in vitro or in vivo T-cell depletion and with the most recent surge in use of haploidentical SCT the administration of post-transplant cyclophosphamide typically on days +3 and +4 after SCT at a dose of 50 mg/kg per day (ptCy). This type of GvHD prophylaxis was pioneered by colleagues from the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center avoiding calcineurin inhibitors prior to cyclophosphamide in order to hit the replicating alloreactive T-cells the most [10]. This regimen was developed following the discovery that cyclophosphamide had little toxicity for hematopoietic stem cells. Other types of GvHD prophylaxis used in haploidentical SCT include combination of immunosuppressive drugs in combination with antithymocyte globulin or different ways of T-cell depletion, the most recent development include alpha/beta and CD19 depletion, attempting to preserve GvL by not depleting the gamma/delta T-cell subsets and not depleting NK cells [17]. Comparisons between the different strategies for rates of rejection, GvHD and GvL effects are difficult as no head to head studies are available. It appears that the ptCy, while associated with lower GvHD risks may allow for more relapse, at least in a phase II study where double cord blood transplantation was compared to haploidentical bone marrow transplantation using ptCy for GvHD prophylaxis [18] as shown in Fig. 7.3. In an observational study by the EBMT comparing ptCy to ATG [19] in haploidentical SCT relapse rates were comparable between the groups although GvHD risks were somewhat lower with ptCy.

In contrast, a study by Wang et al. [20] comparing outcomes of high-risk AML patients with SCT from HLA haploidentical donors (HID, n = 81) or HLA-identical siblings (SIB, n = 36) using comparable regimens except for added ATG in HID group. There was more aGVHD in the HID cohort while cGvHD was similar. The 2-year cumulative incidence of relapse was significantly lower in HID (26%) than in SIB patients (49%) (P = 0.008). Because non relapse mortality was similar, the

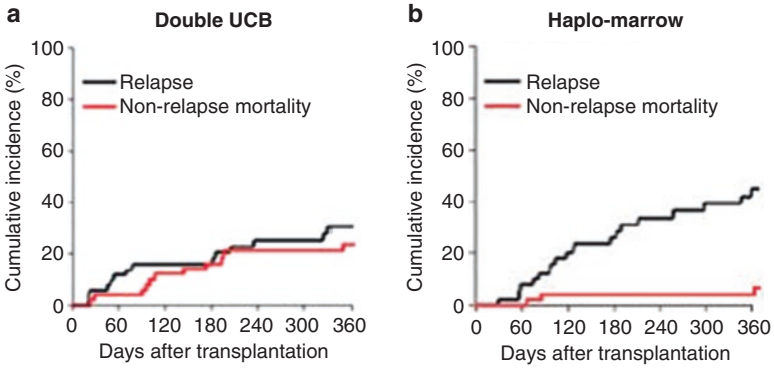

Fig. 7.3 Incidence of relapse and of non relapse mortality in two parallel studies using either double cord blood transplants or haploidentical bone marrow transplant with ptCy for GvHD prophylaxis (from reference Brunstein). In spite of this not being a direct comparison it appears that relapse incidence is somewhat higher with haploidentical SCT than with double cord blood SCT

3-year probability of overall survival was higher in HID patients (42%) than in SIB (20%) ($P = 0.048$) patients. Their data suggests that haploidentical SCT can possibly achieve a stronger GVL effect than identical sibling donors for high-risk acute leukemia patients in the context of GvHD prophylaxis using moderate doses of ATG.

7.9 Donor Lymphocyte Infusions to Induce GvL

The use of donor lymphocyte infusions to induce GvL effects after haploidentical SCT has been reported although the number of reported patients and length of follow up is limited. In one study with 42 patients [21] after haploidentical SCT receiving 10^3–10^7 CD3 cells/kg in escalating doses the rate of GvHD appeared not to be substantially higher than rates reported for matched donor recipient pairs. Response appeared to be particularly good in patients with Hodgkin lymphoma and AML in molecular relapse. This data shows that DLI can be given to exert GvL after haploidentical SCT although data are not sufficient to assess safety conclusively.

7.10 Conclusions

Haploidentical SCT has seen an important increase in popularity with approximately 2000 haploidentical SCT now done annually in Europe [16]. Studies comparing results of haploidentical SCT to unrelated donor SCT and to sibling donor SCT have been instrumental [22–25] in establishing the value of this transplant modality. The ease of ptCy administration has certainly contributed to this rapid evolution. Whether GvL after haploidentical SCT is strong or not is most likely to

be dependent on numerous factors, including the underlying disease, degree and type of GvHD prophylaxis used, the donor, stem cell source and other factors, many of which are not known. Ultimately, all measures to minimize GvHD risks will very likely impact on GvL strength. Best results are obtained by techniques, which find the best balance between these two immunologic effects. Some current studies compare prospectively haploidentical SCT to 1 antigen mismatched unrelated donor SCT or to 10/10 matched donor SCT. Some of these protocols use the identical conditioning and GvHD prophylaxis regimen with the intention to address the issue of GvL strength in the context of otherwise similar protocols.

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Chapter 8
Outcome of Haploidentical SCT in Patients with Acute Leukemia

Albert Esquirol and Jorge Sierra

8.1 Introduction

Acute leukemia (AL) is a heterogeneous group of malignancies characterized by the clonal proliferation of immature hematopoietic cells with disruption of their normal differentiation.

The treatment for acute myeloid leukemia (AML) in fit patients consists of intensive intravenous chemotherapy based on cytarabine (7 days) and anthracyclines (3 days). With this 3 + 7 regimen complete remission (CR) is achieved in 60–80% of young (up to 65 years of age) and 30–60% of elderly patients. Once in CR, consolidation treatment with intermediate or high dose of cytarabine is needed to avoid early relapse (from one to four courses). Following, allogeneic hematopoietic transplantation is indicated or not, depending on risk allocation of the patient and the disease, as well as the availability of a suitable donor. Fit patients with intermediate and adverse risk genetics AML do benefit from allografts [1].

In acute lymphoblastic leukemia (ALL) intensive chemotherapy is also the first choice. Initial treatment includes more agents than in AML, with vincristine, prednisone, daunorubicin, L-asparaginase, cyclophosphamide, and methoretaxe being the backbones of the schemes. Additional drugs are subsequently administered in different sequences. In essence, the treatment consists of an induction phase and several consolidations followed by low-dose maintenance during 2–3 years. Currently, protocols for relatively young adults are based on intensive combinations as those administered in high-risk children. These approaches are not feasible in elderly patients due to the high morbidity and mortality. Allogeneic stem cell transplantation has indication in first complete remission in patients with initial high-risk features, residual disease after chemotherapy, or both, as well as after a relapse [2].

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In short, allogeneic stem cell transplantation is the best option for high-risk AL, but only 30% of patients in need have a matched related donor [3]. The probability to identify a closely HLA-matched unrelated donor is 80% for Spanish and other Caucasian populations [4]. This probability decreases if the patients belong to ethnic minorities; it is 30–40% in the Mexican and Central/South America populations and 15–20% in African Americans and black Caribbean [5]. Other options for patients who lack HLA-compatible adult donors are umbilical cord blood and haploidentical related donor transplantation [6]. These alternatives have the advantage of the rapid availability of hematopoietic stem cells, although cord blood has the limitation of the usually low dose of the graft for adult patients. In any case, if the transplant has to be performed shortly and the patient lacks an HLA-identical sibling, these stem cell sources have to be seriously considered, since unrelated donor search and cell procurement may take several months.

8.2 Historical Perspective and Initial Approaches in Partially HLA-Matched Hematopoietic Transplantation

Historically, hematopoietic stem cell transplantation using a non-fully HLA-matched related donor was associated with a high incidence of graft failure, delayed and incomplete immune recovery, as well as frequent non-relapse mortality (NRM). Powles et al. published in 1983 a series of 35 patients with advanced acute myeloid leukemia (AML – 33 patients) or lymphoblastic leukemia (ALL – 2 patients) who received a mismatched related allogeneic bone marrow transplantation (BMT). Fifteen patients were fully HLA-haploidentical and 20 had one or more coincident antigen in the non-identical haplotype. Cyclophosphamide plus total body irradiation (TBI) was administered as conditioning regimen in 31 patients and melphalan replaced TBI in 4 patients. Cyclosporine and methotrexate were given as graft versus host disease (GVHD) prophylaxis. High incidences of NRM (20 of 35 patients) and graft failure (10 of 35 patients) were observed. Of note, cumulative incidence of relapse was only 11% in the AML group. Eleven of 35 patients were alive between 6 months to 3 years [7].

Beatty et al. described high incidences of grade II-IV acute GVHD and NRM in a series of 105 patients with advanced hematological malignancies who received a partially compatible related donor BMT. This study group was compared to a control group of 728 patients transplanted from an HLA-identical sibling. Myeloablative conditioning (MAC) regimen was administered. Haploidentical transplantation (one, two or three HLA-loci incompatible) was associated with delayed engraftment and high incidences of graft rejection and mortality. The cumulative incidence of II-IV acute GVHD was 70% in the study group versus 42% in control group (Fig. 8.1). However, there was no statistical difference in survival when comparing the mismatched versus the matched donor group [8].

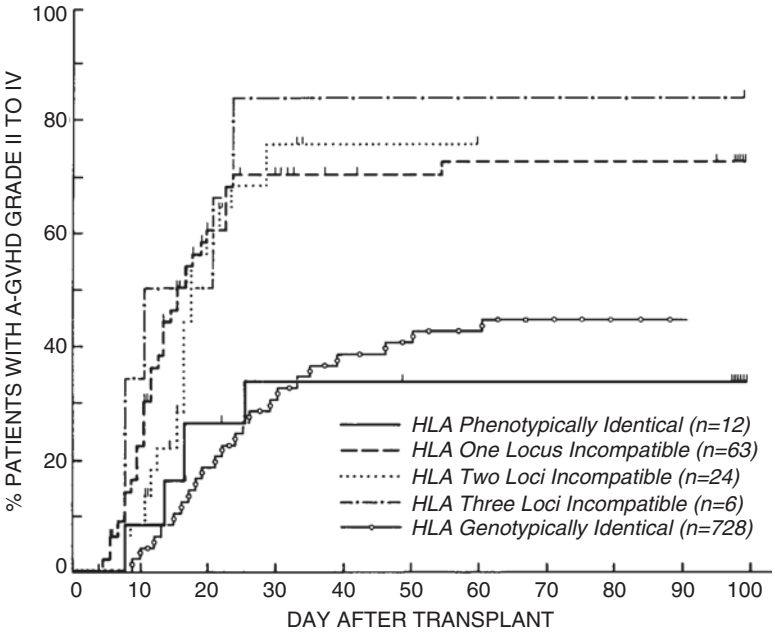

Fig. 8.1 Cumulative Incidence of acute grade II-IV graft versus host disease in relation to the number of disparate loci [8]

Szydlo et al. analysed the impact of HLA-mismatch in the International Bone Marrow Transplant Registry (IBMTR) database. Two-thousand fifty-five patients who received a BMT for AML, ALL and chronic myeloid leukemia (CML) were included in the study. Increased incidence of NRM and decreased leukemia-free survival (LFS) were observed in transplants from one or more antigen-mismatched donors compared to those from an HLA-identical siblings [9].

8.3 "Ex Vivo" T Cell Depleted Haploidentical Transplantation

Due to the poor results after unmanipulated BMT from fully haploidentical donors [7–9] different strategies were developed to decrease graft-versus-host disease (GVHD) and mortality. Extreme "ex vivo" T-cell depletion of the graft was investigated to avoid lethal GVHD. In this regard, a pioneer haploidentical transplant program was established by the Perugia group in the 1990s [10], based on the preclinical experience with mega-dose of stem cells investigated by Raisner et al. in Israel [11]. Initially, 17 patients with advanced AML, ALL or CML in blast crisis received haploidentical related (11 donors) or sibling (6 donors) transplants. All patients

received a MAC regimen based on TBI, thiotepa, antithymocyte globulin (ATG) and cyclophosphamide followed by T-cell depleted hematopoietic stem cells in a very high dose (11.62 ± 4.74 × 10^6 CD34/kg), without postransplant GVHD prophylaxis. Only one patient had graft failure and the incidence of acute GVHD was low (18%), but the high frequency of infection deaths was the major drawback of the platform [12].

The same group subsequently reported 43 patients with poor prognosis AML or ALL, treated with MAC consisting of TBI, thiotepa, ATG and fludarabine (instead of cyclophosphamide) followed by a mega-dose of CD34+ selected progenitors without postransplant GVHD prophylaxis. This study showed a high rate of engraftment, low incidence of graft failure, limited non-haematological toxicity and absence of GVHD. Overall survival (OS) at 18 months was 28% and disease free survivals (DFS) were 36% and 17% for AML and ALL, respectively. Infections were the main cause of the high NRM (40%) due to a slow immune recovery. Leukemia relapse developed in 13 of the 43 patients, being particularly frequent in patients with active disease at transplant [13].

In 2005, the Perugia group published their updated data on 104 patients with AML or ALL. All of them received the conditioning mentioned in the previous paragraph, followed by the infusion of a median of 13.8 × 10^6/kg CD34+ cells. Ninety-one percent of patients achieved a fast engraftment and only 8 of the 100 evaluable patients developed grades 2–4 acute GVHD. In 3 of the 8 patients there was a progression to chronic GVHD. NRM was 36% at 22 months with infections as the main cause. The incidences of relapse for patients in CR versus active disease at transplant were 16% and 51%, respectively. Event free survival (EFS) was better than in their previous experience, 48% in patients with AML and 46% in ALL [14] (Fig. 8.2).

Another approach on using haploidentical donors was the platform reported by Mehta et al. in 201 patients with AML and ALL. Conditioning regimen consisted of TBI, cyclophosphamide, cytarabine, etoposide, anti-thymocyte globulin (ATG) and methylprednisolone, followed by the infusion of bone marrow (BM) with partial T cell depletion using “ex vivo” OKT3 or T10B19. GVHD prophylaxis was the combination of cyclosporine, methylprednisolone, and pretransplant ATG. The cumulative incidence (CI) of grades 2–4 acute GVHD was 13% and the CI of chronic GVHD was 15%. The 5-year OS and DFS were 29% and 34% for patients in first or second CR, and 13% and 14% for the remaining patients, respectively. The CI of relapse and NRM at 5 years were 31% and 51%, respectively, and like in other studies a viral infection was the most common cause of NRM [15].

German investigators developed another haploidentical transplant strategy using CD3 plus CD19 depleted grafts from peripheral blood and reduced intensity conditioning (RIC) instead of MAC [16]. Federman et al. published 61 patients, mostly with acute leukemia (38 AML and 8 ALL). Conditioning included fludarabine, thiotepa, melphalan and OKT-3. CI of NRM was 23% at 100 days and 42% at 2 years, CI of acute and chronic GvHD were 46% and 18%, respectively. CI of relapse was 31%. The OS for patients with AML in CR at transplant was 32% at 2 years.

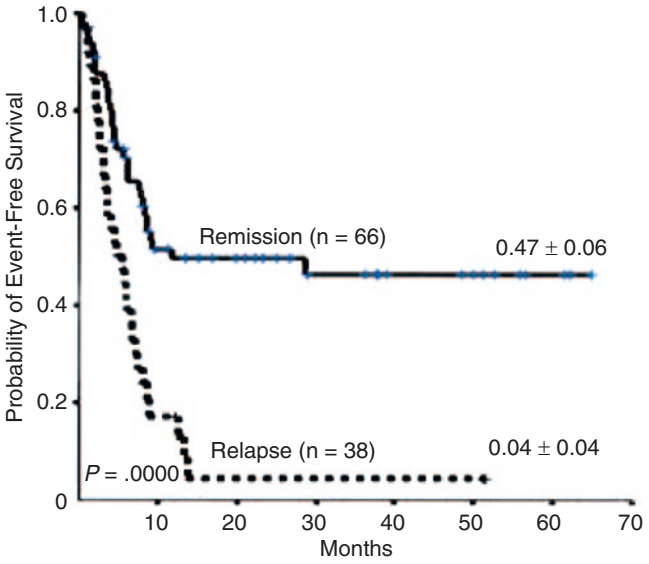

Fig. 8.2 Probability of event-free survival after haploidentical transplantation in acute leukemia: The Perugia experience [14]

8.4 Haplodentical Transplantation with G-CSF Primed BM and PB and Multiagent GVHD Prophylaxis

At the Peking University 250 patients with AL received haploidentical transplants using a homogeneous strategy. Cytosine arabinoside, busulfan, cyclophosphamide, semustine and ATG were used for conditioning. Hematopoietic stem cells were obtained from BM (G-CSF primed) and peripheral blood (PBSC). Postransplant GVHD prophylaxis included four drugs (cyclosporine, methotrexate, mycophenolate mofetil and ATG pre-infusion). CI of grade 2–4 acute GVHD was 46% and of chronic GVHD was 54% (extensive in 23% of patients). CI of relapse at 3 years were 12% and 24% for standard AML and ALL patients, respectively, whereas the CI were 20% and 48% in high risk AML and ALL, respectively. OS were 72% and 55% for standard and high risk AML, respectively, while the corresponding values in ALL were 65% and 26% [17].

The Peking approach was updated in 2012 and included 756 patients with AL (136 patients with CML). CI of grades 2–4 and 3–4 acute GVHD were 43% and 14%, respectively. The CI of overall and extensive chronic GvHD at 2 years after transplantation were 53% and 23%, respectively. The CI of relapse was 18% at 3 years. Of note, the CI of relapse in high-risk disease was 29%, better than in previously described study, probably as a consequence of prophylactic donor infusion lymphocyte that was intended in all these patients. Remarkably, the probabilities of OS and LFS were 67% and 63%, respectively (Fig. 8.3) [18].

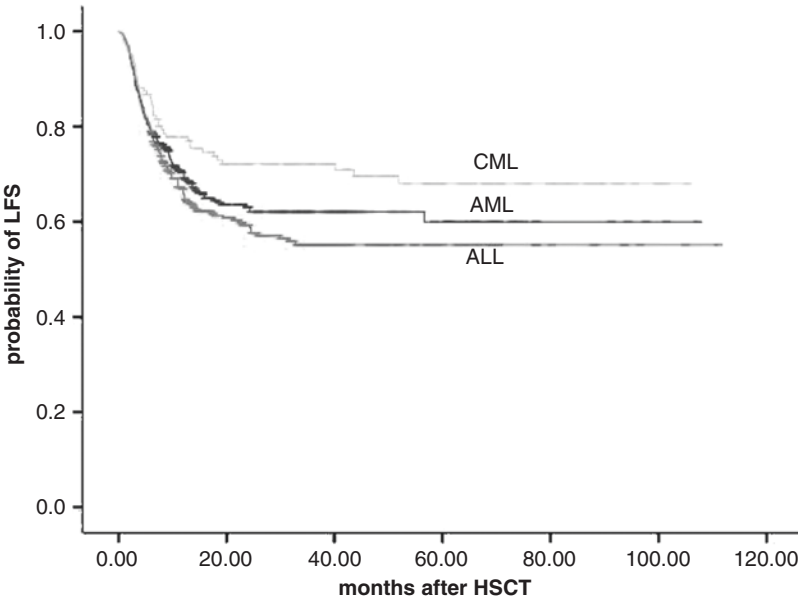

Fig. 8.3 Probabilities of LFS for AML, ALL and CML after HLA-haploidentical transplantation: The Peking experience [18]

8.4.1 Haploidentical Transplant with Post-Transplant High-Dose Cyclophosphamide

A significant advance in the haploidentical transplant setting has been the introduction T-cell repleted transplants with administration of high-dose cyclophosphamide shortly after stem cell infusion. Investigators from the John Hopkins hospital pioneered this approach. The Seattle group joined the protocol after the first experiences from Baltimore.

Cyclophosphamide (CY) is an alkylating agent included in most standard conditioning regimens. Hepatic cytochrome p-450 metabolizes CY to aldophosphamide; when this metabolite enters into quiescent cells their high content of aldehyde-dehydrogenase transforms the metabolite into an inactive compound, in contrast to what happens in proliferating cells. Of note, while aldophosphamide induces apoptosis the inactive form does not [19]. As a result non-dividing hematopoietic stem cells and quiescent lymphoid cells are preserved from the effect of CY whereas this drug kills early alloreactive T-cells (Fig. 8.4) [20].

The surviving donor derived cells will lead to hematopoietic recovery including the post-transplant T cell pool. The balance between effector and regulatory T cells will determine outcomes as graft rejection and GVHD. Further GVHD prophylaxis with a calcineurin inhibitor, mycophenolate mofetil (MMF) or both has to be administered to maintain the balance in a safe range. Subsequently, the full recovery of

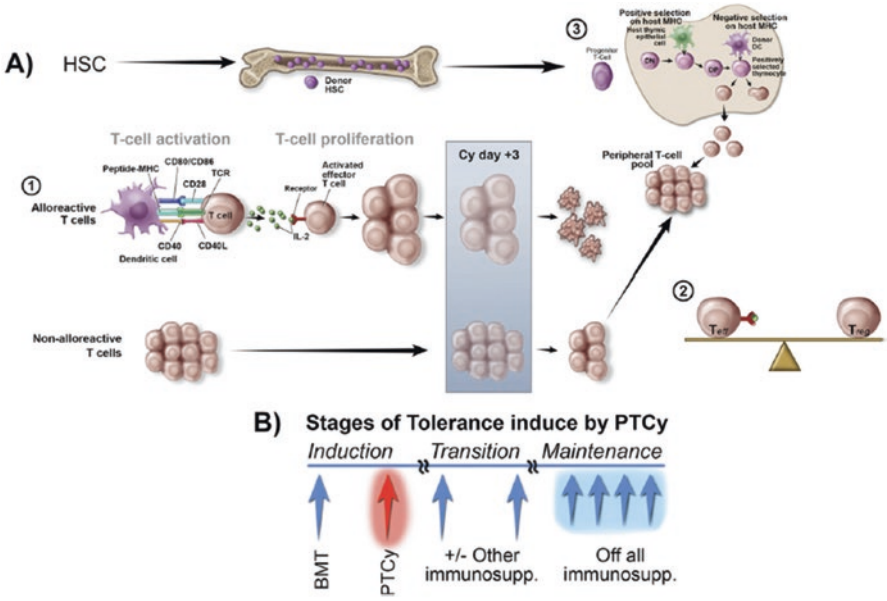

Fig. 8.4 Mechanism for the induction of post-transplantation tolerance by cyclophosphamide [20]. (**a**) Mechanism of tolerance induction with post transplantation cyclophosphamide as GvHD prophylaxis (**b**) Sequential stages of tolerance induced by cyclophosphamide

blood counts, lymphoid subsets, NK cells and the bidirectional immune tolerance after tapering immunosuppressive agents are key elements for the success of the procedure (Fig. 8.4) [20].

The first experience with this approach was published by O'Donnell et al. and included 10 patients with advanced hematological malignancies who received cyclophosphamide, fludarabine, and ATG followed by BM of haploidentical relatives. All patients received a high dose of CY (50 mg/kg/day) on day +3, and MMF and tacrolimus from day +4; 8 of 10 patients achieved donor engraftment (2 patients presented graft rejection). Six of 10 were alive at 284 days and 5 of them were alive and leukemia-free. Six patients developed grades 2 and 3 acute GvHD, (3 for each group). Four patients relapsed and 3 of them died as consequence of relapse whereas infection was the cause of death for the remaining patient [21]. The experience was extended in a cohort of 68 patients transplanted in Baltimore and Seattle. All of them received nonmyeloablative cyclophosphamide, fludarabine, and TBI followed by BM infusion. The only difference between the two transplant centres was the number of days that CY was administered; 28 patients received 1 day of CY (Seattle group) whereas 40 patients received 2 days (Baltimore group) [22]. Overall, 13% of patients had primary graft failure and CI of grades 2–4 and 3–4 acute GVHD were 34% and 6% at 200 days, respectively, without differences between one or 2 days of CY. CI of chronic GVHD was 5% for patients who had received two doses of CY and increased to 25% if they had received only one dose. No other outcome

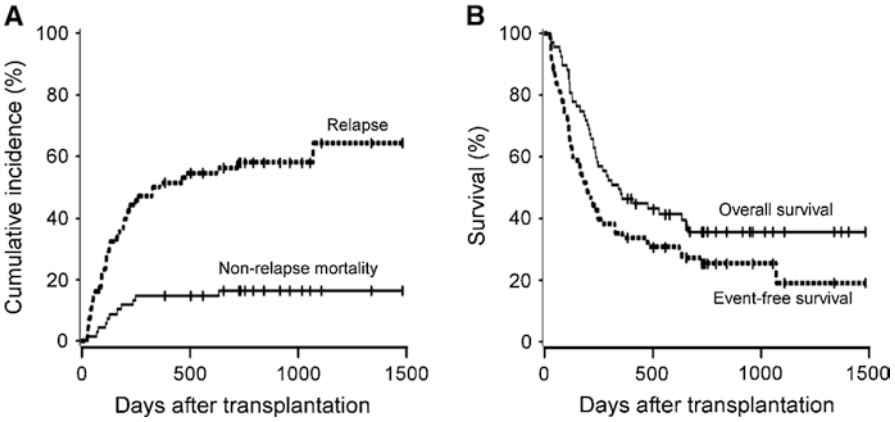

Fig. 8.5 (**a**, **b**) Haploidentical T-cell replete BMT with post-transplant high-dose cyclophosphamide: (**a**) cumulative incidence of relapse and non relapse mortality; (**b**) Probability of overall survival and event free survival [22]

differences were shown. NRM was 4% at 100 days and 15% at 1 year. CI of relapse were 51% and 58% at 1 and 2 years, respectively (Fig. 8.5a). OS and EFS were 46% and 34% at 1 year, and 36% and 26% at 2 years (Fig. 8.5b).

Since these first studies showed a high incidence of disease relapse, Solomon et al. designed a MAC regimen followed by unmanipulated PBSC infusion and post-transplant CY. This prospective study enrolled 20 patients with 14 of 20 being AML or ALL [23]. Conditioning was fludarabine, busulphan and cyclophosphamide followed by administration of PBSC (median number of CD34, $5x10^6$/Kg). High dose of CY (50 mg/kg/day) was administered on days +3 and +4, followed by tacrolimus and MMF from day +5. Engraftment occurred in all patients with rapid full donor chimerism. CI of grades 2–4 and 3–4 acute GVHD were 30% and 10%, respectively. CI of overall and extensive chronic GVHD were 35% and 5%, respectively. NRM at 100 days and 1 year was only 10% without deaths in the standard-risk group. OS, DFS, and relapse at 20 months in the high-risk were 69%, 50% and 40%, respectively, and these outcomes in the standard risk were 88%, 67% and 33% [23].

In a recent retrospective analysis, McCurdy et al. have reported 372 patients with a variety of hematological malignancies, mainly AL or lymphoblastic lymphoma (114 patients, 31%). Three years OS and progression free survival (PFS) for all patients were 50% and 40%, respectively. CI of NRM, grades 2–4 and 3–4 acute GVHD at 180 days were 8%, 32%, and 4%, respectively. The sub-analysis of intermediate-risk AML (n = 64) showed a 41% PFS and 45% CI of relapse at 3 years of follow-up [24].

Also administering MAC, the Genova group published its experience with unmanipulated haploidentical BM transplantation. Twenty-seven patients were treated with thiotepa, fludarabine and busulphan (TBF), a regimen previously described in

the context of cord blood transplantation [25]. In 8 elderly patients (>60 years) the busulphan doses were reduced from three to two or one day and 15 with fludarabine and TBI. High dose CY was administered on days +3 and +5, cyclosporine and MMF were started the day of stem cell infusion and day +1, respectively [26].

AML and ALL were the main diagnosis (25 and 12 of 50, respectively). CI of grades 1, 2 and 3 acute GVHD were 21%, 6% and 6%, respectively, without any patient developing refractory acute GVHD. CI of chronic GVHD was 26% (16% minimal and 10% moderate chronic GVHD). CI of transplant related mortality (TRM) at 6 months was 18%, being notably low (9%) for patients in CR at transplant. CI of relapse was 22% at 20 months (17% for patients in CR and 33% for patients in relapse). OS and DFS at 18 months were 62% and 51%, respectively [26].

An update of T-cell replete haploidentical transplants conditioned with TBF included 148 patients (76 with AML or ALL), again with encouraging results. CI of grades 0–1, 2, 3–4 acute GvHD were 82%, 14% and 4%, respectively, and CI of chronic GVHD was 20% 10 months. Age above 60 years was associated with poor results in terms of acute and chronic GVHD; in elderly patients (>60 years) CI of acute GVHD and chronic GVHD were 28% each compared to 14% and 18% in younger patients, respectively. NRM was 14% at 4 years. CI of relapse was 27% (11% for first CR, 26% for second CR and 40% for patients with active disease). OS were 77%, 49% and 38% for patients in first CR, second CR and active disease, respectively (Fig. 8.6) [27].

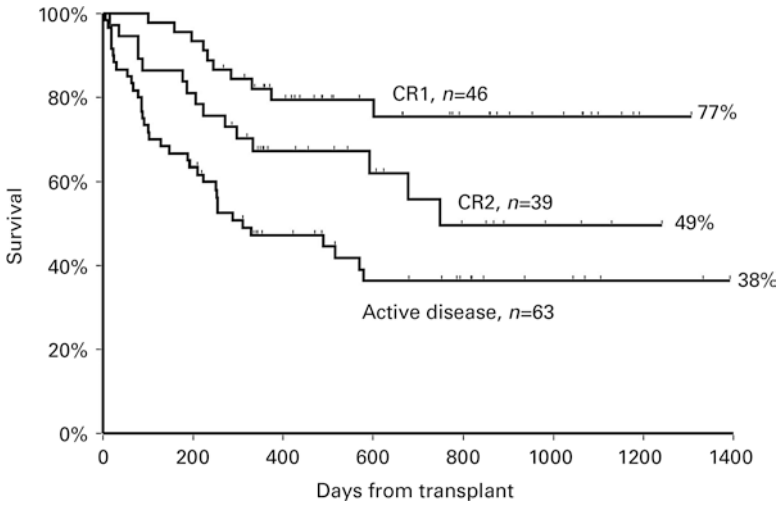

Fig. 8.6 Haploidentical T-cell replete BMT with post-transplant high-dose cyclophosphamide: The Genoa experience. Overall survival for patients in complete remission (first and second) and active disease [27]

8.5 Comparison Between Haploidentical Related Donor Transplantation and Other Modalities

In a retrospective analysis, Raiola et al. analyzed 459 patients with advanced hematologic malignancies, 40% of them with AL, transplanted from different donors and stem cell sources: HLA-identical siblings (SIB), matched unrelated donors (MUD), mismatched unrelated donors (mMUD), umbilical cord blood (UCB) and haploidentical relatives (HRD). CI of grades 2–4 acute GVHD were 31% in the SIB group, 21% in the MUD, 42% in the mMUD, 19% in the UCB and 14% in HRD group ($p < 0.001$). No differences were observed in the CI of grades 3–4 acute GVHD. CI of chronic GVHD in the five groups were 29%, 22%, 19%, 23% and 15% (p 0.053), respectively. No significant differences were observed between the transplant modalities in terms of NRM and relapse. DFS at 4 years were 32%, 36%, 34%, 33% and 43%, respectively and OS were 45%, 43%, 40%, 34% and 52%, respectively (Fig. 8.7) [28] (Table 8.1).

Another comparative study analyzed 227 patients conditioned with fludarabine and melphalan in patients transplanted from a matched related (MRD) (38%) or MUD (48%) donor, with the addition of thiotepa in HRD procedures (14%). Similar results were observed in the three groups: PFS for patients transplanted from MRD, MUD and HRD were 52%, 42%, 43% at 1 year, and 36%, 27%, 30% at 3 years, respectively. Also, no differences were found when the patients in the same disease-stage were compared. CI of grades 2–4 acute GVHD were similar after MRD, MUD and HRD, 24%, 19% and 26%, and CI of grades 3–4 were 4%, 4% and 0%, respec-

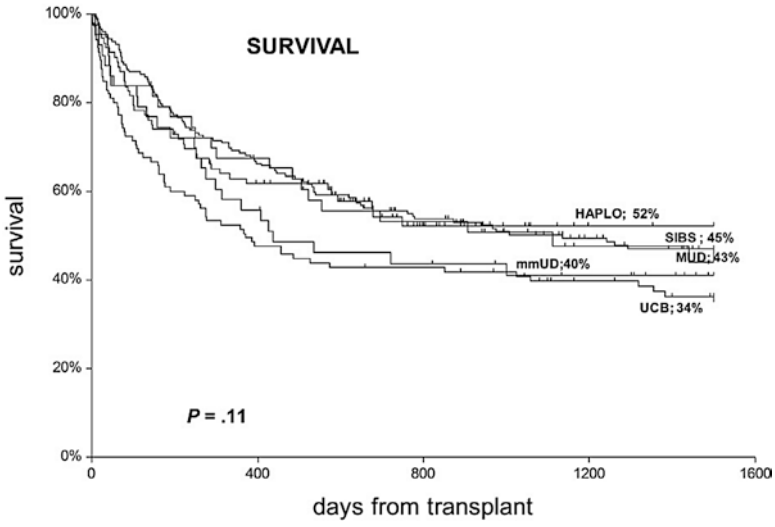

Fig. 8.7 Haploidentical T-cell replete BMT with post-transplant high-dose cyclophosphamide: The Genoa experience: Probability of Overall survival according the type of donor [28]

Table 8.1 Main outcomes depending on the type of transplantation

	Raiola et al. [28] 4 years follow up						DiStasi et al. [29] 1 year follow up (except chronic GvHD that was calculated at 3 years)				Wang et al. [31] 3 years follow up		
	SIB	MUD	mMUD	UCB	HRD	p value	SIB	MUD	HRD	p value	SIB	HRD	p value
OS	45%	43%	40%	34%	52%	0.1	–	–	–	–	82%	79%	0.36
DFS	32%	36%	34%	33%	43%	0.2	52%	42%	43%	0.120	78%	74%	0.34
NRM	24%	33%	35%	35%	18%	0.1	20%	35%	24%	0.099	8%	13%	0.13
Relapse	40%	23%	30%	30%	35%	0.89	28%	23%	33%	0.750	15%	15%	0.98
2–4 acute GvHD	31%	21%	42%	19%	14%	0.001	31%	29%	29%	0.709	13%	36%	0.001
3–4 acute GvHD	7%	3%	9%	1%	4%	0.1	11%	6%	0%	0.044	3%	10%	0.004
Extensive chronic GvHD	29%	22%	19%	23%	15%	0.053	31%	21%	11%	0.125	2%	12%	0.001

OS overall survival, *DFS* disease free survival, *NRM* non relapse mortality, *GvHD* graft versus host disease

tively (p 0.68). Chronic GvHD incidences were not different in the three groups 46%, 42% and 24% (p 0.51) and 29%, 23% and 17% of extensive only (p 0.91) [29] (Table 8.1).

A recent retrospective study compared 192 HRD and 1982 MUD transplants. All patients had AML but other aspects were diverse such as conditioning regimen, stem cell source (BM in HRD and PBSC in MUD). OS at 3 years after HRD was comparable to MUD, 45% vs 50% after MAC and 46% vs 44% after RIC (Fig. 8.8). Acute and chronic GVHD were lower after HRD vs MUD. Thus, CI of grades 2–4 acute GVHD in HRD and MUD were 16% vs 33% after MAC and 19% vs 28% after RIC. At 3 years, chronic GVHD in HRD and MUD were 30% vs 53% after MAC and 34% vs 52% after RIC at 36 months. The lower incidence of chronic GVHD after HRD could be explained because patients received BM and post-infusion CY, two variables associated to low incidence of this complication. No differences in NRM or relapse were observed in the two transplant groups if patients received MAC. When comparing to MAC, RIC led to decreased NRM and increased relapse incidence [30].

Another non-randomized prospective study compared haploidentical versus HLA-identical sibling transplantation in patients with AML. Four-hundred fifty patients with AML in first CR were analyzed. These patients were treated according the Peking approach previously described [18]. At 3 years, no differences were observed in haploidentical versus HLA-identical sibling transplantation in terms of DFS (74% vs 78%) and OS (79% versus 82%) (Fig. 8.9); 3 years CI of NRM were 13% and 8%, respectively. Relapse incidence was identical (15%) in the two groups [31] (Table 8.1).

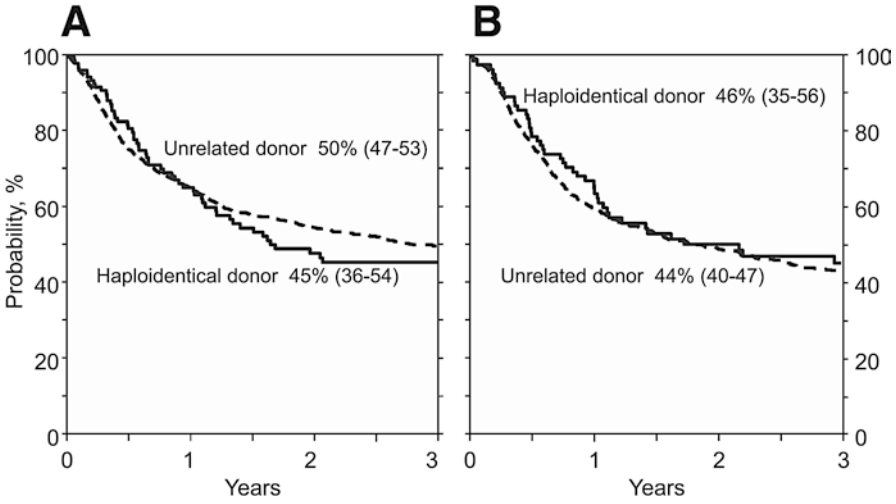

Fig. 8.8 Probability of overall survival after haploidentical or unrelated donor transplantation [30] conditioned by MAC (**a**) and RIC (**b**) transplants

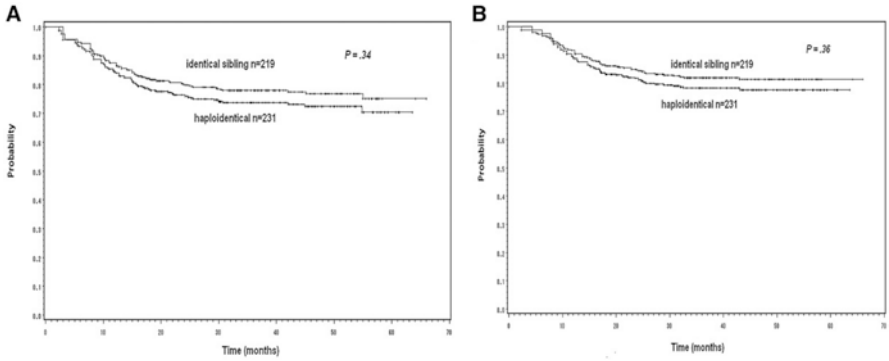

Fig. 8.9 Probability of DFS (**a**) and OS (**b**) after haploidentical or identical sibling donor transplantation [31]

8.6 Conclusions

The initial drawbacks in the haploidentical donor transplantation setting were the high incidences of graft failure and NRM. The infusion of megadoses of CD34 + `cells with lowest amounts of T-cells led to fast engraftment and low incidence of GVHD [6, 32, 33]. However, this approach associated with heavily impaired immune recovery and a high incidence of infections causing NRM. Disease relapses were also frequent after T-cell depleted transplants. Better results were observed, mainly in children, with depletion of specific B and T cell populations such as CD3 and CD19 lymphocytes.[6, 32, 33].

The worldwide application of haploidentical transplants has been the consequence of the strategy pioneered by the Baltimore, Seattle and Genoa groups consisting of T-cell replete BM transplants with high dose CY after stem cell infusion. This approach is also feasible if PB is the stem cell source. Low incidence of GVHD, NRM and promising results in terms of OS and DFS were achieved [6, 32, 33]. Several studies have compared this strategy to HLA-matched transplantation from HLA-identical siblings or MUD demonstrating similar outcomes [34].

In summary, haploidentical transplantation is a valid option for patients who lack an HLA-sibling donor with results so far comparable to those achieved with other alternative stem cell sources and with the fastest availability.

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Chapter 9
Outcome of Haploidentical Stem Cell Transplantation in Patients with Lymphoma

Rocío Parody and Anna Sureda

9.1 The Role of Allogeneic Hematopoietic Stem Cell Transplantation in Lymphoproliferative Disorders

9.1.1 Scientific Background

More than 50% of patients with non-Hodgkin (NHL) and Hodgkin Lymphoma (HL) can be cured with conventional chemotherapy (CT) with or without involved field radiotherapy (RT). However there is a relevant proportion of patients, at least 30–40%, who will relapse, or eventually not respond to first line therapy; these patients have been the focus of innovative and more intensive strategies to try to overcome their dismal prognosis [1–4].

The role of hematopoietic stem cell transplantation (HSCT) has been extensively studied in both HL and NHL. Autologous stem cell transplantation (Auto-HSCT) is generally indicated as consolidation therapy in second complete remissions (CR) for patients with HL or diffuse large B cell lymphoma (DLBCL) who relapse after first line therapy or demonstrate to be primary refractory [3, 4]. Auto-HSCT is considered as consolidation therapy after a first CR or partial remission (PR) in patients with mantle cell lymphoma (MCL) and in patients with peripheral T cell lymphoma (PTCL) even in the absence, in the latter ones, of prospective clinical trials demonstrating the benefit of Auto-HSCT in front of conventional chemotherapy [5–7]. Overall, 3-year progression free survival (PFS) and overall survival (OS) after Auto-HSCT are estimated to be around 40–60% and 50–80% respectively, significantly higher with respect to salvage conventional CT at least in some histologies. Non-relapse mortality (NRM) of Auto-HSCT remains low (<10%), in contrast with that of allogeneic stem cell transplantation (Allo-HSCT) [8–10].

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The rationale for including Allo-HSCT in the treatment strategy of patients with lymphoproliferative disorders is based in the existence of the 'so called' graft versus lymphoma (GVL) effect. Unfortunately, NRM after allo-HSCT has been exceedingly high for many years in the lymphoma setting and this fact has eventually precluded a more meaningful clinical development in this area [9, 10]. First data on myeloablative (MA) conditioning regimen in heavily pre-treated HL patients reported dismal outcomes with a high 3-year NRM of 61% and a low PFS around 15% [11]. A similar although somehow better scenario was initially reported for follicular lymphoma (FL) patients, with a 4-year NRM of 38% [12]. The Center for International Blood and Marrow Transplant Research (CIBMTR) reported on the results of 114 MA Allo-HSCT for lymphoma performed in the 90s; With a lower NRM than previously reported (22% at 3 years), the main problem was a high rate of relapse/progression (52% at 3 years) and a dismal survival (3y–OS and PFS of 33 and 25%, respectively) [13]. With this background, allo-HSCT was relocated to refractory or relapsed patients after auto-HSCT in both HL and NHL, and even nowadays, in the setting of new immunochemotherapy protocols, it is debatable when to proceed to it [14–16]. Nevertheless, different retrospective studies have shown that availability of a donor has a significant impact on both OS and PFS for relapsed HL after auto-HSCT [17]. Moreover, the introduction of less intensive conditioning regimens and a better supportive care over the last decades, together with an accurate selection of patients, have led to better overall outcomes after the procedure [18–21].

Reduced intensity conditioning (RIC) regimens have the aim to get a balance between the cytotoxic effect of conditioning drugs and the immune effect of donor T cells, also known as GVL effect. These regimens have widened the indications of allo-HSCT to older patients, heavily pretreated patients or patients with comorbidities, the daily reality of the vast majority of the patients with lymphoma. In a recent meta-analysis of allo-HSCT in HL, the most important variables associated with better outcomes where chemosensitive disease, a prior auto-HSCT and the period study; A decrease of 5–10% in NRM and relapse together with a 15–20% higher PFS and OS were found in studies that initiated patients' accrual in 2000 or later compared with earlier studies [22]. The European Bone Marrow Transplantation (EBMT) group published the first comparative retrospective analysis between RIC and MA allo-HSCT in patients with relapsed/refractory HL. RIC group had a significantly lower 1 year-NRM (23% vs 46% in MA), a better 5 year OS (28 vs 22%) and a trend for a higher PFS [23]. In line with this study, in which donors were predominantly matched siblings, the CIBMTR published worse outcomes in the setting of allo-RIC from unrelated donors (URD), with 1-year NRM of 30% although similar 2-year PFS and OS of 20% and 37%, respectively, with respect to HLA identical sibling donors [20]. More favorable NRM data came from the MD Anderson patient cohort (n = 58 patients, 48.2% with refractory relapse), where day 100 NRM and 2-year NRM were 7% and 15%, respectively, with no significant difference noted between related and URD donors [24]. A recently published study from the EBMT registry has more up-to-date outcomes after allo-HSCT comparing MA and RIC regimens (study period 2006–2010) with a follow up close to 5 years.

There were no significant differences neither in NRM (11% at 2 years for both groups) nor OS (73 and 62% at 2 years, respectively, p = 0.13) and relapse rate (RR) was slightly lower in the MA group (41% versus 52% at 24 months, P = 0.16), which translated into a trend for a better PFS (48% versus 36% at 2 years, p = 0.09) in this group of patients [25]. As relapse rate continues to be a major problem and the main cause of transplant failure, regardless of conditioning or donor type, the intensity of conditioning regimens still represents a challenge in the setting of relapsed lymphoma undergoing allo-HSCT. Whereas there is a plateau for NRM at 2 years of around 20%, relapse risk continues to steadily increase over time with no apparent plateau up to 3 years after transplant, being at this point higher than 40% in some series [22, 26–32]. Apart from conditioning intensity, the GVL effect is essential for reducing disease progression as it has been demonstrated after the administration of donor lymphocytes (DLI). However some issues must be considered depending on the histological subtype, as it will be discussed later on [33].

For those patients who relapse after allo-HSCT, OS falls to approximately 33% at 3 years and 23% at 5 years, although it may be widely variable depending on the time to relapse and lymphoma histology; early relapses (<1 month after transplant) do significantly worse than later ones (> 6 months) (1-year OS of 24% vs 77%) [34]. Lymphoma histology is another prognostic factor; 3-year OS is estimated to be 16% for patients with aggressive NHL relapsing after allo-HSCT vs 40% for indolent NHL and 47% for patients with HL. Others prognosis factors for OS of relapsed lymphoma after allo-HSCT are good Karnofsky performance status (KPS), normal lactate dehydrogenase, early stage disease (stage I-III) and isolated extranodal organ involvement at the time of relapse [35].

Overall, retrospective studies of allo-RIC report a 2–3 years-PFS of 20–51%, including all donor types, and OS of 37–64% for patients with HL [26, 30, 36–38]. Overall outcomes are better for patients with indolent NHL, with 2–3 year PFS rates of 54–59% and 65–81%, respectively, and the worse outcomes have been reported for high-grade NHL, with a 2–3 years- PFS and OS of 35–44% and 45–49%, respectively [27–29, 39, 40]. Most important risk factors affecting PFS and OS have been extensively analyzed in retrospective analysis and are related to disease status before allo-HSCT, chemosensitive disease, extranodal involvement, poor KPS, female donor for a male recipient and occurrence of chronic GVHD [17, 20, 23, 26]. The key issues that have to be taken into consideration to maximize both PFS and OS in patients with lymphoma undergoing allo-HSCT are based on the appropriate selection of salvage therapy after auto-HSCT and before allo-HSCT, accuracy in the conditioning regimen and donor selection, and post-transplant management.

Finally, published results of haploidentical stem cell transplantation (haplo-HSCT) in lymphoma are still limited compared with those of matched sibling and URD (Table 9.1), and are mostly based on the use of nonmyeloablative (NMA) or RIC conditioning within the platform of T-replete graft with immunosuppression after SCT (against the classical strategy of T-cell depleted grafts and CD34 mega-dose following Perugia protocol, mostly applied for acute leukemia). Increasing experience suggest a strong therapeutic benefit, even comparable with classical donors, as it will be discussed below.

Table 9.1 Haploidentical transplantation in lymphoma: review of the literature

Study	n	Study period	Lymphoma type	Conditioning	2-year OS	2-year PFS	1-year NRM	RR
Dodero	24	2003–2007	HL&NHL	Tt-Cy-Flu-Alem-TBI CD34 + &DLIpost	44	45	15	50
Luznik*	23	1999–2006	HL&NHL	Flu-Cy-TBI	36	34	15	51
Kasamon	68	<2010	HL&NHL	Flu-Cy-TBI	–	35(1y)	15	55–60
Burroughs	28	1998–2007	HL	Flu-Cy-TBI	58	51	5	40
Raiola	26	2009–2011	HL	Flu-Cy-TBI	77	63	4	31
Castagna	49	2009–2012	HL&NHL	Flu-Cy-TBI	71	63	16	18.7
Kanakry	69	2009–2013	NHL	Flu-Cy-TBI	76	60	10	27
Kanakry	83	2009–2013	B-NHL	Flu-Cy-TBI +Rituximab post	86 (1y)	71(1y)	8	20
Kasamon Indolent Aggressive	148	2003–2013	NHL	Flu-Cy-TBI	47 (3y) 46 (3y)	39 (3y) 37 (3y)	12 (whole group)	40 (3y) 38 (3y)
Kanate	185	2008–2013	HL&NHL	Flu-Cy-TBI	63	50	11	36
Gosh	180	2008–2013	HL&NHL	Flu-Cy-TBI	65	51	10	37
Brammer	22	2009–2015	HL&NHL	Flu-Mel/Tt vs TBI	54	54	19	27
Bacigalupo*	15	2010–2014	HL&NHL	Tt-Flu-Bu/TBI-Flu	38–49	–	14 (4y)	26–40
Dietrich	59	2007–2013	NHL	Variable	56	50	23 (2y)	27
Martinez	98	2010–2013	HL	Variable	67	43	17	39

OS Overall Survival, *PFS* Progression-free Survival, *NRM* Non-relapse mortality, *RR* Relapse Rate, *HL* Hodgkin Lymphoma, *NHL* non-Hodgkin lymphoma, *Tt* Thiotepa, *Cy* Cyclophosphamide, *Flu* Fludarabine, *Alem* Alemtuzumab, *TBI* Total Body Irradiation, *DLIpost* Donor Lymphocyte Infusion post transplantation, *Bu* Busulfan
*overall outcomes for the whole series including myeloid malignancies

and relapse rate in specific subtypes of lymphoma (n = 2611 patients included). In the setting of RIC /NMA protocols, chronic GVHD was associated with a significant lower relapse rate in FL and MCL, but not on other subtypes as HL, DLBCL and PTCL. The occurrence of both acute and chronic GVHD was associated with a lower risk of relapse in FL and MCL. The GVL effect has also been demonstrated in the setting of DLI for FL and MCL, results were less conclusive for both HL and PTCL and the authors could not demonstrate any relationship at all for DLBCL

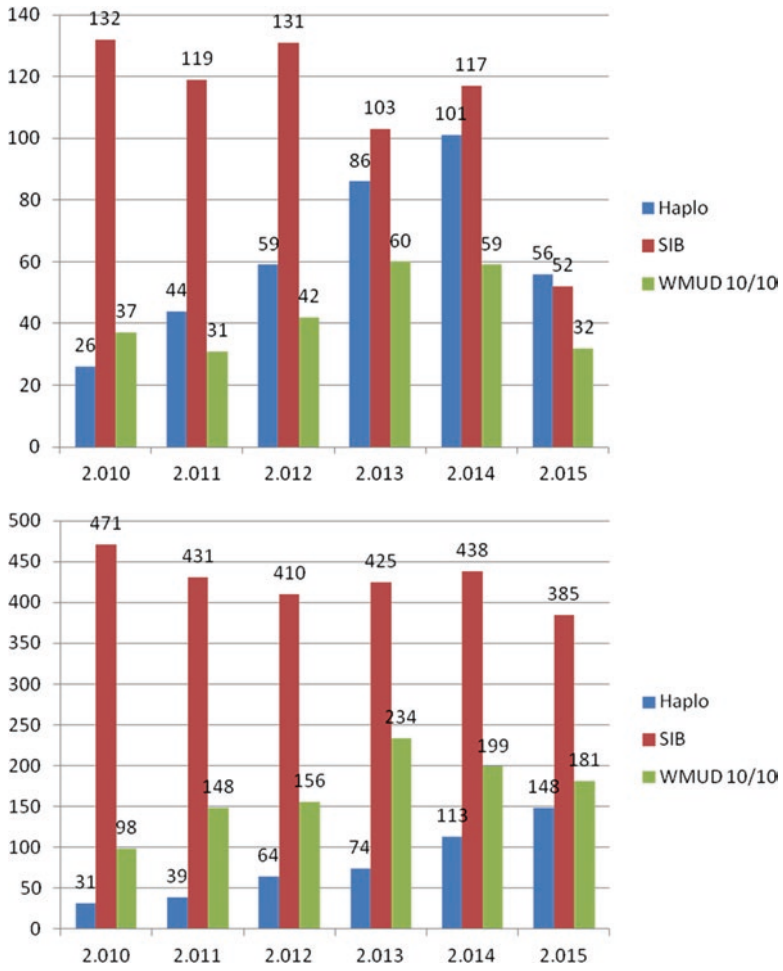

Fig. 9.1 (**a**) Donor type in HL (EBMT data). There has been a significant increase in the number of patients being allografted from a haploidentical donor in recent years. (**b**) Donor type in NHL (EBMT data). As opposed to HL, HLA identical siblings and WMUD still represent the two most frequently used donors in NHL patients; haploidentical donors nevertheless have also increased in numbers over time

9.1.2 EBMT HSCT Activity for HL and NHL

According to the recently published EBMT activity [41], NHL accounts for 30% of indications for auto-HSCT, 3 more times than HL (9.5%), whereas less than 10% of both NHL and HL are indications for allo-HSCT (8 and 3%, respectively). Consecutive retrospective multicenter studies have been published over the time showing overall outcomes of auto and alloHSCT in the setting of lymphoproliferative diseases, as it was mentioned in the previous section [12, 23, 25, 26, 36].

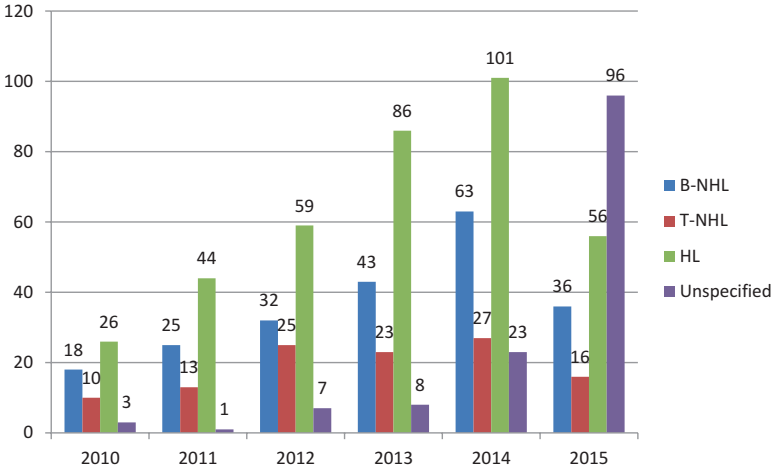

Fig. 9.2 Haplo-HSCT by lymphoma sutype: Hodgkin Lymphoma is the most common subtype from 2010 to 2015 in Europe (EBMT data)

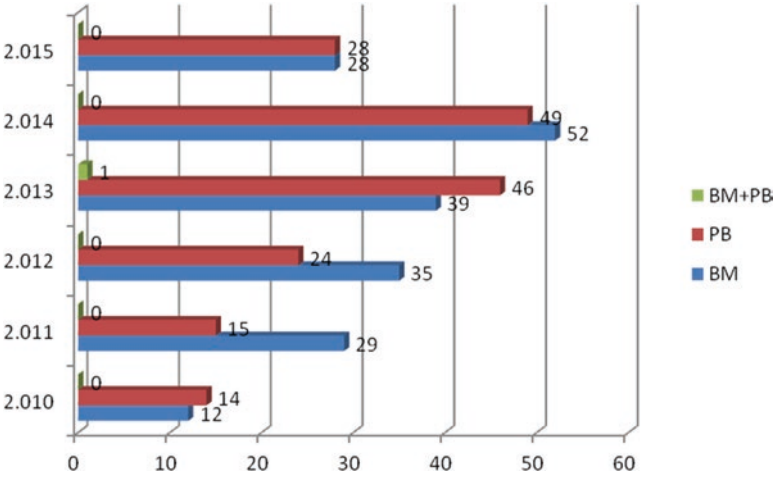

Fig. 9.3 Stem cell source in haplo-HSCT, from 2010 to 2015 (EBMT data)

With respect to donor type, there has been a switch along the last 5 years (Fig. 9.1a, b): In HL, haplo-HSCT has replaced URD as stem cell transplantation source (number of haplo-donor has duplicated in 5 years). In NHL, although activity in haploHSCT is 5 times higher, it is still the third donor type in frequency.

In the setting of haploHSCT, HL represents the most frequent histological subtype (Fig. 9.2), whereas peripheral blood (PB) is increasingly used as stem cell source (Fig. 9.3). RIC protocols are the most frequently used ones, as the experience

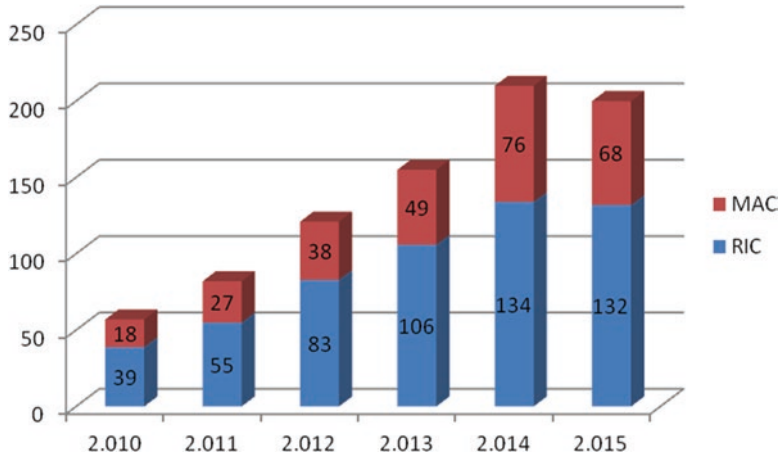

Fig. 9.4 Conditioning intensity in haplo-HSCT for lymhoma (EBMT data). Reduced intensity conditioning protocols are the most frequently used in Europe (78% in HL patients/57% in NHL patients)

with MA was associated with unexpectedly high NRM for allo-HSCT overall in the setting of lymphoproliferative diseases (Fig. 9.4).

9.1.3 The Graft Versus Lymphoma Effect

The GVL effect is the rationale basis of allo-HSCT. The first evidence of this immunological effect in the setting of lymphoid malignancies was reported by 1990 [42]. A comparative analysis between auto-HSCT and allo-HSCT in a series of 118 patients with lymphoid malignancies showed a probability of relapse of 18% after allo-HSCT vs 46% after auto-HSCT in chemosensitive patients. However, the higher NRM associated with allo-HSCT (mostly due to GVHD) did not justify the indication of allo-HSCT instead of auto-HSCT outside from prospective clinical trials. Some years later, Peggs et al. [38] reported the favorable results of DLI in a total of 16 out of 49 patients with multiply relapsed HL (median of 5 prior treatments) after allo-HSCT in both related and unrelated settings, with documented response in 9 (56%) and a 4 year-PFS of 39%. More recently, the same UK group analyzed the results of 76 patients with relapsed/refractory HL after allo-RIC with alemtuzumab as *in vivo* T-cell depletion. DLI was effective in reverting mixed to complete donor chimerism in 86% of the patients and durable responses to DLI were observed in 79% of patients treated because of disease relapse [43].

Subsequently, other studies have reported similar results after immunosuppression withdrawal and DLI in relapsed lymphoma after allo-HSCT, but a potential risk of moderate-severe GHVD, that would negatively affect survival, must be taken into consideration [44–48]. A recent CIBMTR study explored the association of GVHD

patients. The low proliferation rate of the former types could explain, at least in part, a higher effectiveness of immunotherapy [33].

With these caveats in mind, promising results have been shown with novel therapeutic approaches augmenting the anti-tumor efficacy of DLI while dissociating the GVL effect from GVHD: antiCD20 and DLI, brentuximab vedotin plus DLI (overall response rate of 69.2%, 1 year OS 81.2%), bendamustine and DLI (overall response rate of 55%, with 1-year-OS 70% in responders) [35, 49–51].

In the setting of haplo-HSCT, both efficacy and toxicity of DLI have been less commonly tested. The Baltimore series [52] supports the use of haplo-DLI taking into consideration the results obtained in 40 patients, including 11 patients with lymphoma, who received one to four consecutive DLI at escalated doses (first haplo-DLI at a dose of 1×10^5 CD3+ cells/kg). Unexpectedly, a low rate of acute GVHD was observed (25%), grade III-IV in 60% of cases. Twelve (30%) patients achieved a CR with a median duration of response of 11.8 months highlighting the fact that cytoreduction before DLI was associated with better response rates. Recently, a short series of 43 patients, including 10 patients with HL, received a median of 2.6 DLI at escalating doses (from 1×10^5 to 1 x10^7/kg) in the setting of relapse after haplo-HSCT, usually after salvage CT. For HL, the efficacy of DLI was significantly higher with a 70% of overall responses, versus 33% in acute leukemia with hematological relapse. Moreover, a lower incidence of GVHD and a higher 2-year OS was observed in HL with respect to the other group (10% vs 17% and 80% vs 19% respectively) [53].

9.2 Haploidentical Hematopoietic Stem Cell Transplantation (Haplo-HSCT) and Lymphomas

9.2.1 Conditioning Regimen and Immunoprophylaxis in Haplo-HSCT for Lymphoma

The most common conditioning regimen in this setting is the Baltimore pioneer protocol which is a NMA combination with cyclophosphamide (Cy) 14.5 mg/kg on day −5 and day, − 6, fludarabine (Flu) 30 mg/m2 from day −2 to day −6 and low dose TBI (2 Gy) on day −1, with post-transplant Cy 50 mg/kg on days +3 and +4, a calcineurin inhibitor (CNI), usually tacrolimus, and mycophenolate mofetil (MMF) [54–59], and T repleted allograft. This regimen was initially described by Luznik et al. [55] in 68 patients with high-risk hematological malignancies (including 13 HL and 10 NHL), using bone marrow (BM) as stem cell source. One-year NRM was 15% and risk of both acute and extensive chronic GVHD was low (34 and 5% respectively), However, relapse rate was high (51%) and this was translated into a 2 year-OS and DFS of 36% and 26%, respectively, mainly attributed to the poor risk features of the underlying disease. Interestingly, patients with lymphoid malignancies had better outcome in terms of event free survival (EFS) than those with

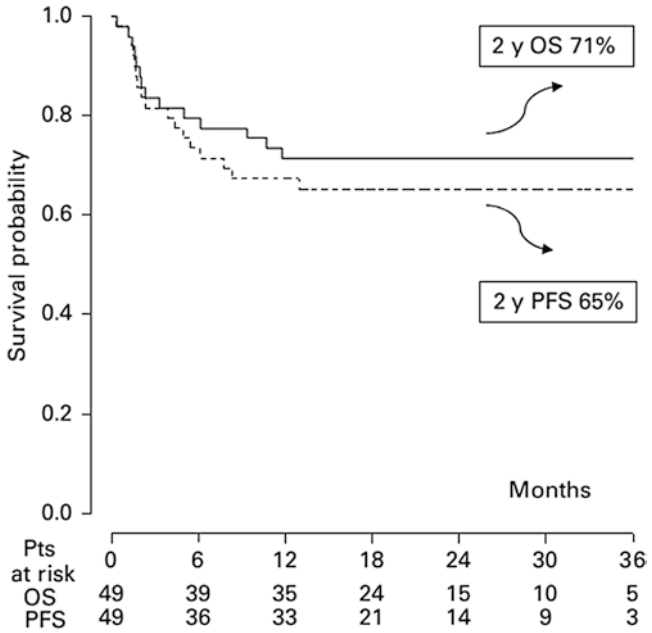

Fig. 9.5 OS and PFS after NMA haploSCT for Lymphoma (Castagna et al.)

myeloid malignancies (p: 0.02). Since more recently significantly better overall outcomes have been shown with this NMA conditioning, the initially reported poor results are not supported at least in certain subgroups of patients: Castagna et al. found a 2-year OS and PFS of 71% and 65% in a series of 49 patients with lymphoma (Fig. 9.5).

A melphalan-based conditioning with fludarabine 160 mg/m2 and melphalan 100 mg/m2 (instead of Cy) and thiotepa 5 mg/kg or 2 Gy TBI was proposed by the MD Anderson group with somewhat better outcomes than the previous one [60]. They presented the results of a short series of 22 patients (11 patients with NHL, 7 patients HL, 4 chronic lymphocytic leukemia), 67% not in remission at transplant, with a 2-year PFS and OS of 54% (no differences between NHL and HL), 1-year NRM of 19%, and 3 year-relapse rate of 27%.

More recently, and looking for more intensive conditioning protocols, the Spanish group proposed the replacement of low dose TBI by IV busulfan (BU), ranging from 3.2–9.6 mg/kg total dose (1–3 doses) depending on the intensity required, in the setting of HL [61, 62]; results were comparable with the MD Anderson series. Finally, the Italian group proposed the use of a MA conditioning, based on the combination of fludarabine and BU (6.4–9.6 mg/kg total dose) plus thiotepa (instead of Cy), 10 mg/kg total dose, Overall 148 patients were included, 92 receiving this regimen (15 lymphomas), 42% with active disease at transplant; results were promising with overall outcomes in terms of a low NRM (13%) and 2 year-OS (from 77% for patients in first CR to 38% for patients in relapse) [63, 64].

Although initial studies used T-replete BM with the aim of avoiding chronic GVHD, there is an increasing experience with PB, with apparent no disadvantages with respect to BM [65–68]; However comparative studies in this setting are scarce. Raj et al. reported on the results of 55 patients with high-risk malignancies (25 patients with lymphoproliferative diseases) and T-replete PB haplo-HSCT. The 2-year cumulative incidence of chronic GHVD was 18%, whereas OS and EFS at 2 years were 48% and 51%, respectively. The 2-year cumulative incidences of NRM and relapse were 23% and 28%, respectively [65].

Chinese groups have substituted Cy after haplo-HSCT by antithymocyte globulin (ATG) [69–72]. They have recently updated their experience with ATG as GVHD prophylaxis in a BU-based conditioning regimen (mostly myeloablative) and PB as stem cell source [72] in a series of 130 patients (18 patients with lymphoproliferative disease). Cumulative incidence of II-IV acute GVHD was 33.4%, overall and extensive chronic GVHD were 38.6% and 16.5%, respectively, with a 3 year- NRM of 24.1% and 3 year- OS and PFS of 45.6% and 44.2%, respectively. Relapse rate varied from 26.9% in patients in CR at transplant to 59.3% if not in complete remission. It was noteworthy that infectious complications were the leading cause of NRM accounting for 27.8% of overall causes, most probably in relation to the use of ATG. On the other hand, an Italian group conducted a phase II prospective clinical trial that included 121 patients with high risk hematological malignancies (21 patients with HL and NHL) undergoing an haplo-HSCT with a novel platform based on treosulfan and fludarabine as MA regimen, together with ATG Fresenius, rituximab, sirolimus and MMF as GVHD prophylaxis, with PB as stem cell source in all cases. With respect to the previous study, they found a similar incidence of II-IV acute GVHD of 35% and chronic GVHD of 47%, but worse overall outcomes with 3 year- NRM of 31%, relapse incidence of 48% and 3 year- OS of 25% [73].

The experience with haplo-HSCT in lymphoproliferative disorders in paediatrics is limited due to the fact that this indication is very infrequent [74–77]. Two strategies of haplo-HSCT have been used for paediatrics, based on *ex vivo* T cell depletion (specifically removal of donor αβ T and B cells), on one hand, and T replete haplo-HSCT with Cy post HSCT, on the other, although lymphomas are rarely included. An Italian multicenter study that included 33 children undergoing T replete haplo-HSCT (5 patients with lymphoma) showed promising outcomes with 1-year OS and PFS of 72% and 61% respectively, a low NRM of 9%, together with low rates of acute and chronic GVHD [77]; relapse was also the main cause of death in this patient population.

9.2.2 Haploidentical Hematopoietic Stem Cell Transplantation and Hodgkin Lymphoma

The most relevant study that established the basis of haplo-HSCT for HL was published in 2008 by Burroughs et al. [56]. In a population of heavily pre-treated HL patients (n = 90, median of four prior lines of therapy, 21% chemorefractory

patients), results of haplo-HSCT compared favorably to those of HLA identical siblings and URD. Two-year cumulative incidence of relapse/progression and PFS were 40% and 51%, respectively for haplo-HSCT, versus 56% and 23% for HLA-matched related and 63% and 29% for URD. Although the retrospective nature of the study precludes supporting consistently that a haploidentical donor is better than others, it can at least be concluded that it is a valid alternative option.

More recently, the Italian group reported promising results in a series of 26 patients with advanced HL (65% with active disease at transplant) who underwent NMA haplo-HSCT using the Baltimore protocol. They found an unexpected low NRM of 4% that translated into an OS of 77% at 4 years. Relapse rate was comparable to other sources (31%, median time of 5 months after transplant) and PFS was around 63%, significantly associated with disease status at transplant (1 year-PFS of 100%, 67% and 37% for CR, PR and refractory disease, respectively) [58]. Six out of 8 patients who relapsed were treated with chemotherapy and DLI and five (83%) responded (CR in 50%), supporting the existence of a clinically relevant GVL effect for HL.

In line with prior reports, the Spanish group recently analyzed the results of haplo-HSCT with BU-based conditioning protocols in 43 patients with advanced HL. Cumulative incidences for both grade II-IV acute and chronic GVHD were low (39% and 19%, respectively), 1 year-NRM was 21% and 2 year-relapse was 24%, bearing in mind that 67.5% of the patients were PET positive at transplant. Disease status at the time of transplant was the strongest predictor for PFS (78.5% if CR vs 33.5%; $p = 0.015$) and OS (86% if CR vs 46%, $p = 0.044$) [62].

HL seems to be more sensitive to alloreactivity in comparison to NHL in the setting of haplo-HSCT; Castagna et al. compared the results of haplo-HSCT vs related donor in lymphoma, and the only significant factor predicting for a better OS and PFS was histology (HL against NHL). Two year-OS and PFS were 85.2% and 73.9%, respectively, for HL, versus 54.6% (both OS and PFS) for NHL ($p = 0.02$ and $p = 0.1$, respectively) [59]. More recently the same group has reported the results on 62 patients with advancedtHL (35% refractory) undergoing to haplo-HSCT with Baltimore protocol or thiotepa-based conditioning: 3-year OS and PFS were 63% and 59%, respectively (84% and 76% in case of CR at transplant, respectively, versus 0% in case of active disease); The risk factor affecting OS were refractory disease and high comorbidity index, whereas PB as stem cell source was protective; 1 year NRM was 20% (even less for Baltimore protocol, as low as 11%) and relapse rate 21% [78].

9.2.3 Haploidentical Hematopoietic Stem Cell Transplantation for Non-Hodgkin Lymphoma

There is less experience with haplo-HSCT in NHL with respect to HL. Some case reports have been published and multicenter studies of haplo-HSCT and hematological malignancies have included no more than 10–20 patients with NHL. One of the most relevant series has been reported by Castagna et al., with 17 patients, 71%

with aggressive histology, and overall outcomes at least similar than with others donor types [59].

Kasamon et al. [57] analyzed the outcomes of haplo-HSCT with NMA conditioning, in older patients above 50 years, including a total of 148 patients with NHL. Equivalent overall outcomes were reported for both aggressive and indolent lymphomas: 3-year cumulative incidences of OS, PFS and relapse were 47%, 39% and 40%, respectively, for aggressive NHL, and 46%, 37% and 38%, respectively, for indolent NHL. NRM was unexpectedly low for the whole group, around 12%, bearing in mind the median age (61, range 50–75). There was no apparent decrement in PFS or OS in older patients. The most important risk factor for OS and PFS was disease-risk index (DRI), a validated tool based on type and status of disease at the time of transplantation that stratifies patients into different risk groups [79]: for low, intermediate and high-very high DRI, 3-year PFS were 62%, 36% and 15% ($p < 0.001$), respectively, and the 3-year OS were 68%, 44% and 31% ($p < 0.001$), respectively. Relapse rather than toxicity was the leading cause of treatment failure.

The group from Johns Hopkins's analyzed a poor risk cohort of 44 T-cell lymphoma patients that underwent allo-HSCT from related donors, half of them from haploidentical donors [80]. This series is very heterogeneous in the intensity of conditioning regimen (both MA and RIC were included) and immunoprophylaxis (including Cy post SCT in 68% of patients). The aim of the study was not to compare both donor types and overall outcomes were presented for the whole group, with a 2-year PFS of 40% and OS of 43%. For those patients being allografted using RIC protocols (18 out of 24 haplo-HSCT), 2-year PFS and OS were 37% and 44%, respectively. Again disease status at transplant had an impact on survival, with a trend for a higher PFS in first CR versus any other (2-year PFS of 53% versus 29% $p = 0.08$).

9.3 Comparative Studies of Haploidentical Hematopoietic Stem Cell Transplantation with Other Donor Types

Since overall activity in haplo-HSCT is dramatically increasing óver the last years, different retrospective comparative studies of donor type have been published (haplo vs related and URD) in the setting of hematological malignancies overall [81–83]. Bearing in mind the biases associated with the retrospective nature and heterogeneity of the groups, all of them achieve similar conclusions; comparable overall outcomes can be achieved regardless of donor type, in terms of OS, PFS, NRM (in some studies higher for URD) and relapse. Just chronic GVHD, at least extensive forms, seems to be less frequent in haplo-HSCT, maybe related with the predominant use of BM as stem cell source and Cy post SCT as GVHD prophylaxis.

The CIBMTR registry published in 2016 the two largest multicenter retrospective studies in the setting of lymphoma [84, 85], comparing the long term outcome of haplo-HSCT and that of allo-HSCT using HLA matched related donors (MRD)

and URD, during the same period of time (2008–2013). In both studies, conditioning regimen for haplo-HSCT was based in the Flu-Cy-TBI combination mentioned before, with a homogenous RIC conditioning for both related and URD, based on Flu plus an alkylating drug +/− 2Gy TBI. GVHD prophylaxis consisted on post-HSCT Cy plus a CNI and MMF for haplo-HSCT, versus a CNI in combination with MTX, MMF or others for the two other sources. Both studies reported similar hematological recovery after transplant between haplo-HSCT and the two other "more standard" donor types. In addition to that, comparable overall outcomes were obtained with one and other donor type. Even acute GVHD that has been a classical concern for haplo-HSCT, has been overcome with the use of post HSCT Cy. None of these two studies has led to any conclusion about potential advantages of haploidentical donors for any specific lymphoma histological subtype.

The EBMT reported on a retrospective series of 59 patients with NHL who underwent haplo-HSCT with Cy post, and compared the results with both MRD (n = 2024) and URD (n = 437). Haplo-HSCT was associated with similar 2 year-OS (56%), PFS (50%), NRM (23%), relapse rate (27%), acute and chronic GVHD (although lower extensive GVHD) than the other ones. Multivariate analysis for OS, PFS and relapse identified as significant risk factors older age, refractory disease, poor KPS and prior auto-HSCT. Regarding lymphoma subtype, DLBCL showed the worst outcomes for OS and relapse [86]. In line with this study, a shorter series of 79 patients with NHL undergoing to allo-HSCT has been reported, to compare the results of haplo-HSCT (n = 26) with MRD (n = 25) and URD (n = 28); No significant differences were found in OS, PFS nor relapse rate. NRM was significantly lower for MRD [87].

The second EBMT retrospective study aimed to compare Haplo-HSCT (n = 98) with MRD (n = 338) and URD (n = 273) for HL. The former one showed similar NRM and relapse rate (17 and 37% at 1 year, respectively), OS ad PFS (67 and 43% at 2 years, respectively) than MRD and URD, comparable acute GVHD although lower chronic GVHD than URD. The worst results were seen in URD in comparison to MRD in terms of NRM and OS. Risk factors that remained statistically significant for both OS and NRM were again older age, refractory HL and poor KPS. URD had a negative impact or NRM and OS with respect to MRD [88].

9.3.1 Haploidentical Hematopoietic Stem Cell Transplantation and Unrelated Donors

The CIBMTR analyzed a total of 917 adult patients with lymphoma who received haploidentical (n = 185) or HLA-matched URD transplantation either with (n = 491) or without ATG (n = 241) [85]. A higher proportion of patients in the haploidentical group had a significantly better KPS and intermediate or high DRI at HSCT. DLBCL was the most common histology in the haploidentical and URD with ATG cohorts, whereas FL was the most common in the URD without ATG. The risk of both III-IV acute and chronic GVHD was lower for haplo-HSCT with respect to URD with or

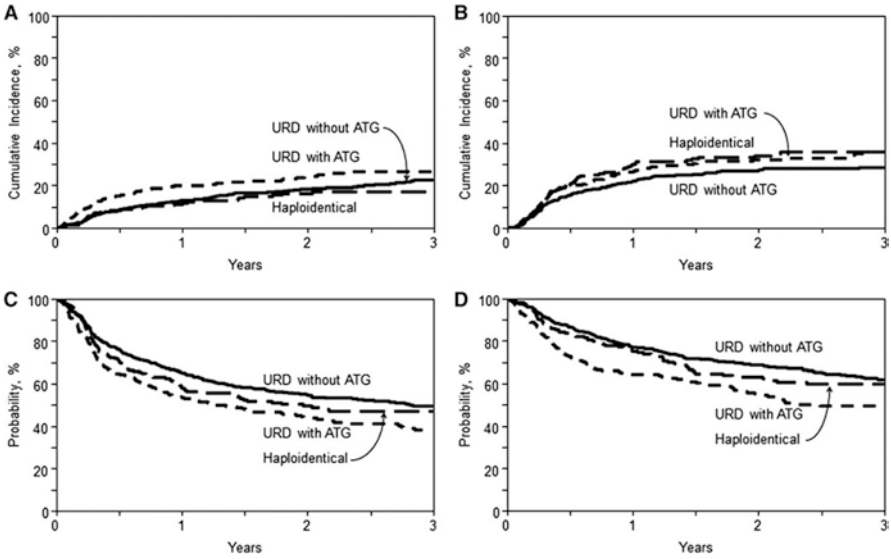

Fig. 9.6 Haplo vs Unrelated donor in Lymphoma (Kanate et al.)

without ATG (8%, 12% and 17%, respectively, p = 0.44, and 13, 51 and 33%, respectively). Multivariate analysis showed URD as the only significant risk factor for both 3–4 acute GVHD and chronic GVHD. This lower risk of GVHD in haplo-HSCT was attributed to the more frequent use of BM as source graft (>90% of haplo, against PB in >90% of URD), and post-HSCT Cy instead of a CNI combined with MTX, MMF or others, with or without ATG. Although some differences were seen in NRM (11, 13 and 20%, p = 0.03), PFS (47, 49 and 38%, p = 0.02) and OS (60, 62 and 50%, p:0.02) in univariate analysis for haplo-HSCT (Fig. 9.6), URD-HSCT without and with ATG, respectively, they were not translated in significant differences in the multivariate analysis for any of the four major outcomes after transplantation. Variables associated with a lower OS included recipient age > 60 years (RR 5, 1.91), KPS <90 (RR 5, 1.47), lymphoma histology other than FL, absence of CR prior to transplantation, intermediate and high DRI. The two latter variables were also associated with a lower PFS, together with lymphoma histology (other than HL and FL within the first 7 months) and prior auto-HSCT. No differences were either seen in relapse rate among the groups (36, 28 and 36%). A higher relapse/progression risk was observed for all disease histologies other than FL and HL within the first 7 months of allo-HSCT and for PTCL and HL beyond 7 months. The most common cause of death in all three cohorts was recurrent/progressive lymphoma and among non-relapse causes of death, infectious complications accounted for 7%, 8% and 11% of cases in haplo-HSCT, URD-HSCT without and with ATG respectively. The study concluded that in the absence of randomized studies and bearing in mind its limitations due to the retrospective nature of the series, a RIC/NMA haplo-HSCT with post SCT Cy might be a valid alternative option in case of no HLA-identical sibling.

These results are in line with another recently published retrospective study: Baker et al. that compared 54 haplo-HSCT (11 lymphomas) with 59 URD (8 lymphomas), with a higher proportion of PB in the haplo group unlike the previous study (56% against 7% in the previous one). There were no statistically significant differences regarding NRM, relapse, PFS and OS. Unlike the previous study, a similar risk of both acute (II-IV and III-IV) and chronic GVHD was found, probably related with a higher proportion of PB in the whole group. The most common cause of death was disease relapse in both groups [89].

A French retrospective study has just published the results of 98 allo-HSCT using an alternative donor (haplo vs mismatched UD and cord blood) in the setting of HL: apart from no significant differences for OS and PFS among the three groups, and a lower risk of chronic GVHD for haplo-HSCT, the most relevant finding is a significantly higher survival free of relapse and GVHD for haplo-HSCT (HR 2, $p = 0.03$ and HR:2.43, $p = 0.009$, for mismatched UD and cord blood, respectively, $p < 0.01$) [90].

9.3.2 Haploidentical Hematopoietic Stem Cell Transplantation and Matched Sibling Related Donors

The CIBMTR has also compared the results of haplo-HSCT with MRD in 987 patients with lymphoma (180 and 807 patients in each group respectively) [84]. The haplo group included a higher proportion of older patients and better KPS, earlier stage of disease but more frequent intermediate or high DRI in relation to the MRD cohort. Most common histologies were DLBCL and FL in the haplo and MRD groups, respectively. Both groups had comparable risk of II-IV and III-IV acute GVHD, whereas haplo-HSCT was associated with a significant lower risk of chronic GVHD at 1 year (12 vs 45% in MRD). No statistically significant differences were seen regarding 1-year NRM (10 and 9%), 3 year-relapse/progression rate (37 and 40%), 3-year PFS (48% for both groups) and 3-year OS (62 and 61%) in both univariate and multivariate analysis (Fig. 9.7). Factors associated with higher risk of disease progression/relapse were lymphoma histology other than FL, not being in CR at allo-HSCT, presence of bulky or extranodal disease at transplant, HSCT performed before 2010, and intermediate or high DRI. The same factors but also histology other than FL or T-NHL were associated with a higher risk of mortality. The most common cause of death in both groups was recurrent disease/progression, in 47% and 52% in haplo and MRD respectively).

These results compare favorably with those of a prior CIBMTR study for patients with lymphoma undergoing a mismatched URD-HSCT (3-year NRM, 44%; OS, 37%) or a cord blood transplant (3-year NRM, 37%; OS, 41%), suggesting that haploidentical donor should be considered as one of the first options in the absence of a MRD or the immediate need of the transplant procedure [91].

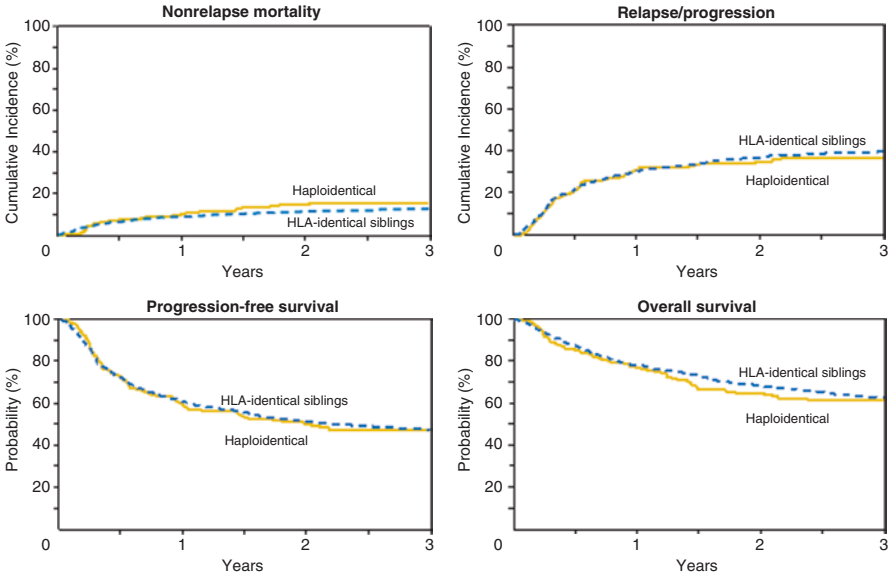

Fig. 9.7 Haplo vs Related donor in Lymphoma (Gosh et al.). (**a**) NRM (p: 0.08, 3 y); (**b**) Relapse/progression (p: 0.07, 3y); (**c**) PFS (p: 0.02, 3y); (**d**) OS (p: 0.02, 3y)

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Chapter 10
Applications of Haploidentical SCT in Patients with Non-malignant Diseases

Nicolaus Kröger

10.1 Introduction

Allogeneic stem cell transplantation has become an established treatment procedure for patients with hematological malignancies as well as for non-malignant diseases. Because of the increasing numbers of volunteer donors the number of unrelated stem cell transplantations has been steadily increased during the last years [1]. About 25% of the patients will have an HLA-identical sibling and about 70–80% will find a suitable HLA-compatible unrelated donor or an umbilical cord blood unit [1]. However, the likelihood to find a suitable unrelated donor strongly depends on patients' ethnic background and for some ethnic populations this likelihood is less than 20% [2]. In contrast most of those patients will have a family member identical for one HLA haplotype and fully mismatched for the other haplotype which can be used immediately without any further delay as an haploidentical donor.

The major problem in haploidentical stem cell transplantation is the higher risk of graft failure and because of the HLA-disparity a high risk of severe graft-versus-host disease (GvHD). This high risk of GvHD resulted in attempts to deplete T-cells from the graft by ex-vivo graft manipulation which however is costly and time-consuming. Furthermore a higher risk of infectious complications was noted mainly because of delay in immune reconstitution [3]. Despite more sophisticated methods to deplete T-cells by ex-vivo engineering interest in haploidentical stem cell transplantation increased by introduction of so-called T-repleted strategies using either anti-thymocyte globulin or post-transplant cyclophosphamide as effective GvHD prophylaxis [4, 5].

The European Society for Blood and Marrow Transplantation (EBMT) reported an increase of 291% in haploidentical since 2005 [1]. This increase in using

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haploidentical donors for allogeneic stem cell transplantation was seen in myeloid and lymphoid malignancies as well as in non-malignant disorders [1]. Here we describe the results of haploidentical stem cell transplantation in non-malignant diseases.

10.1.1 Aplastic Anemia

Only few cases of aplastic anemia with ex-vivo T-cell depletion and haploidentical stem cell transplantation have been reported. Two out of four severe aplastic anemia patients who received ex-vivo α/β + T- and B-cell depletion of the haploidentical graft experienced primary graft failure [6]. More data on haploidentical stem cell transplantation are available for T-cell repleted approaches either by using ATG or post-transplant cyclophosphamide. (see Table 10.1).

In a small series including 4 patients with aplastic anemia and 4 patients with graft failure after unrelated donor stem cell transplantation by using post-transplant cyclophosphamide as GvHD prophylaxis in combination with tacrolimus and MMF in 6 patients a sustained engraftment could be achieved with low incidence of acute GvHD (11%) and 75% overall survival at 1 year [7].

Sixteen patients with AA who received post-transplant cyclophosphamide as GvHD prophylaxis in combination with tacrolimus and MMF after haploidentical stem cell transplantation have been reported from a Brazilian multicenter study. Two graft failures and 13% acute GvHD was reported with a 1-year overall survival of 67% [8].

The Peking University approach for haploidentical stem cell transplantation by using G-CSF primed bone marrow and peripheral blood stem cells and ATG was reported first in 26 patients after conditioning with fludarabine, cyclophosphamide,

Table 10.1 Selcted study of haploidentical stem cell transplantation in patients with aplastic anemia

Author	Number	Conditioning regiment	GvHD prophylaxis	aGvHD II – IV (%)	OS (%)
Clay et al. [7]	n = 8	Fludarabine/ cyclophosphamide/2Gy TBI	Cyclophosphamide day +3, +4, tacrolimus, MMF	11	75 (1 y)
Esteves et al. [8]	n = 16	Fludarabine/ cyclophosphamide/2–6 Gy TBI	Cyclophosphamide day +3, +4, tacrolimus, MMF	13	60 (1 y)
Xu et al. [9]	n = 101	Busulfan/cyclophosphamide/ ATG	Cyclosporine A, MMF, MTX	34	89 (3 y)
Gao et al. [10]	n = 26	Fludarabine/ cyclophosphamide/ATG	Cyclosporine A, MMF, MTX	12	85 (2 y)

and ATG including GvHD prophylaxis with cyclosporine A, MMF, and MTX. Acute and chronic GvHD was observed in 12% and 40%, respectively [10], and an overall survival of 85% at 2 years was reported.

In another large multicenter Chinese study with similar GvHD prophylaxis including 101 patients with aplastic anemia a higher acute GvHD rate of 34% was reported with a 3-year OS rate of 89% [9]. In a recent comparison of haploidentical vs. HLA-identical sibling transplantation a similar 3-year OS rate was reported (86% vs. 91%, p = 0.4) [11].

10.1.2 Thalassemia

One of the first large series of haploidentical stem cell transplantation reported on 22 patients with thalassemia major who received stem cell graft after CD34 positive selection or CD3+/CD19+ negative depletion from the mother after busulfan-based conditioning regimen. The graft failure rate was high with 29% and a therapy-related mortality of 14% resulting in a disease-free survival of 61% [12]. Using post transplant cyclophosphamide as GvHD prophylaxis in combination with tacrolimus, sirolimus, and MMF after conditioning with busulfan, ATG, and fludarabine followed by haploidentical stem cell transplantation has been in investigated in 31 patients with β-thalassemia and β-thalassemia/hemoglobin E. Two graft failures were observed and the incidence of acute GvHD was 30%. 2-year OS and EFS were 95% and 94%, respectively [13] (see Table 10.2).

10.1.3 Sickle Cell Disease

Sickle cell disease is a major health problem. As an inherited disorder of human hemoglobin the disease can result in chronic pain, stroke, and end-organ failure. In the recent years encouraging data of allogeneic stem cell transplantation as curative treatment has been reported. However, still a substantial number of affected patients is lacking HLA-compatible donor.

Haploidentical stem cell transplantation can overcome the lack of HLA-compatible family or unrelated donor. A CD34 selected haploidentical stem cell transplant was investigated in 8 patients with sickle cell disease. Graft failure occurred in 3 patients (38%) and two patients died from chronic GvHD resulting in a DFS and OS of 38% and 75% respectively [14].

Haploidentical stem cell transplantation with post cyclophosphamide as GvhD prophylaxis first was reported in 1 patient with sickle cell disease and 1 patient with PNH after a non-myeloablative conditioning regimen. Both patients experienced rapid engraftment without any signs of GvHD [15].

A large series of 14 patients with sickle cell disease was reported from the same group using haploidentical donors after ATG, fludarabine, cyclophosphamide, and

Table 10.2 Selected study of haploidentical stem cell transplantation in patients with thalassemia

Author	Number	Conditioning regiment	GvHD prophylaxis	aGvHD II – IV (%)	OS (%)
Sodani et al. [12]	n = 22	Busulfan/ fludarabine/ thiotepa/ cyclophosphamide/ ATG	CD34 selection or CD3/CD19 depletion + cyclosporine	0	96 (3 y)
Anurathapan et al. [13]	n = 31	Busulfan/ fludarabine	Cyclophosphamide day +3, +4, tacrolimus / sirolimus, MMF	30	95 (1 y)

Table 10.3 Selcted study of haploidentical stem cell transplantation in patients with sickle cell anemia

Author	Number	Conditioning regiment	GvHD prophylaxis	aGvHD II – IV (%)	OS (%)
Dallas et al. [14]	n = 8	Fludarabine, thiotepa or busulfan, ATG, and OKT3 or 5-azacytidine, fludarabine, cyclophosphamide, thiotepa, and OKT3	CD34 selection	40 (grade II/IV: None)	75 (5 y)
Bolanos-Meade et al. [15]	n = 14	Fludarabine/ cyclophosphamide, ATG, and 2 Gy TBI	Post cyclophosphamide day +3, +4, tacrolimus, MMF	0	100 (2 y)

2 Gy TBI as conditioning regimen followed by post cyclophosphamide as GvHD prophylaxis with tacrolimus or sirolimus and MMF. No GvHD was observed and a 100% overall survival at 2 years. The major problem was graft failure which was noted in 6 patients (45%) [16] (see Table 10.3).

10.1.4 Severe Combined Immunodeficiency (SCID)

A first successful report on haploidentical stem cell transplantation for SCID used agglutination with soybean agglutination (SBA) followed by E-rosette depletion (SBA-E) [17]. The SBA-E approach for T-cell depletion has been extended in 145 patients with SCID [18]. In this study only 10% of the patients developed acute GvHD. Seventy five percentage of the patients survived with T-cell and 41% with B-cell reconstitution.

In a European trial using bone marrow as stem cell source and also SBA-E as T-cell depletion the overall survival was 52%. The major cause for non-relapse mortality were infections and GvHD [19] (see Table 10.4).

CD34 selection as GvHD prophylaxis followed by haploidentical stem cell transplantation was reported in 175 patients with SCID resulting in 76% sustained engraftment, 22% acute GvHD, and 54% overall survival at 3 years [20].

In a retrospective collection of 240 infants with SCID a large population of the patients (n = 138) received haploidentical stem cell transplantation after different or no conditioning regimen. GvHD rate was higher after CD34 selection (34%) than after SBA-E (n = 13%). Long-term overall survival was 74% [21].

10.2 Outlook

Haploidentical stem cell transplantation has become a reasonable treatment option for patients with non-malignant disease lacking an HLA-compatible donor. T-cell depletion strategies resulted in relative high risk of graft failure with exception of SCIDs. Further refinement has been reported for selected depletion of α/β T-cells and CD19/B-lymphocytes. In a smaller study of different non-malignant diseases

Table 10.4 Selected trials of haploidentical stem cell transplantation for SCID

Author	Number	Conditioning regiment		GvHD prophylaxis	aGvHD II – IV (%)	OS (%)
Railey et al. [18]	n = 145	No conditioning		Soybean agglutinin and subsequent E-rosette depletion (SBA-E) plus cyclosporine A	10	75 (8 y)
Fernandes et al. [20]	n = 175	No conditioning MAC RIC	(n = 30) (n = 81) (n = 64)	CD34 selection	22	62 (5 y)
Antoine et al. [19]	n = 294	No conditioning (n = 87) or busulfan/cyclophosphamide (n = 207)		SBA-E, monoclonal α/β or CD34 selection	30–40 (<1996) 22 (>1996)	54 (3 y)
Pai et al. [21]	n = 138	No conditioning MAC RIC Immunosuppression	(n = 87) (n = 25) (n = 16) (n = 10)	SBA-E (n = 70) CD34 selection (n = 50) Others (n = 12)	13 (SBA-E) 31 (CD34 selected)	74

such as SCID, aplastic anemia, osteoporosis. Fanconi anemia, Shwachman-Diamond syndrome, congenital amegakaryocytic thrombocytopenia, thalassemia major, and hemophagocytic lymphohistiocytosis the GvHD was low with 13% and the OS was impressive with 91% but the rate of rejection was still high with 16% [6]. Including suicide gene-modified T-cells after α/β T-cell depletion haploidentical stem cell transplant may reduce the risk of graft failure [22]. T-cell repleted stem cell transplant is an alternative method either with ATG or post-transplant cyclophosphamide for haploidentical stem cell transplantation. However, here the incidence of GvHD seems to be higher and some concern has been raised for children less than 10 years [23].

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Chapter 11
Applications of Haploidentical SCT in Pediatric Patients

Marco Zecca and Patrizia Comoli

11.1 Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment for children and adolescents with malignant and non-malignant diseases. Recent progress in HSCT contributed to the improvement of outcomes for patients with diseases curable by HSCT. Human leukocyte antigen (HLA)-identical sibling is the preferred donor choice, however the probability of having such a donor correlates to the number of siblings within the family, and is approximately 25%. HLA-matched unrelated volunteer donor (MUD) is also a good option for successful HSCT, but the probability to find a HLA-matched donor correlates with race and ethnicity, and, in the current multiethnic context, identifying a MUD in a timely manner remains a challenge. Indeed, the search for a HLA-matched volunteer donor may result in unacceptable delay in certain diseases, such as very high risk acute leukemias, or severe combined immunodeficiency (SCID), for which the goal is to proceed to transplantation as early as possible after diagnosis. For those without a HLA matched donor, alternative hematopoietic progenitor cell sources include mismatched unrelated donors, umbilical cord blood, and haploidentical related donors.

Transplantation from a full HLA-haplotype mismatched family member (haplo-HSCT), in addition to ensuring a donor for the large majority of patients, offers several other advantages, including prompt availability of the stem cell source, the possibility to select the best donor from a pool of family candidates, and immediate access to donor-derived cellular therapies either for the prevention of relapse or the treatment of infections after HSCT. Despite these advantages, widespread use of

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haplo-HSCT has been limited for many years by relevant complications mediated by bidirectional alloreactivity responsible for unacceptably high rates of graft rejection and severe graft-versus-host disease (GvHD).

The continuous development of graft engineering and pharmacologic GVHD prevention strategies, better supportive care, and optimal conditioning regimens have significantly improved the outcomes of haploidentical HSCT, and this progress has led to establishment of haplo-HSCT as a standard therapeutic option for patients with both malignant and non malignant disease needing a HSCT procedure and lacking a HLA-identical or compatible donor.

11.2 Donor Selection

Most patients requiring HSCT have more than one haploidentical donor. In the majority of pediatric patients, however, the best donor will have to be selected from one of the parents. Ideally, the goal is to employ a graft that will enable complete and permanent engraftment of donor hematopoiesis, ensure rapid immune reconstitution, and exert effective graft-versus-leukemia (GVL) effect in the absence of GvHD. Several studies investigated donor/recipient characteristics that influenced haplo-HSCT outcome.

The role of HLA disparity degree was evaluated in large clinical trials, mainly in the T-cell replete context [85, 146], and it did not influence the incidence of acute GVHD and treatment related mortality (TRM). In the study by Wang et al., conducted on 1210 pediatric and adult patients transplanted with G-CSF mobilized T-cell replete bone marrow and peripheral stem cells, donor age less than 30 years was associated with a lower incidence of acute GVHD compared to older donor age, this observation being also confirmed in the pediatric T-cell depleted setting [67]; younger donor age and male gender were also associated with less TRM and better overall survival (OS). Moreover, having a maternal donor was associated with a higher GVHD incidence and TRM than having a paternal donor. These findings are in contrast to observations from other studies demonstrating a lower risk of relapse and survival advantage for grafts from maternal donors [136], in which the anti-leukemic effect of maternal donor HSCT had been explained by maternal immune system exposure to fetal antigens during pregnancy. When analyzing the role of non-inherited maternal antigen (NIMA) disparities, also Wang et al. found that NIMA mismatched sibling haplo donors conferred a lower incidence of acute GVHD compared to non-inherited paternal antigen (NIPA) mismatched donors [146].

The use of highly purified CD34+ stem cells in haploidentical HSCT has allowed deep insights into the biology of NK cells and into the understanding of NK alloreactivity [110, 129]. Among adult patients affected by AML, a subgroup of patients given T cell depleted HSCT from an HLA-disparate relative having alloreactive NK cells showed a low risk of leukemia relapse [9]. Cytotoxic activity of NK cells is under the negative feedback control from inhibitory killer immunoglobulin-like

receptors (KIRs), that recognize epitopes shared by HLA class I alleles. KIR-KIR ligand mismatches in the donor-recipient direction lead to loss of the inhibitory feedback and activation of donor NK cells targeting recipient hematopoietic cells and leukemic cells [130]. In contrast to alloreactive T-lymphocytes, NK cells are thought to be capable of inducing GVL effect without promoting GvHD, through elimination of residual recipient antigen-presenting cells in addition to leukemia blasts [130]. Accordingly, KIR analysis to identify donor/recipient KIR mismatch has been proposed as a tool for donor selection prior to T-cell depleted haplo-HSCT [131].

Notably, other factors may contribute to NK alloreactivity. In particular, killing of target cells may also depend on the surface density of activating receptors on NK cells and on the expression of their ligands on target cells [47]; indeed, activating KIRs (in particular KIR2DS1) were shown to play a substantial role in mediating alloreactivity [36, 123]. Thus, phenotypic identification of the alloreactive NK cell subset and assessment of the NK cytolytic activity against leukemic cells represent important criteria in donor selection [123]. Moreover, recent reports have proposed a novel approach for optimal donor selection based on the KIR genotype analysis. Among these, a study in a haplo-HSCT setting provided evidence that the selection of donors with KIR B haplotypes was associated with significant improvements in both overall and relapse free survival [114, 140].

KIR mismatch between recipient and donor has been associated with improved outcomes in both T-cell deplete and T-cell replete haplo-HSCTs [103, 123, 129, 130, 140]; however, some studies failed to find an association between the presence of donor NK alloreactivity and a favorable clinical outcome of transplanted patients [75, 113, 143]. The apparent discrepancies are likely due to the different clinical settings, in particular the type of graft (manipulated vs unmanipulated), the conditioning regimen, stem cell source and number (i.e. CD34+ HSC megadose), the type of GVHD prophylaxis, and the disease status of the patient at the time of HSCT, and these variables have to be taken into account in the donor selection algorithm.

Finally, another factor that may have a negative influence on haplo-HSCT outcome is the presence of recipient antibodies directed to donor HLA antigens (DSAs), as DSAs have been associated to graft rejection [40]. Thus, pre-transplant DSA analysis may help donor selection, and, in case DSAs to antigens of all available donors are present, may guide antibody removal by anti-B cell monoclonal antibodies or plasma exchange.

11.3 Haploidentical HSCT Strategies

11.3.1 T-Cell Depletet Graft

HLA-haploidentical HSCT had been experimented with varying success since mid-seventies [42], at first in the setting of acute leukemia and aplastic anemia. The greatest challenge to performing haplo-HSCT had been high rates of graft failure

and severe GVHD. Notable success came with the demonstration that in children with SCID it was possible to reconstitute a functional immune system with HSCT without inducing life-threatening acute GVHD by performing a T-cell depletion using soybean agglutinin and sheep red blood cell rosette formation technique [31, 61, 62, 126]. Primary immune deficiencies were an ideal setting for haplo-HSCT, as, in contrast to other disorders, the profound immunodeficiency in SCID minimized the risk of immunologic graft rejection, conceptually eliminating the need for immunosuppressive conditioning before HSCT and, consequently, reducing the toxicity of the procedure.

In contrast to SCID, haplo-HSCT was less successful in the setting of acute leukemia owing to a high rate of graft failure, attributed to host derived T-lymphocytes that survived the conditioning regimen [87]. A major breakthrough intervened when preclinical studies in murine models demonstrated that infusion of large numbers of donor hematopoietic stem cells (HSC "mega dose") could overcome the major histocompatibility complex (MHC) barrier and promote engraftment [14]. Seminal clinical studies showed that transplantation of mega doses of stem cells ($>10 \times 10^6$ CD34+ cells/kg), obtained by supplementing T cell-depleted bone marrow transplants with granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood stem cells (PBSC), after a conditioning regimen consisting of single fraction total body irradiation (TBI), thiotepa, cyclophosphamide (CY) and rabbit antithymocyte globulin (rATG), allowed for successful primary engraftment and relatively low incidence of acute and chronic GVHD despite the use of T-cell depletion as the only GVHD prophylaxis [10, 11]. The initial protocol was implemented with time, as highly purified CD34+ cells selected from mobilized PBSC by magnetic sorting replaced soybean agglutination and E-rosetting T cell depleted inoculum, and fludarabine replaced CY in the conditioning regimen [12]. In the pediatric setting, different versions of the "Perugia protocol" were applied successfully to treat both malignant and non-malignant hematologic disorders [6, 44, 72, 104].

Despite acceptable rates of engraftment and GVHD, TRM due to infectious complications and malignancy relapse remained a major problem after CD34+ HSC-selected haplo-HSCT. In the attempt to ameliorate immune reconstitution, attempts were made to switch from positive CD34 HSC selection to negative T and B cell depletion, in order retain, besides the CD34+ stem cells, large numbers of other cells including γδ and NK cells, monocytes, and dendritic cells. With introduction of automated devices, simultaneous depletion of CD3+ T cells and CD19+ B cells allowed to prevent GVHD and posttransplant lymphoproliferative disease (PTLD) [18, 35]. In order to reduce toxicity, a non-myeloablative conditioning regimen based on the use of fludarabine, thiotepa, melphalan and the anti-CD3 T monoclonal antibody OKT3 was employed. T-cell reduction did not reach the 4.5–5 log depletion obtained with CD34+ selection, and despite post-HSCT GVHD prophylaxis, the rate of acute and chronic GVHD were higher than those observed in the CD34+ T cell-depleted haplo-HSCT; moreover, notwithstanding a faster immune recovery, leukemia relapse remained a major problem.

A more effective approach to negative depletion of T cells is the more recently described negative depletion of T-cell receptor (TcR) αβ + T lymphocytes from

mobilized peripheral stem cell grafts, coupled with B cell depletion [34]. With this technique, Bertaina et al. reported high OS and disease-free survival (DFS) (91%) coupled with a low incidence of acute GvHD (13%) and chronic GvHD in 23 children with a variety of non-malignant disorders, including SCID, Fanconi anemia (FA), severe aplastic anemia (SAA), osteopetrosis, and primary immunodeficiencies (PIDs) [22]. Recently, a multicenter Italian study comparing the outcome of Tαβ/B cell depleted haplo-HSCT vs UD-HSCT in children with acute leukemia transplanted with a myeloablative regimen reported primary engraftment in 95 of 97 patients receving haplo-HSCT and, with the only pharmacologic GVHD prophylaxis of pretransplant ATG in the haplo-HSCT setting, 16% and 0% grade II–IV and III–IV acute GVHD, respectively, as compared to 39% and 12% in UD-HSCT recipients [23]. After a median follow-up of 3.3 years, the 3-year leukemia-free survival was 63% vs 62% in the UD-HSCT setting, with chronic GVHD rates of 6% vs 20%, respectively. Encouraging results in the setting of ALL and AML were also reported from other groups [94, 106].

Different means to deplete alloreactive T cells within the graft have been experimented in the setting of haplo-HSCT. Triggering of alloreactivity in vitro through a mixed lymphocyte reaction (MLR) obtained by co-culturing donor T cells with recipient antigen-presenting cells has been generally followed by depletion of the activated donor T cells through surface activation markers or photoactive dyes. Cavazzana-Calvo et al. designed a protocol to allo-activate donor T cells responsible for GVHD and eliminate them with an immunotoxin that reacted with the cell surface activation antigen CD25, and demonstrated in a group of pediatric patients receiving haplo-HSCT the ability of an allodepleted T-cell "add-back" to exert an anti-viral infection effect without causing GVHD [5, 108]. Using a similar approach, Amrolia et al. infused donor allodepleted lymphocytes in 16 pediatric recipients of T-cell depleted haplo-HSCT, showing a low rate of GVHD, but inability to prevent posttransplant leukemia relapse and viral infections [4]. A method to deplete alloactivated T cells, based on the use TH9402, a phototoxic dye that accumulates in activated T cells due to their inability to efflux rhodamidelike drugs, was also developed and employed in the setting of haplo-HSCT [19].

An alternative approach to prevent GVHD while preserving anti-leukemia and anti-infectious immunity is to functionally inactivate alloreactive T cells by inducing alloantigen-specific anergy. Several groups demonstrated how blockade with antibodies directed to costimulatory molecules during allostimulation in MLR could induce anergy directed to the specific alloantigen, while preserving other immune responses [29, 45]. In a pilot trial conducted in 12 pediatric haplo-HSCT recipients, Guinan et al. incubated donor marrow cells with CTLA-4–Ig, an agent that inhibits B7-CD28 costimulatory signal, in the presence of irradiated mononuclear cells from the recipient. Primary engraftment after myeloablative conditioning was demonstrated in 10 of the 11 evaluable patients, and acute GVHD of the gastrointestinal tract developed in three patients, despite posttransplant prophylaxis with cyclosporine and short-course methotrexate. Five of the 12 patients were alive and in remission 4.5–29 months after transplantation [70]. In a follow-up study, a 50% rate of TRM was observed, and 8 of 24 reported patients developed acute GVHD [49].

Finally, studies conducted in animal models had suggested that coinfusion of CD4 + CD25+ regulatory T cells (Tregs) and conventional donor T cells could inhibit lethal GVHD after allogeneic HSCT across MHC, while preserving GVL surveillance [56].

Di Ianni et al. were able to prevent GVHD, improve immune reconstitution, and induce a strong GVL effect after infusion of donor-derived Tregs, followed by conventional T cells, in 28 recipients of CD34+ selected haplo-HSCT, in the absence of any post-transplant immunosuppression [52]. Despite prompt immune reconstitution, however, the rate of opportunistic infections, and thus TRM, remained high, perhaps due to Tregs hampering immunity to infectious agents.

11.3.2 T-Cell Replete Graft

Haplo-HSCT with ex-vivo T cell depletion ensures the best mean to prevent GVHD. However, it requires specific cell processing expertise, a myeloablative conditioning regimen to eradicate residual recipient immune cells and support engraftment, and posttransplant immunologic interventions to boost immune recovery. Being costly and technically demanding, its application has been generally confined to highly experienced centers, consequently limiting widespread application of haplo-HSCT. Recently, this scenario has dramatically changed thanks to the impressive results obtained with unmanipulated haplo-HSCT strategies in patients affected by malignant diseases.

Historical attempts at using unmanipulated haploidentical allografts were associated with an unacceptably high rate of GVHD. To overcome this hurdle, efforts have been made to increase the intensity of posttransplant GVHD prophylaxis regimen. The first unmanipulated approach, pioneered by the Johns Hopkins group, relied on the use of post-transplantation cyclophosphamide (PTCY) [102]. The concept behind this strategy relied on the observation that CY could induce skin graft tolerance [20]. It was hypothesized that in vivo donor and recipient alloreactive T cell depletion could be obtained by exposing to PTCY donor/recipient lymphocytes proliferating to reciprocal alloantigens within the first posttransplant days. This procedure could ensure both engraftment and GVHD prevention, while sparing quiescent T cells, that are less sensitive to CY due to high levels of aldehyde dehydrogenase, an enzyme responsible for CY metabolism [81]. Using a reduced-intensity conditioning regimen including fludarabine, CY and 200 Gy TBI, and PTCY, Luznik et al. obtained a 87% rate of engraftment, with 6% grade III–IV acute GVHD, and demonstrated a significantly higher rate of chronic GVHD with the use of PTCY on day +3 vs day +3 and +4 (25% vs 5%, respectively). The 2-year overall survival and event-free survival (EFS) rates were as low as 36% and 26%, due to high incidence of relapse (58% at 2 years) [102]. Similar results of acceptable incidence of GVHD (32% acute grade II–IV and 13% chronic GVHD) and very low TRM were obtained in a large multicenter trial conducted in patients with malignancies, in which relapse was a major cause of mortality and was primarily attributed

to the use of nonmyeloablative conditioning for patients with acute leukemias [30]. To address this obstacle, the use of myeloablative preparative regimens was explored and found successful. Raiola et al. showed a 4-year disease-free survival of 43% in 92 adult patients transplanted for hematological malignancies [125].

Data on the feasibility of this approach in children are scarce, although the procedure is increasingly employed [21, 78].

A different unmaninuplated haplo-HSCT strategy was applied in 250 pediatric and adult patients with acute leukemia, based on the use of myeloablative haplo-HSCT with non-T-cell depleted, cytokine-primed marrow and peripheral blood grafts, associated with in vivo T-cell depletion by ATG and GVHD prophylaxis consisting of cyclosporine, short-course methotrexate and mycophenolate mofetil (MMF) [74]. All but one patients engrafted. The cumulative incidence of grade II-IV acute GVHD was 46%, grade III-IV was 13%, and incidence of chronic GVHD was 54%. At 3 years after HSCT, the cumulative incidence of opportunistic infections was 49%, and 141 of 250 patients were alive and disease free. An Italian study reported a significantly lower incidence of acute and chronic GVHD with a similar strategy, but with bone marrow as the only source of HSC [51].

A multicenter Italian trial explored the feasibility of a haplo-HSCT protocol consisting of unmanipulated PBSC infusion after a treosulfan and fludarabine conditioning regimen, and GVHD prophylaxis based on antithymocyte globulin Fresenius (ATG-F), rituximab and oral administration of sirolimus and mycophenolate [121]. Incidence of acute GvHD grade II-IV was 35%, and correlated negatively with Treg frequency, while that of chronic GvHD was 47%. At 3 years after HSCT, TRM was 31%, with 48% relapse incidence and 25% OS. The high rate of chronic GVHD might have been partly due to the use of PBSC rather than BM as stem cell source.

11.4 Indications for Haplo-HSCT in Children

11.4.1 Haplo-HSCT in Childhood Malignancies

At present, allogeneic HSCT in children with ALL is reserved to patients who experience an early or very early marrow relapse after first line chemotherapy, or to the subpopulation of high-risk ALL in first complete remission (CR1), i.e. those with known molecular biological markers or chromosomal abnormalities, and clinical factors such as poor prednisone response and resistance to initial chemotherapy including persistence of minimal residual disease (MRD) [138] (Table 11.1). Likewise, in the setting of pediatric AML, indications for allo-HSCT are high/very high risk disease (infant AML and children with unfavorable karyotype, or FAB M0, M6 or M7), or patients in CR2 [138]. Conversely, childhood myelodysplastic syndromes (MDS), including juvenile myelomonocytic leukemia (JMML) without germ line PTPN11 and CBL mutations, myelodysplasia-related AML, advanced MDS and refractory cytopenia of childhood (RCC) with high risk of disease

progression (monosomy 7 or >2 chromosomal abnormalities), have an indication to HSCT [84, 99, 137] (Table 11.1). In JMML and in children ≤12 years with advanced MDS, a myeloablative conditioning regimen of busulfan (16 g/kg over 4 days), cyclophosphamide (120 mg/kg over 2 days) and melphalan (140 mg/m^2 in single dose) and rATG is indicated. In older patients with advanced MDS, who experience a higher TRM, and in high risk RCC children with normo/hypercellular bone marrow, a myeloablative conditioning based on thiotepa (4–5 mg/kg/day for 2 days), treosulfan (14 g/m^2/day for 3 days), fludarabine (40 mg/m^2/day for 4 days,) and rATG may be employed. Finally, RCC children with hypocellular bone marrow may receive a reduced-intensity conditioning with fludarabine (40 mg/m^2/day for 4 days), thiotepa (5 mg/kg/day for 2 days) and rATG.

In the setting of Hodgkin disease (HD) or non-hodgkin lymphoma (NHL), allogeneic HSCT may be considered in patients with relapsed/refractory disease or disease relapsed after autologous HSCT [3, 32, 69, 132].

In all cases, haplo-HSCT can be considered when a matched sibling donor or a well-matched unrelated donor are not available. However, with haplo-HSCT results constantly improving [23], it is now debatable if UD-HSCT is more indicated than haplo-HSCT in refractory malignancy, a setting where haplo-HSCT (especially Tαβ/B cell depleted haplo-HSCT without posttransplant GVHD prophylaxis), may be an ideal platform for strategies to boost immune surveillance and control disease outgrowth.

In childhood acute leukemia, early studies of haplo-HSCT were heterogeneous and carried out on small cohorts. Moreover, as the procedure was considered experimental, the patients enrolled were mostly very high risk patients or refractory ALL with dismal outcome. With the development of the T-cell depleted, CD34+ megadose haplo-HSCT, the Perugia group obtained for the first time encouraging results [10, 11]. With a strategy based on fractionated TBI, thiotepa, fludatabine and rATG conditioning and no posttransplant GVHD prophylaxis, in their larger cohort that included also pediatric patients, they obtained 95% primary engraftment, with 8% and 7% acute and chronic GVHD; in children, a 15% TRM was observed, with a DFS probability of 38% in ALL and 62% in AML in any CR. For patients transplanted in relapse, DFS was 5% in ALL and 38% in AML [12]. In 47 pediatric patients transplanted with the same strategy, 5% graft failure was observed, with grade III-IV acute and extensive chronic GVHD of 6% and 3%, and 25% TRM (10% in patients transplanted after 2005). The 5-year estimate of DFS for the whole cohort was 50%; interestingly, 70% DFS was observed in children with ALL, 75% in MDS, while only 20% in AML patients. The DFS of the 18 patients with ALL transplanted from an NK-alloreactive donor was 81% [100, 123]. In 2006, Chen et al. published data providing evidence that a CD3+/CD19+ depletion strategy using PBSCs, in combination with a reduced intensity conditioning based on fludarabine, melphalan, thiotepa and OKT-3 monoclonal antibody, was a feasible option for children with hematological malignancy [35]. Although higher rates of acute GVHD were seen in comparison to CD34+ selected grafts, due to lower T cell depletion, TRM was low. However, the 2-year disease-free survival was 25%. A retraspective study by

Table 11.1 Indications to haplo-HSCT in children

Disease	Disease status	Alternative donor allo-HSCT	Evidence grading
Hematological malignancies			
AML	CR1 (low risk)	Generally not recommended	III
	CR1 (high risk)	Clinical option	III
	CR1 (very high risk)	Clinical option	II
	CR2	Standard of care	II
	>CR2	Standard of care	II
ALL	CR1 (low risk)	Generally not recommended	III
	CR1 (high risk)	Clinical option	II
	CR2	Clinical option	II
	>CR2	Clinical option	II
CML	Chronic phase	Clinical option	II
	Advanced phase	Clinical option	II
NHL	CR1 (low risk)	Generally not recommended	II
	CR1 (high risk)	Clinical option	II
	CR2	Clinical option	II
HL	CR1	Generally not recommended	II
	First relapse, CR2	Clinical option	III
MDS		Clinical option	III
Non-malignant disorders and solid tumors			
Primary immunodeficiencies		Standard of care	II
Thalassemia		Clinical option	III
Sickle cell disease (high risk)		Clinical option	III
Aplastic anemia		Clinical option	II
Fanconi anemia		Clinical option	II
Blackfan-diamond anemia		Clinical option	III
Chronic granulomatous disease		Clinical option	III
Kostman's disease		Clinical option	III
MPS-1H Hurler		Clinical option	II
MPS-1H Hurler Scheie (severe)		Generally not recommended	III
MPS-VI Maroteaux-Lamy		Clinical option	II
Osteopetrosis		Standard of care	II
Other storage diseases		Generally not recommended	III
Autoimmune diseases		Generally not recommended	II
Germ cell tumor		Clinical option	II
Ewing's sarcoma (high risk or >CR1)		Developmental	III
Soft tissue sarcoma (high risk or >CR1)		Developmental	III
Neuroblastoma (high risk)		Developmental	III
Neuroblastoma (>CR1)		Developmental	III
Wilm's tumor (>CR1)		Generally not recommended	III
Osteogenic sarcoma		Generally not recommended	III
Brain tumors		Generally not recommended	III

Modified from Sureda A. et al. [138]

the European Blood and Marrow Transplant group on the outcome of haplo-HSCT in children transplanted for very high risk ALL, showed five-year TRM, relapse incidence, and DFS of 37%, 36%, and 27%, respectively. The study highlighted the importance of performing the transplant in remission, using CD34+ cell megadose, and indicated a significant impact of center experience (centers performing large numbers of allo-HSCT: DFS of 39% vs 15% in small centers) [90]. Recently, a multicenter Italian study comparing the outcome of Tαβ/B cell depleted haplo-HSCT vs UD-HSCT in children with acute leukemia transplanted with a myeloablative regimen reported primary engraftment in 95 of 97 patients receving haplo-HSCT and, with the only pharmacologic GVHD prophylaxis of pretransplant ATG in the haplo-HSCT setting, 16% and 0% grade II-IV and III–IV acute GVHD, respectively, as compared to 39% and 12% in UD-HSCT recipients [23]. After a median follow-up of 3.3 years, the 3-year leukemia-free survival was 63% vs 62% in the UD-HSCT setting, with chronic GVHD rates of 6% vs 20%, respectively. Encouraging results in the setting of ALL and AML were also reported from other groups. Lang et al. reported on 41 children with acute leukemia, MDS and nonmalignant diseases receiving Tαβ/B cell depleted haplo-HSCT with conditioning regimens consisting of fludarabine or clofarabine, thiotepa, melphalan and serotherapy with OKT3 or ATG-Fresenius [94]. Primary engraftment occurred in 88%, acute GvHD grades II and III-IV occurred in 10% and 15%, respectively. The 1.6 year survival rate was 51%, with 41% relapse being the major cause of death. With the same manipulation approach, and a conditioning based on treosulfan, melphalan, fludarabine and ATG, Maschan et al. reported 100% primary engraftment and 39% acute GVHD II-IV in 33 children transplanted with UD-HSCT or haplo-HSCT for AML. At 2 years, the cumulative incidence of relapse was 40% in the haplo group, with a DSF of 59%, whereas TRM was 0% [106].

Regarding the T-cell replete approach, a recent pilot study of PTCY-based haplo-HSCT, showed a cumulative incidence of disease progression of 26% in children with acute leukemia, with 24% NRM and 40% aGVHD. Out of a total of ten grades II–IV acute GVHD cases, severe GVHD occurred exclusively in children below the age of 10 years, and the authors hypothesize defective clearance of alloreactive T cells due to altered CY metabolism in the young age group [78]. A multicenter Italian study of T-cell replete haplo-HSCT based on myeloablative or reduced-intensity conditioning, and GVHD prophylaxis with PTCY, MMF and calcineurin inhibitor, conducted in 33 children with high-risk hematologic malignancies and lacking a match-related or -unrelated donor, showed aGVHD and cGVHD rates of 22% and 4%, respectively, with a DFS rate of 61%, 24% cumulative incidence of relapse and 9% TRM [21].

Regarding lymphoma, the experience in children is limited. Broader use has been hampered for a long time mainly by high TRM, offsetting the advantage of a GVL effect. However, since the use of nonmyeloablative conditioning and T-cell replete haplo-HSCT with PTCY, results in adult patients have dramatically

improved. In a cohort that included also adolescents, Burroughs and colleague first observed an advantage of haplo-HSCT on matched related and unrelated donor HSCT in HD, as, due to a lower TRM with comparable disease control, the haplo group showed a 51% DFS compared to <30% in the other two groups [33]. Recent data from two large multicenter, retrospective, registry studies showed similar results in adult patients with HD and NHL transplanted with PTCY haplo-HSCT. The analysis by Kanate et al. registered the same relapse rate of 36% compared with UD HSCT, despite haplo cohort having higher disease risk index scores, but less acute and chronic GVHD, with a OS of 60% vs 50%, respectively [82]. Gosh et al. compared haplo-HSCT with matched sibling donor HSCT in adults, finding superimposable TRM and PFS rates, but significantly less chronic GVHD in the haplo-HSCT cohort [65]. As relapse remains the major cause of treatment failure, it will be important to use the haplo-HSCT platform as a basis for GVL effect, by integrating transplant with novel immunological therapies. An example in the pediatric setting is the use of DLIs modified by insertion of the inducible caspase 9 suicide gene, that proved of efficacy in two patients with lymphoma enrolled in a phase I trial [155].

11.4.2 Haplo-HSCT in Severe Aplastic Anemia

Currently, haplo-HSCT in acquired severe aplastic anemia (SAA) is reserved to children who have failed previous immunosuppressive treatment with ATG and cyclosporine-A and who do not have a suitable matched family or unrelated donor or cord blood unit, or to patients who have rejected a previous unrelated donor transplant [15, 16].

In the recent years, several series of children [59, 76, 147, 148, 150, 151, 153] and adults [41, 50, 57, 97] with SAA and given haplo-HSCT have been reported. However, the number of pediatric patients in each study is often relatively small, and the preparative regimens and GVHD prophylaxis are different. Nevertheless, the reported average 1-year EFS is good and in the order of 75% or more. Both T-cell depletion and unmanipulated bone marrow or peripheral blood stem cells have been successfully used. Because of the high risk of rejection, unmanipulated bone marrow or peripheral blood stem cells have been preferred in several cases, usually in combination with high-dose PTCY or with monoclonal antibodies or ATG as GVHD prophylaxis.

In conclusion, even if it is still in the experimental stage, haplo-HSCT should be considered in patients with SAA failing first-line immune suppressive therapy and lacking an HLA-matched related or unrelated donor. Unfortunately, the data available so far do not allow to make strong recommendations regarding the best conditioning regimen, the optimal composition of the graft, and the best GVHD prophylaxis strategy. Both *ex vivo* T-cell-depleted and unmanipulated graft strategies have been explored; results show comparable efficacy and acceptable toxicities of both these approaches [15, 39, 64].

11.4.3 Haplo-HSCT in Constitutional Cytopenias

Constitutional bone marrow failure syndromes represent a group of rare genetically and phenotypically heterogeneous disorders characterized by the variable presence of multiple congenital somatic abnormalities, the gradual onset of bone marrow failure involving one or more hematopoietic cell lineages, and the predisposizion to develop clonal hematopoietic disorders as well as, in some cases, solid tumors [24]. The bone marrow insufficiency can be uni-linear, such as usually in Diamond-Blackfan anemia or in congenital amegakaryocytic thrombocytopenia, or it can involve all the three lineages, such as in Fanconi anemia, diskeratosis congenita or Shwackmann-Diamond syndrome. Also the degree of cytopenia is variable among the different disease and can worsen over time: in Fanconi anemia the cytopenia is typically absent at birth and usually appears during childhood, while in Diamond-Blackfan anemia the hyporegenerative anemia appears in infancy [24].

Optimized supportive care, including red blood cell and platelet transfusions, and prevention of infectious complications, are critical for the conservative management of these patients [139]. Some children, namely those affected by Fanconi anemia and dyskeratosis congenital, can benefit from treatment with androgens, while those with Diamond-Blackfan anemia can improve anemia with steroid treatment. Allogeneic HSCT is currently the only curative treatment able to restore normal hematopoiesis. Nevertheless, the underlying defect in DNA repair, typical for example of Fanconi anemia, is responsible of the hypersensitivity to the treatment with irradiation and alkylating agents as cyclophosphamide, leading to excessive regimen-related toxicity and severe acute GVHD [66]. Furthermore, a strong association between chronic GVHD and the development of secondary malignancies (squamous cell carcinoma) has been demonstrated [26, 122], thus increasing the risk of late mortality notwithstanding the cure of bone marrow insufficiency.

Current evidence in the medical literature on the use of haplo-HSCT in this particular setting is often limited to case reports and small retrospective case series [2, 54, 101, 141]. Recently, Zecca et al. described 12 children with Fanconi anemia treated with haplo-HSCT, who received T cell-depleted, CD34+ positively selected stem cells after a conditioning regimen including fludarabine (30 mg/m^2/day for 4 days), cyclophosphamide (300 mg/m^2/day for 4 days), rATG (10 mg/kg/day for 4 days) and single dose TBI (200 cGy). Survival and DFS were 83%, while the cumulative incidence of TRM was 17%, with no fatal regimen-related toxicity. The incidence of acute and chronic GVHD was limited. Low infused CD34+ cell dose seemed to correlate with graft rejection [152]. Bertaina et al. described four further patients who received the same conditioning regimen, and were successfully transplanted using T-cell depleted PBSC after T α/β + and B CD19+ negative selection [22]. Furthermore, Bonfim et al. reported 30 children with Fanconi anemia given haplo-HSCT with unmanipulated bone marrow and post-transplant cyclophosphamide (25 mg/kg/day on day +3 and +4) [27]. The conditioning regimen included fludarabine (150 mg/m^2), cyclophosphamide (10 mg/kg) and single dose TBI (200 cGy). Pre-transplant rATG (4–5 mg/kg) was added to the conditioning regimen

after the first 12 transplants, because of the high incidence of severe acute and chronic GVHD. Hemorrhagic cystitis occurred in 50% of the patients, but overall survival was 73% with all surviving patients achieving full donor chimerism.

Taken together, these results demonstrate the feasibility of haplo-HSCT also for Fanconi anemia patients and, more in general, for children with constitutional bone marrow failure syndromes. However, because of the peculiar frailty of this heterogeneous patient population, particular attention must be payed to the choice of the conditioning regimen, because of the high regimen-related toxicity. Furthermore, a very effective GVHD prophylaxis should be adopted, in view of the strong association between chronic GVHD and secondary malignancies in otherwise cured long-term survivors.

11.4.4 Haplo-HSCT in PID

Primary immunodeficiencies (PID) are a group of heterogeneous diseases, many of which are caused by monogenic defects, resulting in susceptibility to life threatening infections, uncontrolled inflammation, or autoimmunity. Historically, allogeneic HSCT has been a curative option for several primary PID, including severe combined immunodeficiency (SCID), Wiskott-Aldrich syndrome (WAS), chronic granulomatous disease (CGD) hemophagocytic lymphohistiocytosis (HLH) and many others [13, 63]. This field has rapidly expanded over the last years. Currently, more than 300 PIDs have been genetically defined and 34 new genetic disorders have been added to the International Union of Immunological Societies (PIDTC) PID classification in the last 2 years [124]. Many of these diseases can be cured by allogeneic HSCT even if, given the heterogeneity and rarity of some diseases, in some cases the indication to HSCT can be controversial. Table 11.2 summarizes the most important, established or still debated indications [71].

HSCT in PID can be a challenge. Comorbidities such as chronic infections and severe pulmonary dysfunction, that could make patients ineligible to the procedure, are common. Myeloablation may be avoided in order to reduce excessive toxicity, but reduced-intensity regimens could lead to higher rejection rate or to increased mixed chimerism. Also the degree of donor engraftment necessary for disease cure is yet not completely understood. In children with SCID, HSCT is considered an urgent and life-saving procedure, while in other forms of PID, where the immune defect does not result in an imminent risk, the transplant could be delayed until a properly matched donor is found. Indeed, a causative molecular defect can be identified in many patients with PID, leading to formulation of a definitive diagnosis. In this case, the decisional process is relatively simple and allows to rapidly proceed to the transplant on the basis of the existing knowledge about the underlying disease. Unfortunately, in other cases, in which a genetic diagnosis cannot be achieved, the decision to transplant is often delayed until the susceptibility to severe recurrent infections or autoimmunity are clearly demonstrated.

Table 11.2 Indications to HSCT in immunofeficiency disorders

Efficacious and recommended
Chronic granulomatous disease
DOCK8 deficiency
GATA2 deficiency
Griscelli syndrome, type II (RAB27A deficiency)
Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX)
Leukocyte adhesion deficiency type I
Perforin deficiency
Severe combined immunodeficiency syndrome
Severe congenital neutropenia
Wiskott Aldrich syndrome
X-linked hyper-IgM syndrome
X-linked lymphoproliferative disease type I
X-linked lymphoproliferative disease type II
May be efficacious but still limited evidence
Adenosine deaminase type II deficiency
Autosomal dominant hyper IgE syndrome
C1Q deficiency
CD25 deficiency
CTLA-4 haploinsufficiency
IL-10 deficiency
IL-10 receptor deficiency
LRBA deficiency
Nijmegen breakage syndrome
PGM3 deficiency
STAT1-gain of function
STAT3-gain of function
Warts, hypogammaglobulinemia, infections, myelokathexis (WHIM)
Still controversial
Common variable immunodeficiency
Di George syndrome
IκBα deficiency (NFKBIA deficiency)
NEMO deficiency (IKBKG deficiency)
X-linked agammaglobulinemia
X-linked thrombocytopenia

Modified from Hagin D. et al. [71]

The major advantage of using a haplo-HSCT is that a healthy donor, usually a parent, is immediately available so that the transplant can be performed very quickly. In the study of the Primary Immunodeficiency Treatment Consortium (PIDTC), reporting 240 infants with SCID transplanted between 2009 and 2009, more than 50% of the patients (138/240) received a transplant from a partially matched related

donor [118]. Children who received a T-cell depleted graft from partially matched related donors and did not received any conditioning regimen had a survival probability of 79% while those receiving any type of conditioning had a survival probability of 66%. However, the use of a reduced intensity or myeloablative conditioning was associated with improved T-cell count and better B-cell function. Older age (>3.5 years) and active infection at time of HSCT were associated with lower survival rate, while children transplanted in early infancy (<3.5 months) had an excellent outcome, similar to that of patients transplanted from a matched sibling, even if grafted from an alternative donor.

Also some patients with WAS and given haplo-HSCT, either with or without T-cell depletion, have been reported in different series of patients usually including also transplants from matched family or unrelated donors [17, 77, 88, 93, 109, 112, 133]. These studies show that haplo-HSCT can be an effective form of treatment. However, it must be noted that in the setting of WAS a mixed chimerism appeared to have a strong detrimental effect on EFS because of an increased incidence of autoimmunity [117]. For this reason, a stable multilineage donor engraftment is required to fully correct the disease [109] and this consideration supports the use of fully myeloablative conditioning regimens, in order to minimize the chance of autologous reconstitution and recurrence or persistence of the WAS phenotype.

Also rare cases of haplo-HSCT in children with CGD have been recently reported [73, 111, 120, 154]. However, the experience with CGD is still too limited to give specific recommendations and haplo-HSCT in CGD should sill be considered experimental.

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening hyperinflammatory clinical syndrome with uncontrolled immune response which results in hypercytokinemia due to an underlying primary or secondary immune defect. HCT is recommended in patients with documented familial HLH, recurrent or progressive HLH despite chemoimmunotherapy, and CNS involvement [80]. The selection of the optimal stem cell donor and source, as well as of the conditioning regimen, is important for HLH patients undergoing HSCT, because of the high risk of rejection and unstable mixed chimerism reported in this disease [116]. HSCT using haploidentical donors for HLH patients who do not have matched donor was demonstrated to be feasible, and the outcome has improved over time [55, 96, 98, 115, 116].

Conventional myeloablative conditioning regimens, mostly including busulfan, cyclophosphamide and etoposide with or without ATG have been usually adopted. However, it has been reported that the outcome of myeloablative conditioning for HLH can be impaired by high early TRM. The increased TRM has prompted the use of less toxic approaches adopting reduced intensity regimens. The combination of alemtuzumab, fludarabine, and melphalan has demonstrated good efficacy [46, 105]. Also melphalan or treosulfan are promising alternatives. Fludarabine, treosulfan, alemtuzumab, and thiotepa could represent a conditioning regimen with a high rate of disease-free survival and low toxicity [96]. Reduced intensity

conditioning before haplo-HSCT was reported to sufficiently restore immune regulation in infants with FHL, while decreasing TRM and long-term sequelae [115]. However, the high incidences of mixed chimerism and graft loss still remains a significant concern in HLH patients.

11.4.5 Haplo-HSCT in Hemoglobinopathies

Allogeneic HSCT offers a potentially curative treatment for patients with hemoglobinopathies, mainly thalassemia major (TM) and severe sickle cell disease (SCD). Nevertheless, so far the applicability of HSCT has been limited mainly by donor availability, with a less than 20% of eligible patients having a HLA-matched sibling donor [1]. Furthermore, the probability of finding a potential MUD is largely dependent on the ethnic and racial background [68].

In TM, transfusion-dependency is an indication for HSCT, especially in younger patients before development of iron-related tissue damage. In SCD, allogeneic HSCT is currently limited to patients with a severe phenotype, with frequent vaso-occlusive crises and acute chest syndrome that are unresponsive to hydroxyurea, with end organ damage such as stroke or osteonecrosis of multiple joints, and with severe disease that has been associated with an increased risk of early mortality and/or requiring regular transfusion therapy [7, 89].

Haplo-HSCT could significantly increase the donor pool for patients with SCD, who historically have limited donor options. In adult patients with SCD (age 15–46 years), a single-center experience using a novel non myeloablative haploidentical or HLA-mismatched donor regimen employing PTCY showed the feasibility of this approach: more than 80% of eligible patients had suitable related haploidentical donors, and in the cohort of 14 haploidentical recipients with SCD, 60% of the patients engrafted with no deaths or significant GVHD; however, the graft failure rate was 43%. So, the experience using this approach is still relatively limited and remains under clinical investigation [25]. In 2013, Dallas et al. reported eight children with SCD given unmanipulated haploidentical HSCT after a myeloablative conditioning regimen and a GVHD prophylaxis based on the use of an anti-CD3 monoclonal antibody. However, only three of the eight patients achieved a sustained engraftment and are alive and disease-free, while graft failure and SCD recurrence were observed in 38% of the patients [48]. A further study was published in 2017 by Foell et al., describing nine children or adolescents with SCD and given haplo-HSCT [60]. The conditioning regimen included thiotepa, treosulfan, fludarabine and ATG, and GVHD prophylaxis consisted of CD3+/CD19+ depletion followed by the administration of cyclosporine-A and mycophenolate until day +120 post-HSCT. All nine children achieved stable engraftment with only one patient dying of transplant-related complications.

Moving to TM patients, two studies evaluated the role of haplo-HSCT in children. In 2010, Sodani et al. presented 22 patients with TM given HSCT from their haploidentical mother after an intensive conditioning regimen and a GVHD prophylaxis based on T-cell depletion. The reported survival probability was 90%, the thalassemia-free survival was 61% and the cumulative incidence of the rejection was 29% [134]. More recently, in 2016, Anurathapan et al. described 31 patients (median age 10 years, range 2–20) transplanted after a myeloablative conditioning regimen (busulfan + fludarabine + ATG) and with a GVHD prophylaxis including PTCY, tacrolimus and mycophenolate mophetil [8]. The authors reported a survival probability of 95%, an EFS probability of 94%, with only 2 out of the 31 patients rejecting the transplant. In both studies, all patient received an intensive pre-conditioning treatment, including high-dose hydroxyurea, azathioprine, hematopoietic growth factors, hypertransfusion regimen and intensive iron chelation [134] or 2 cycles of fludarabine (40 mg/m^2/day for 5 days) and dexamethasone (25 mg/m^2/day for 5 days) [8]. The pre-conditioning treatment was administered in order to decrease marrow cellularity and to suppress erythropoiesis, with the aim of reducing the risk of rejection and graft failure. The pre-conditioning immunosuppressive and myelo- and erithrosuppressive treatment could play a relevant role in the success of the transplant in this otherwise difficult disease, and deserves further investigation.

Overall, despite the higher incidence of graft rejection as compared to other diseases, these recent results are encouraging because of the low toxicity of the procedure. Only few patients with TM and, still less with SCD, often belonging to ethnic minorities with rare HLA phenotype [68], are able to receive a HLA compatible donor transplant. The use of haploidentical donors could extend the use of HSCT in a setting where this procedure is still largely underutilized. Prospective trials are needed to determine the risk-benefit ratio of this approach, and many such studies are currently ongoing.

11.5 Strategies to Enhance Immune Reconstitution and GVL After Haplo-HSCT

As we have seen in previous sections, transplant-related mortality (in the pediatric setting mostly ascribable to opportunistic infections), and relapse in patients transplanted for malignancy, are the major causes of failure after T-cell depleted haplo-HSCT. These complications are likely related to the delayed immune reconstitution, and, in order to overcome their development, different means to boost immune surveillance have been implemented.

Proof of principle studies had demonstrated the feasibility to administer unmanipulated donor lymphocytes (DLI) to treat viral complications or leukemia relapse after T-cell depleted HSCT [91, 119]. The rate of acute GVHD developing after the procedure, however, prompted manipulation of donor lymphocytes to

reduce alloreactivity while maintaining immune surveillance potency. Two strategies have been explored to reduce the risks derived from alloreactivity associated with DLI. The first approach was based on transduction of nonspecific T cells with a retroviral construct containing suicide genes, to induce susceptibility to drug-mediated lysis in case of development of alloreactive response [28]. Infusion of HSV-thymidine kinase gene-marked lymphocytes has proved safe and devoid of adverse effects [38]. However, its mechanism of action requires interference with DNA synthesis so that cell killing may take several days and be incomplete, resulting in a delay in clinical benefit. Recently, an alternative strategy that relies on inducible caspase proteins (iCasp9) to exploit the mitochondrial apoptotic pathway has been explored. The use of DLI modified by iCasp9 cell-suicide system in a small cohort of children transplanted for acute leukemia demonstrated the potential advantages in terms of rapid and consistent cell removal in case of GVHD development [53]. Escalating doses of iCasp9-modified DLI have been employed in 20 pediatric patients receiving Tα/β depleted haplo-HSCT for PID, and proved safe (25% cumulative incidence of aGVHD, no TRM) and able to provide prompt immune reconstitution [83].

An alternate strategy consists in delivering infectious/leukemia antigen-specific T cells selected by cell culture or by sorting. A major breakthrough was achieved by the adoptive transfer of virus-specific cytotoxic T lymphocytes reactivated from the peripheral blood of HSCT donors as prophylaxis/treatment against CMV disease or EBV-positive post-transplant lymphoproliferative disease in patients given T-cell depleted, HLA-disparate, unrelated HSCT [127, 145]. This approach has been successful in preventing and treating infectious complications after T-cell depleted haplo-HSCT, both in the pediatric and adult setting, while limiting the risk of inducing GVHD [44].

In the setting of leukemia, attempts have been made to boost tumor-specific responses and control leukemia relapse by post-transplant add-backs of donor cytotoxic T cells (CTLs) directed towards patients blasts [58], minor histocompatibility antigens [149], or leukemia-related antigens [43]. One of the main limitations is that CTL antigen recognition is major histocompatibility complex (MHC)- restricted. Moreover, in many cases, tumor-specific antigens able to elicit protective immune responses have not been identified.

To extend the recognition specificity of T lymphocytes beyond their classical MHC-peptide complexes, a gene-therapeutic strategy has been developed that allows redirecting T cells to defined tumor cell surface antigens, by the transfer of an antigen-binding moiety, most commonly a single chain variable fragment derived from a monoclonal antibody, together with an activating T-cell receptor (chimeric antigen receptors, CARs). Recently, CARs directed to the CD19 molecule, expressed on B-cell malignancies, have been employed in pediatric and adult patients with refractory ALL and proven highly efficient, with CR rates of 70–90% [92, 95, 107, 142]. These studies included patients with a prior history of allogeneic HSCT, and no GVHD was recorded. A phase I study of CD19 CAR T cell infusion

after autologous and allogeneic HSCT included also 8 haplo-HSCT recipients, and the OS and DFS at 12 months for the haplo group were 100% and 75%, respectively. In the allogeneic setting, CAR T cell doses up to $108/m^2$ were safe and did not exacerbate GVHD [86].

It has been shown that leukemia blasts may escape immune control mediated by T cells and cause relapse by losing HLA mismatched alleles after HSCT, due to an acquired uniparental disomy, with consecutive total loss of the HLA-mismatched haplotype [144]. In this case, infusion of selected and/or activated NK cells may help control leukemia relapse. In addition, NK cells mostly target hematopoietic cells sparing solid organs, suggesting that an NK-mediated antitumor effect can be achieved in the absence of GVHD.

Studies have shown that infusion of haploidentical NK cells to exploit KIR/HLA alloreactivity is safe and can mediate impressive clinical activity in some patients with AML [128], and donor NK cells have been infused after haplo-HSCT with some evidence of efficacy [37, 79, 135]. Despite reports of clinical efficacy, a number of factors limit the application of NK cell immunotherapy for the treatment of cancer, such as the failure of infused NK cells to expand and persist in vivo. Therefore, means to maximize NK persistence and efficacy are currently being implemented.

11.6 Conclusions

Dramatic progress in the outcomes of haplo-HSCT in pediatric patients has been registered over the past decade, providing a chance to cure the children and adolescents in need of a HSCT.

Although the optimal strategy to overcome the HLA–histoincompatibility barrier is still debated, results in the pediatric populations appear equally encouraging with both T-cell depleted and T-replete HSCT approaches. In order to evaluate which strategy may be more appropriate in the different disease settings, multicentre controlled/randomized trials will have to be eventually conducted. Haplo-HSCT with PTCY has the potential to be the preferred transplant option for patients without HLA-matched donors in developing countries, where cell processing laboratories with specialized expertise and unrelated donor registries may be difficult to establish and maintain.

The excellent results obtained with Tαβ/B cell depleted haplo-HSCT, as well as with T-replete HSCT with PTCY, could challenge, in the near future, the current hierarchical algorithm in which MUD and unrelated cord blood are preferred to haploidentical donors, also in view of the possibility to exploit postransplant immune interventions in malignancy. Recent studies comparing haplo-HSCT to other types of allo-HSCT in both adult and children suggest that such a step may not be far to come.

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Chapter 12
Innovative Approaches to Increase the Success of the Haploidentical SCT

Ulas D. Bayraktar and Stefan O. Ciurea

12.1 Introduction

While T cells in the donor graft facilitate engraftment, contribute significantly to immune reconstitution, and eliminate residual disease after allogeneic hematopoietic stem cell transplantation (AlloSCT), they are the primary culprit in the development of graft-versus-host disease (GVHD). Consequently, too much suppression of T cells may derail immune recovery and lessen graft-versus-disease (GVD) effect leading to higher transplant-related mortality (TRM) and relapse rates, while too less may not prevent GVHD. This is particularly true after HLA-haploidentical hematopoietic stem cell transplantation (HaploSCT), in which a high HLA-histoincompatibility barrier needs to be crossed. Consequently, two primary strategies utilized in HaploSCT, ex vivo T-cell depletion of grafts and intensification of pharmacological immunosuppression, led to control of GVHD and improvements in TRM with relapse of the disease becoming the leading cause of treatment failure. Both methods indiscriminately eliminate T cells. Although post-transplant cyclophosphamide is thought to primarily eliminate alloreactive T cells, it is likely that T cells directed against leukemia are also affected hence relatively high relapse rates seen in leukemia patients after HaploSCT using posttransplant cyclophosphamide.

Preserving immune cells directed against infectious agents and underlying disease while eliminating alloreactive ones with potential to induce GVHD has been the holy grail of transplantation. This requires either ex vivo manipulation of the cell

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products or in vivo pharmacological targeting of alloreactive cells. The current focus is on the former since there is no proven agent to specifically target alloreactive cells although drugs such as bortezomib [1] and ibrutinib [2] may be promising. As per manipulation of the grafts, ex vivo allodepletion of the graft using anti-CD25 antibodies [3] or photodepletion methods [4] show mixed results. While pathogen-specific T cells were shown to improve treatment of CMV [5] and EBV [6] disease post-transplant, they might not be practical for prophylaxis.

With recent advances in immunology and better understanding of T cell development, the content of cell products and their infusion timing may soon be tailored to promote immune surveillance against leukemia and infectious agents while keeping GVHD at bay. Regulatory T cells, memory T cells, natural killer (NK) cells, and γδ T cells are current targets to enrich in cell products. Engineering T cells to express chimeric antigen receptors (CARs) or suicide genes are other innovative approaches to boost immune surveillance post-transplant without invoking GVHD.

12.2 Co-Infusion of Regulatory and Conventional T Cells

Regulatory T cell (Tregs) maintain immune homeostasis and immune self-tolerance. In murine models of mismatched transplantation, Tregs were shown to suppress lethal GVHD [7] and favor post-transplant immune reconstitution when coinfused with conventional T-cells (Tcon) [8]. While the role of Tregs on GVL has been debated, recent evidence suggests co-infusion of Tregs and Tcons protected mice from GVHD while preserving GVD effect in mismatched transplant models [9–11]. To improve GVD effect and immunologic reconstitution with Tcons while preventing GVHD with Tregs, the Perugia group infused donor Tregs before the infusion of mega-doses of T cell depleted peripheral blood progenitor cells and donor Tcons without any post-transplant immunosuppression [10]. Tregs were selected by first depleting CD8+/CD19+ cells in the leukapheresis product and then selecting CD25+ cells. Only 2 of 28 patients developed aGVHD and none developed cGVHD. Although a wide T-cell repertoire developed rapidly, 8 patients still died of opportunistic infections. This study suggested that adoptive immunotherapy with Tregs counteracted the GVHD potential of conventional T-cells in HaploHCT, however, the high incidence of opportunistic infections and treatment-related mortality remained a concern [10].

Long-term results of Tcon-Treg co-infusion was then compared to those of historical controls by the Perugia group [11]. Forty-three patients with high-risk leukemia (10 ALL, 33 AML), underwent HaploHCT after a myeloablative conditioning regimen consisting of total body irradiation (8 Gy), fludarabine, thiotepa, and cyclophosphamide. Cyclophosphamide was latter changed to alemtuzumab/ATG to decrease rates of veno-occlusive disease and extra-hematological toxicities. Patients received freshly isolated 2×10^6/kg Tregs on day-4, followed by a megadose of CD34+ cells and 1×10^6/kg Tcons on day 0. Six patients (15%) developed

grade ≥2 acute GVHD which was similar to 11% in historical controls. At a median follow-up of 46 months, non-relapse mortality (NRM) was 40% overall and 21% in 18 patients who received anti-T antibodies instead of cyclophosphamide during conditioning. Of 41 evaluable patients, only 2 have relapsed with a significantly lower cumulative incidence of relapse compared to that of historical controls (5% vs. 21%, p = 0.03). To further demonstrate anti-leukemic effect of Treg-Tcon infusion, several murine studies were performed that revealed all mice that received human leukemic cells with or without Tregs died of leukemia while all mice that received leukemic cells with both Tregs and Tcons were rescued from leukemia without GVHD. Those that received leukemic cells with Tcons died of GVHD. This study demonstrated that Tregs protected most of patients against GVHD while preventing leukemia relapse. Although accompanying immunological studies showed earlier emerging of various virus and fungi specific T cells compared to historical controls, NRM rate was still unsatisfactorily high.

12.3 Alpha-Beta T Cell Depletion

Selection of T cells by T cell receptor (TCR) phenotype has proven useful in discriminating T cells capable of eliciting GVHD from others. γδ T cells, with TCRs made up of one γ (gamma) and one δ (delta) chain, are a unique population of lymphocytes possessing properties of both innate and adaptive immune system with rearranged TCRs producing diversity and rapid, innate-like responses [12]. Importantly, it has been suggested that γδ T cells do not require antigen processing and HLA presentation of antigens rendering them unlikely to generate GVHD [13]. Moreover, a faster recovery of γδ T cells after SCT has been associated with longer disease-free survival [14]. Similarly, natural killer (NK) cells which are involved in innate immune system [15], attack primarily hematopoietic cells sparing the solid organs, rendering them almost incapable of causing GVHD [16]. According to the widely used "missing self" model, a NK cell recognizes a cell as foreign when the particular cell lacks one or more HLA class I alleles specific to the inhibitory receptors (killer immunoglobulin-like receptors, KIRs) on the NK cell [17, 18]. After HaploSCT, if donor NK cells express KIRs that are not engaged by any of the class I MHC molecules on the recipient cells, these "alloreactive" NK cells may help to eradicate the remaining leukemia cells after the conditioning regimen and to clear residual lymphocytes and APCs, potentially preventing graft rejection and GVHD [19].

Accordingly, methods to deplete αβ T cells preserving γδ T cells and NK cells have been developed [20]. Bertaina et al. reported their results in 45 children (median age of 10 years) with acute leukemia who underwent HaploHCT with TCR-αβ and CD19 depleted PB grafts [21]. Pre-transplant anti-thymocyte globulin was the only pharmacologic GVHD prophylaxis used. Primary engraftment was achieved in 44 patients and only observed acute GVHD were grade I-II skin-only in

13 children. Two patients died of infectious complications. With a median follow-up of 11 months, the 2-year leukemia-free survival was 75%. Similar results were obtained in 23 children (age 0/0.4–12) with non-malignant hematological diseases of whom 21 engrafted after ablative conditioning [22]. Acute GVHD occurred only in 3 patients with skin involvement grade 1–2. Two patients died of infectious complications. With a median survival of 18 months, overall survival at two-years was 91%. On the other hand, the Tuebingen group observed grade II-IV aGVHD in 10 (25%) of 41 children with malignant and non-malignant hematological diseases [23]. Larger studies are needed to better assess outcomes of patients who receive TCR-αβ depleted grafts.

12.4 CD45RA Depletion

T cells differ in their functional activity and various classification schemes exist according to their cell surface phenotype [24–26]. Majority of T cells that can respond to minor H antigens and cause GVHD are thought to be naïve (T_N, never exposed to their cognate antigen) with a $CD45RA^+CD62L^+$ surface phenotype [27]. Several in vitro and mouse studies support this hypothesis [28–32]. Consequently, depletion of $CD45RA^+$ naïve T cells has been explored using CliniMACS magnetic bead separation system [33, 34]. Because a subset of CD34+ hematopoietic progenitor cells express CD45RA [35], Bleakley et al. devised a 2-step procedure in which first donor pheresed PB is selected for CD34+ cells and then CD34-negative fraction was depleted for CD45RA to preserve all CD34+ cell subsets [34]. Additionally, investigators at St. Jude reported their experience with HaploHCT using CD45RA depleted grafts in 17 patients with hematological malignancies (ages 8–19). HPCs were obtained after G-CSF mobilization from peripheral blood. The product on 5th day of G-CSF was positively selected for CD34 while the product on 6th day was depleted for CD45RA. Five days after the second collection, leukapheresis was performed again and NK cells were enriched through CD3 depletion and CD56 selection [36]. Patients received ablative conditioning with total lymphoid irradiation, fludarabine, cyclophosphamide, thiotepa, and melphalan followed by CD34+ selected product on day 0, CD45RA depleted product on day +1, and NK cells on day +6. Sirolimus or mycophenolate mofetil was started on day +13. A 3.6 log depletion in CD45RA+ cells was achieved in the final product to be infused. All patients engrafted successfully. Three patients had CMV reactivation but none have progressed to CMV disease. Three patients (18%) developed aGVHD. On post-transplant day 30, almost all T cells were negative for CD45RA suggesting that T cells in the early post-transplant period were adoptively transferred. After a median follow-up of 223 days, 13 patients were alive. None of the patients died of infectious complications.

12.5 Engineered Donor Lymphocytes with a Safety Switch

Infusion of engineered T cells with safety switches may promote post-transplant immune reconstitution with a safety switch to counteract GVHD should it develop post-infusion. In 2009, Ciceri et al. reported Milan experience in 28 patients who underwent HaploHCT with T cell depleted peripheral blood grafts and received donor lymphocytes engineered to express herpes simplex virus-thymidine kinase suicide gene (TK-cells) monthly for four times post-transplant [37]. TK cells' suicide could be triggered by the use of ganciclovir. In 22 patients, TK cells were engrafted successfully. Immune responses against CMV and EBV improved after TK-cell infusions. Without any GVHD prophylaxis, 10 patients developed acute GVHD and required ganciclovir resulting in abrogation of GVHD in all. There were no GVHD related deaths or long-term complications [37]. Despite the promising results, ganciclovir is not the optimal drug to use as a trigger after transplant since it is a commonly used drug to treat CMV.

An alternate approach was developed by the Baylor group using a bio-inert molecule, AP1903 to trigger suicide in donor lymphocytes engineered to express an inducible caspase-9 transgene (iC9) [38]. In all 10 pediatric patients (age 3–17) who underwent HaploHCT with T cell depleted grafts and were infused iC9-T cells between 30 and 90 days after transplantation, iC9-T cells were engrafted successfully [39]. No immediate toxicities related to infusion were observed. Five patients developed GVHD within 2–6 weeks of infusion and received one dose of AP1903. Within 2 h of AP1903 administration, iC9-T cells were >90% eliminated and GVHD was rapidly reversed without subsequent recurrence. AP1903 did not affect T-cell immune reconstitution in these patients. In four patients with evidence of viral reactivation or disease before infusion of iC9-T cells, viral replication resolved within 4 weeks of iC9-T cell infusion. Furthermore, AP1903 administration did not significantly affect anti-viral immune reconstitution in three patients with active viral disease who received AP1903 to control acute GVHD [39]. Clinical trials using this approach are ongoing. Although feasible and interesting, engineering T cells is expensive and available only at select centers.

12.6 T Cells with Chimeric Antigen Receptors (CAR)

Infusion of donor lymphocytes expressing suicide genes promotes immune reconstitution broadly and non-specifically that may not prevent relapses post-transplant significantly. CARs are fusion proteins with an extracellular antigen recognition moiety and intracellular T-cell activation domain that can direct T cells against specific antigens. Infusion of T cells engineered to express CARs (CAR T cells) may decrease relapse incidence after HaploSCT. Kochenderfer et al. reported their findings in 10 patients who had B cell malignancies that persisted after AlloSCT from matched donors and donor lymphocyte infusions. All patients

received a single dose of anti-CD19 CAR T cells [40]. Two patients achieved response lasting >3 and >9 months after CAR T cell infusion, while 6 patients achieved stable disease lasting between 1 to more than 11 months. None of the patients developed GVHD after infusion. Extending the use of CAR T cells after HaploHCT is also feasible, with cells generated from the same donors as progenitor cells. The MD Anderson group recently published results of a phase 1 study using CAR T cells manufactured using the Sleeping Beauty System [41]. Eight patients with B-cell lymphoma or B-lineage acute lymphoblastic leukemia received CD19-specific CAR T cells after haploidentical transplant manufactured from the same donor. All patients tolerated the infusions well with no significant GVHD. Progression-free survival at 1 year was 75%. These are the first haploidentical transplant patients treated with CAR T cells. Although very limited experience, prevention of disease relapse post-transplant for high-risk ALL patients appears to be the most important therapeutic benefit at the present time.

12.7 Natural Killer Cells

NK cell infusions after HaploHCT may boost GVD effect through innate immunity [42–44]. Yoon et al. reported 41 patients who were infused with donor NK cells twice at 2 and 3 weeks after T cell replete HaploHCT [45]. NK cells were generated by culturing CD3-depleted leukapheresis products with IL-15 and IL-21. The median NK cell dose given was 2×10^8/kg. No acute side effects after NK cell infusions were observed. Nine patients developed acute GVHD, while six patients experienced severe chronic GVHD. Of 37 patients who had refractory acute leukemia or lymphoma at the time of transplant, 25 (68%) achieved CR. Compared to historical controls from the same institution, cumulative incidences of GVHD, TRM, and engraftment were similar while relapse incidence was lower in the NK cell study group. A phase 1 clinical trial for haploidentical transplant patients with advanced hematologic malignancies was recently completed at MD Anderson using *ex vivo* expanded NK cells using the mbIL-21 method [46] with the goal to decrease the rate of disease relapse post-transplant. Results of the phase 1 study were recently report in abstract format at ASH 2016 [47]. Thirteen patients with myeloid malignancies received up to 1×10^8 NK cells on days −2, +7, and +28 of HaploHCT. No grade III-IV aGVHD was observed. Compared with historical controls, patients treated with NK cells had significantly improved NK cell function and cytotoxicity. Moreover, a lower relapse rate was observed, although the difference was not statistically significant.

12.8 Conclusions and Future Directions

Outcomes of haploidentical transplants have improved dramatically in the last two decades due to improved immunosuppression. Unfortunately, this also led to delayed post-transplant immune reconstitution. Novel approaches are being developed to preserve immune cells crucial for healthy immune reconstitution while getting rid of those with the potential to induce GVHD. In near future, we may see designed grafts for individual patients and diseases, i.e. a mix of NK cells, γδ T cells, memory T cells, and T cells engineered to express both CARs and suicide genes. These promising advances, which are all exciting, encourage future development of haplodentical transplantation, providing not only a cost advantage over unrelated donor transplants but also the promise that disease relapse and infectious complications can be controlled in the near future.

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Chapter 13
Future Perspectives for Haploidentical SCT

Ugur Sahin and Taner Demirer

13.1 Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) remains as the sole curative option for many malignant and non-malignant hematological disorders. The selection of proper donor among the available candidates is a crucial step during the initial work-up. Within this context, haploidentical donors offer many advantages, including higher availability, lower operational costs and a relatively shorter work-up period, when compared to traditional HLA-matched sibling donors (MSD) and HLA-matched unrelated donors (MUD) [1, 2]. Despite promising results with haploidentical HSCT, there are still several unresolved issues. Ongoing efforts to overcome these unmet needs mainly target the optimization of the procedure in order to enhance immune reconstitution and decrease complications, including graft versus host disease (GvHD), infections, graft failure and relapse [3, 4].

13.2 Haploidentical Donor Selection

The potential haploidentical donor candidates include the biological parents, biological children and full or half siblings of the recipient. The presence of more than one donor candidate is a common situation and necessitates the determination of the best available haploidentical donor. The traditional donor selection criteria

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including ABO blood type, CMV serostatus of the donor and recipient, sex mismatch, donor age and parity are valid for haploidentical donors [5–8]. Besides, there are several additinal unique criteria for haploidentical donor selection, such as the presence of donor-specific HLA antibodies, donor-recipient HLA mismatch, non-inherited maternal antigens (NIMA) and natural killer cell (NK cell) alloreactivity.

The presence of donor-specific HLA antibodies in the recipient significantly increases the graft failure risk and should be avoided. In the absence of a readily available alternative donor, successful desensitization procedures employing plasmapheresis, intravenous immunoglobulin, tacrolimus and mycophenolate mofetil have been described [9].

Historically, the extent of donor-recipient HLA mismatch showed an inverse relationship with HSCT outcomes. The deleterious effects of HLA-mismatch have been substantially eliminated after the advent of modern conditioning regimens and GvHD prophylaxis strategies. Recent studies using post-transplantation cyclophosphamide (PTCy) for GvHD prophylaxis have reported similar overall (OS) and disease-free survival (DFS) rates for haploidentical grafts when compared to HLA-matched sibling grafts [10, 11].

When the haploidentical donor candidate is a sibling, the mismatch for paternal or maternal haplotype becomes an issue. Haploidentical siblings who are matched for paternal antigens are mismatched for both inherited maternal HLA antigens and NIMA. Mismatching for NIMA, which leads to a reduced alloreactivity through an immunologic hyporesponsiveness induced in utero and during the early neonatal period, is better tolerated. The results of earlier retrospective studies were conflicting, however, mostly in favor of mother-to-child transplants [12–14]. In the later studies, NIMA-mismatch was reported to have a reduced risk of GvHD when compared with non-inherited paternal antigen (NIPA) mismatch [15, 16].

NK cells regulate alloreactivity via T-cell independent pathways and exert their effects through the interaction between killer-cell immunoglobulin-like receptor (KIR) and their ligands. According to KIR ligand incompatibility model, NK cell allo-reactivity is defined as the absence of a donor KIR ligand in the recipient, or vice versa [17, 18]. KIR ligand incompatibility in T-cell depleted haploidentical SCT resulted in decreased frequency of relapse and GvHD as well as improved survival [19–22]. However, other studies had conflicting results [23–29]. The differences across the studies, such as heterogeneity of diagnoses, conditioning regimens and GvHD prophylaxis methods, the presence of T-cell depletion impair comparison of their results.

Currently, unless the donor is medically or psychologically unfit, the presence of donor-specific HLA antibodies in the recipient is the only major contraindication for a specific haploidentical donor. Donor-specific HLA antibodies should be screened during the work-up by flow cytometry or complement-dependent cytotoxicity assays. ABO compatible donors, donors with matched CMV serologic status, younger adults rather than elderly or children and male or nulliparous female donors for male recipients are generally favored during haploidentical donor selection. Donor selection strategies according to NIMA mismatch and KIR gene and ligand interactions should be further studied.

13.3 Stem Cell Source

Peripheral blood stem cell (PBSC) grafts are commonly used in allogeneic HSCT due to ease of collection, higher $CD34^+$ cell yields and earlier immune reconstitution. However, significantly increased amount of $CD3^+$ cell content leads to a higher incidence of acute and chronic GvHD. Thus, bone marrow harvested (BM) grafts have been preferred for haploidentical SCT [30]. The initial studies of PBSC grafts in haploidentical SCT employed myeloablative conditioning regimens [31]. Successful results were also obtained subsequently with non-myeloablative regimens [32, 33]. The choice of stem cell source should be evaluated together with GvHD prophylaxis strategies and T-cell depletion techniques. PBSC grafts may eventually replace BM grafts after the results of ongoing research on graft tailoring.

13.4 T-Cell Depletion Techniques and Conditioning Regimens

Bidirectional alloreactivity between donor and recipient is a double edged sword in haploidentical HSCT; one edge being the beneficial graft versus leukemia (GvL) and unwanted GvHD the other. T-cell depletion (TCD) is inevitably required in order to limit this intense alloreactivity. T-cell depletion can be done either in-vivo (T-cell replete HSCT) or ex-vivo (T-cell depleted HSCT).

Ex-vivo TCD strategies involve manipulation of the graft and include modifications of TCD techniques with infusion of mega-dose $CD34^+$ cells. Non-selective TCD leads to increased risk of graft failure, prolonged immunosupression and increased morbidity and mortality due to infections [34]. TCD can be done via positive selection of $CD34^+$ cells or direct removal of $CD3^+$ cells. The latter may improve immune reconstitution since other immunomodulating cells including NK cells and monocytes are kept in the graft [3]. Selective depletion of $\alpha\beta^+$ T-cells, which are mainly responsible from GvHD, may further improve outcomes. Thus, the preservation of $\gamma\delta^+$ T-cells and NK cells in the graft may exert beneficial effects on infectious complications and immune reconstitution [35, 36].

Mega-dose $CD34^+$ cells ($>10 \times 10^6$/kg) are infused in order to overcome the increased risk of graft failure in the ex-vivo TCD setting [37]. This strategy may enable to achieve engraftment rates of 90–95% and acute and chronic GvHD rates of <10%. However, non-relapse mortality (NRM), especially due to infections, is yet reported to be as high as 37–53% [38–40]. The initial conditioning regimens included 8 Gy of total body irradiation (TBI) in single fraction, thiotepa, cyclophosphamide, and rabbit anti-thymocyte globulin (ATG) [39]. Subsequent studies replaced fludarabine with Cy and alemtuzumab was used instead of thymoglobulin in selected patients [39, 40].

In-vivo TCD strategies involve unmanipulated grafts and includes various combinations of post-transplant immunosupression and use of ATG. The most successful and popular in-vivo strategy is high dose PTCy. It has been proven to decrease the incidence of severe acute GvHD and chronic GvHD and improve survival [41, 42]. The most widely used Seattle-based regimens include low-dose pre-transplant Cy, non-myeloablative conditioning with fludarabine and low-dose TBI and GvHD prophylaxis with PTCy (50 mg/kg/day on days +3 and +4), MMF (from day +5 to +35) and tacrolimus (from day +5 to +180) [10, 42]. Although non-myeloablative regimens ensure acceptably low relapse rates for lymphoid malignanices, studies incorporating myeloablative regimens for selected patients with myeloid malignancies demonstrated superior relapse rates without increasing GvHD and NRM [43, 44]. Recent studies on haploidentical SCT with PTCy in in the treatment of advanced Hodgkin lymphoma had promising survival rates without increasing toxicities [45]. The choice of optimal conditioning regimen for different diseases is a subject of future research. In addition, the exact role of haploidentical SCT with PTCy for each disease has to be compared to allogeneic HSCT from other alternative donors in head-to-head randomized clinical trials.

The "GIAC" strategy, which was developed and commonly experienced in China, is another in-vivo TCD strategy and has four main components: **G**CSF-stimulation of the donor; **I**ntensified immunosuppression through post-transplantation cyclosporine (CsA), mycophenolate mofetil (MMF) and short-course methotrexate; addition of **A**TG to conditioning; and **C**ombination of PBSC and BM grafts. Conditioning is usually involves a modified busulfan plus cyclophosphamide regimen with ATG, cytarabine, and semustine (Me-CCNU) [46–48]. This approach is associated with relatively high rates of severe acute and chronic GvHD. Modification of this strategy by using only BM grafts and adding basiliximab may reduce GvHD rates [49]. Alternative modifications aiming to decrease GvHD rates should be further studied. The comparisons of GIAC and selective TCD strategies with PTCy based regimens should also be made within the context of future prospective clinical trials.

13.5 Delayed Immune-Reconstitution and Infections

The increasing amount of donor T-cell depletion is directly associated with slower immune reconstitution. Thus, delayed immune reconstitution and higher rates of infectious mortality have been frequently reported after non-selective TCD with infusion of mega-dose $CD34^+$ cells [39]. Immune reconstitution after GIAC or PTCy strategies is also slightly slower when compared to matched sibling HSCT. However, this does not cause significant impairment in NRM [11, 50].

Viral infections, especially human cytomegalovirus (CMV) and Ebstein-Barr virus (EBV), are commonly seen after haploidentical SCT and may lead to important complications, such as poor graft function, loss of engraftment and post-transplant lymphoproliferative disorder. The infusion of pathogen-specific cytotoxic T-cells in order to overcome the adverse effects of delayed immune reconstitution is

a promising approach for the prevention or treatment of viral infections [51, 52]. Novel anti-CMV agents including letermovir and maribavir also exert potent antiviral activity without significant side effects in both poth prophylaxis and treatment of resistant disease [53–56]. Although current evidence does not suggest an increased infectious risk, there are ongoing efforts to minimize the negative impact of infectious agents after haploidentical HSCT, in particular CMV reactivation.

Adoptive immunotherapy with T-regulatory cells in order to control the alloreactivity of T-cells is promising and may enable to produce designed grafts, which contain predetermined amounts of conventional T-cells, T-regulatory cells, $\gamma\delta^+$ T-cells and NK cells, for haploidentical SCT in the near future [57]. Various other cellular engineering techniques aiming at enhancing and modifying immune reconstitution have also been described. The infusion of polyclonal T-cells after depletion of alloreactive T-cells or infusion of polyclonal T-cells engineered with suicide genes that can be activated in case of GvHD are among the most interesting methods [58–60].

13.6 Engraftment Failure and Relapse After Haploidentical HSCT

Relapse after haploidentical SCT is still an important problem. The research on several novel approaches including the early use of donor lymphocyte infusions (DLI), post-transplant NK cell infusion and post-transplant consolidation with hypomethylating agents for acute myeloid leukemia and myelodysplastic syndrome have yielded promising results [2].

Loss of the mismatched HLA haplotype expression has been described recently as a mechanism of leukemia escape from immune surveillance and reported to be as high as 25% after haploidentical SCT [61, 62]. This relapse mechanism is commonly observed among patients relapsing nine or more months after HLA haploidentical SCT and does not respond to standard DLI. Thus, these patients should be performed a second haploidentical SCT from a relative who is HLA-mismatched to the original donor [63].

13.7 Other Issues

Disease Risk Index (DRI) has been developed for stratifying the outcomes of haploidentical SCT according to histological diagnosis and conditioning regimens [64, 65]. Low/intermediate risk disease has excellent outcomes. On the contrary, it is still not clear how to manage patients with high risk disease [66].

The results of haploidentical SCT studies, as well as other allogeneic HSCT settings, have demonstrated inferior outcomes for patients with relapsed disease when compared to those transplanted in remission. The management of relapsed disease before haploidentical SCT remains an important issue to be solved.

In conclusion, it is yet hard to interprete and compare each of the above given approaches and methods, since the current data about haploidentical SCT mainly come form the results of non-randomised trials with retrospective comparison. Thus, current recommendations for haploidentical SCT substantially depend on expert opinions. Future studies should particularly focus on head-to-head comparisons of other donor sources with haploidentical donors (such as MSD, MUD, umbilical cord, and haploidentical donor), conditioning regimens and strategies involving graft manipulation. Further research with higher quality features (i.e.; randomised, homogenous population and larger sample size) are needed before recommending haploidentical SCT for a more extended list of indications.

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