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Modern approaches to HLA-haploidentical blood or marrow transplantation

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Abstract

Allogeneic blood or bone-marrow transplantation (alloBMT) is a potentially curative treatment for a variety of haematological malignancies and nonmalignant diseases. Historically, human leukocyte antigen (HLA)-matched siblings have been the preferred source of donor cells owing to superior outcomes compared with alloBMT using other donors. Although only approximately one-third of patients have an HLA-matched sibling, nearly all patients have HLA-haploidentical related donors. Early studies using HLA-haploidentical alloBMT resulted in unacceptably high rates of graft rejection and graft-versus-host disease (GVHD), leading to high nonrelapse mortality and consequently poor survival. Several novel approaches to HLA-haploidentical alloBMT have yielded encouraging results with high rates of successful engraftment, effective GVHD control and favourable outcomes. In fact, outcomes of several retrospective comparative studies seem similar to those seen using other allograft sources, including those of HLA-matched-sibling alloBMT. In this Review, we provide an overview of the three most-developed approaches to HLA-haploidentical alloBMT: T-cell depletion with 'megadose' $CD34^+$ cells; granulocyte colony-stimulating factor-primed allografts combined with intensive pharmacological immunosuppression, including antithymocyte globulin; and high-dose, post-transplantation cyclophosphamide. We review the preclinical and biological data supporting each approach, results from major clinical studies, and completed or ongoing clinical studies comparing these approaches with other alloBMT platforms.

Introduction

Allogeneic blood or bone-marrow transplantation (alloBMT) can be a curative therapy for a variety of haematological malignancies and nonmalignant diseases.[1] Despite the current widespread use of this approach, early studies suggested that alloBMT was only feasible when using donors who were completely matched with the recipient at the human leukocyte antigen (HLA) loci on both copies of chromosome 6 (Box 1).[1] Only one-third of patients, however, have an HLA-matched-sibling donor,[2] and shrinking family sizes in many

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societies are further reducing this probability. Despite coordinated efforts, such as the creation of international registries of more than 20 million volunteer donors, HLA-matched-unrelated donors are unavailable for many individuals, particularly those who are members of certain ethnic groups.[3] Furthermore, the search for an HLA-matched-unrelated donor can pose an unacceptable delay in commencing alloBMT for many patients with aggressive haematological malignancies.

Conversely, HLA-haploidentical (haplo) donors—that is, related donors who share with the patient a single identical copy of chromosome 6, containing the HLA loci—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 (Figure 1). However, use of HLA-mismatched allografts is associated with intense bidirectional allo-reactivity, wherein the host immune system seeks to eliminate donor cells (graft rejection) and the donor immune system seeks to eliminate the host (graft-versus-host disease, GVHD; Box 1). Consequently, early attempts at HLA-haploidentical alloBMT (haploBMT) were limited by unacceptably high treatment-related toxicity.[4–8]

Over the past two decades, new approaches to haplo-BMT have effectively controlled this intense alloreactivity, resulting in markedly improved outcomes, and other promising haploBMT strategies continue to be pursued.[9–16] Herein, we review the three most developed approaches to haploBMT (Figure 2 and Box 2): T-cell depletion (TCD) with ‘megadose’ $CD34^+$ cells; T-cell modulation with granulocyte colony-stimulating factor (GCSF)-primed grafts, antithymocyte globulin (ATG), and intensive post-grafting immunosuppression; and high-dose, post-transplantation cyclophosphamide (PTCy). Using these modern approaches, some reports even suggest that the degree of HLA disparity might no longer be a risk factor for GVHD or adversely affect patient survival after haploBMT.[17,18] Moreover, the results of retrospective analyses published within the past 10 years have demonstrated similar patient survival after haploBMT and HLA-matched-related or HLA-matched-unrelated alloBMT.[19–23] Following these advances, the utility of alloBMT in treating patients with haematological malignancies has been extended to nearly all patients who might require this treatment.

Early studies of haploBMT

After the initial successes achieved using HLA-matched-donor alloBMT for the treatment of patients with advanced haematological malignancies, the feasibility of haploBMT was explored. Following similar reports in children with severe combined immunodeficiency,[24] outcomes of 39 patients who received haploBMT between 1974–1979 using bone marrow from donors who were partially to fully phenotypically matched with the recipients in terms of the unshared haplotypes were published (Figure 1, Box 3).[25] Among 10 patients with aplastic anaemia, five had graft failure, four died of GVHD, and one died of infection. Among the 29 patients with haematological malignancies, nonrelapse mortality (NRM) was 41%, with three patients (10%) dying of graft failure or poor graft function and two (7%) dying of GVHD. The lone surviving patient with aplastic anaemia and five of the 12 surviving patients with haematological malignancies were all serologically HLA-matched with their donors.[25]

In 1983, results of the first study investigating haplo-BMT using unselected first-degree relatives who were not HLA-matched were published.[4] Of the 35 patients treated, 10 (29%) experienced graft failure. GVHD itself was a contributing cause of death in six patients (17%), and 12 additional patients (34%) died of an inflammatory syndrome suggestive of hyperacute GVHD. None of the 12 patients over 30 years of age survived.[4]

Between 1985–1990, investigators at the Fred Hutchinson Cancer Research Center (FHCRC) published their results using haploBMT.[5–7] In the first report, outcomes of 105 patients with haematological malignancies who received haploBMT were compared with 728 patients contemporaneously treated with HLA-matched-sibling alloBMT.[5] Notably, only six of the 105 patients who received haploBMT shared only a single haplotype with their donor (Figure 1, Box 3); 71% of this group were phenotypically identical or had only one antigen–HLA mismatch on six-antigen serological typing (HLA-A, HLA-B and HLA-DR). Regardless of this limited mismatch, graft failure or delayed engraftment (24% versus 14%; P <0.005) and grade II–IV acute GVHD (70% versus 42%; P <0.001) were both significantly more common in patients treated with haploBMT compared with those who received HLA-matched-sibling alloBMT;[5] these results were confirmed in two subsequent reports from successively larger patient cohorts.[6,7] Graft failure was particularly high in patients who were mismatched at both HLA-B and HLA-DR loci,[6] and the risk of GVHD increased significantly for every incompatible HLA locus between donor and recipient (relative risk 1.95 per locus, 95% CI 1.52–2.5; P <0.0001).[7] Even when using combination GVHD prophylaxis with ciclosporin-A (CsA) and methotrexate, which is still widely considered a standard-of-care, the rates of grade III–IV acute GVHD (Box 4)[26] were exceptionally high (28% and 47% for one-antigen and two-antigen-HLA-mismatched haploBMT, respectively).[7] A trend towards a lower incidence of relapse after haploBMT was observed; however, this was more than outweighed by the excess toxicity, resulting in markedly worse survival for patients receiving two-antigen or three-antigen-HLA-mismatched haploBMT.[7]

Results of an International Bone Marrow Transplant Registry study, which included 2,055 patients treated between 1985–1991 with alloBMT using various donor types, were published in 1997.[8] Use of alloBMT from HLA-matched-sibling donors was associated with significantly better rates of successful engraftment, grade II–IV and III–IV acute GVHD and chronic GVHD than any alternative donor cohort, including one-antigen or two-antigen-mismatched HLA-haploidentical donors (P <0.001 for all comparisons with HLA-matched-sibling donor alloBMT). Furthermore, rates of 3-year NRM were greater than 50% for all alternative donor cohorts, compared with 21% in early-stage patients receiving HLA-matched alloBMT. The findings of these studies showed that treatment with haploBMT resulted in survival in a subset of patients, but the high toxicity relative to HLA-matched alloBMT was deemed prohibitive and haploBMT was largely abandoned.

TCD with ‘megadose’ CD34+ grafts

Biological and early clinical data

T cells are considered key mediators of GVHD and graft rejection, with the T-cell content of the graft having a clear association with the risk of GVHD.[27,28] Over a series of publications

from 1976–1980, investigators at the Weizmann Institute of Science and the Memorial Sloan Kettering Cancer Center developed techniques to remove T cells from the allograft before infusion.[29–31] Using soybean agglutinin and erythrocyte-rosetting with sheep erythrocytes, investigators were able to physically separate T cells from B cells, haematopoietic stem cells and other haematopoietic progenitors, providing TCD allografts, which abrogated lethal GVHD in murine alloBMT models.[29–31] Clinical haploBMT using this approach resulted in sustained engraftment in three of four patients without any detectable GVHD.[32,33]

Despite this apparent success, subsequent studies revealed that graft failure remained a persistent problem, affecting more than 20% of patients receiving TCD– alloBMT from donors other than HLA-matched siblings.[34] Unfortunately, graft failure was a direct adverse effect of TCD, as removing T cells decreased the graft-versus-host response and rendered the donor graft more susceptible to rejection by the host. Graft failure was particularly problematic in patients who received HLA-mismatched alloBMT,[35] presumably owing to a much greater abundance of circulating alloreactive T cells in these individuals compared with patients receiving HLA-matched alloBMT.[36] Indeed, graft failure was found to be associated with the emergence of conditioning-resistant, anti-donor T cells in the host.[37,38] However, graft failure in mice was found to be reduced simply by augmenting the conditioning in one of several ways: through increased doses of total body irradiation (TBI) or booster splenic irradiation;[39,40] the addition of thiotepa or another alkylating agent;[41,42] or the use of monoclonal antibodies to further deplete levels of residual host T cells.[43]

Beyond immune-mediated rejection, graft failure appeared to be related to the dose of stem cells infused into the host. Additional mouse studies showed that full donor engraftment without GVHD could be achieved by infusion of ‘megadoses’ of TCD bone marrow.[44,45] These effects of higher non-T-cell doses of bone marrow were subsequently found not simply to be related to better competition for the stem-cell niche in the marrow, but also were related to a ‘veto effect’ in which $CD34^+$ cells directly inhibited T-cell alloreactivity.[46,47]

Clinical outcomes

In an effort to improve engraftment in patients receiving TCD–haploBMT, investigators in Perugia, Italy, integrated several of these important preclinical insights into a novel transplantation platform.[48] To eliminate host T cells, these investigators intensified the patient-conditioning protocol to incorporate thiotepa, cyclophosphamide and TBI as well as adding ATG. A combination of donor bone marrow and GCSF-mobilized peripheral-blood stem cells (PBSCs) was used to augment the stem-cell dose, which together were able to produce 7–10-fold higher levels (‘megadoses’) of haematopoietic progenitors than were found in bone-marrow allografts alone. After collection, the allografts were depleted of T cells using soybean agglutination and erythrocyte rosetting. No post-grafting immunosuppression was given. Early, sustained engraftment was observed in 16 of the first 17 patients who received transplants using this approach. Grade II–IV acute GVHD only occurred in the patient receiving the graft containing the highest number of T cells. Just two patients in this study relapsed, although NRM occurred in nine of the 17 patients.[48] This study showed that the historical barriers to haploBMT of graft failure and GVHD both could

be overcome by intensive myeloablative and immunosuppressive conditioning followed by TCD ‘megadose’ allografts, without any need for a dditional GVHD prophylaxis.

This platform was refined in subsequent studies, firstly by replacing cyclophosphamide with fludarabine[49] and subsequently by transitioning from soybean agglutination and erythrocyte rosetting of bone marrow and PBSC allografts to immunomagnetic selection of $CD34^+$ cells from PBSCs alone[50] (Figure 2 and Box 2). In both of these studies, which investigated the treatment of patients with advanced acute leukaemias, graft failure was infrequent (5–7%) and rates of acute and chronic GVHD were low (<10%).[49,50] Despite the finding that $CD4^+$ T-cell levels remained low for more than a year post-transplantation, rapid natural killer (NK)-cell recovery was seen within 2–4 weeks after transplantation.[49] Patients receiving TCD–haploBMT from NK-cell alloreactive donors seemed to have a lower risk of relapse.[50] Overall, NRM remained high at ∼40%, with 65–71% of these deaths being a result of infections—particularly viral infections.[49,50] Meanwhile, relapse was infrequent in patients who received transplants while in remission (16%).[50]

In 2008, a report on the cumulative European experience of TCD–haploBMT was published.[51] Between 1995–2004, 266 adults with *de novo* acute leukaemia were transplanted with TCD–haploBMT using immunomagnetically selected PBSCs. Successful engraftment was achieved in 91% of the patients who received TCD– haploBMT while in remission. Grade II–IV acute GVHD and grade III–IV acute GVHD occurred in 10% and 6% of patients, respectively. Chronic GVHD was seen in 14% of patients who survived beyond 100 days post-transplantation. 2-year NRM ranged from 36–66% depending on the disease type and stage at haploBMT. In this study, patients with acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL) who underwent transplantation while in first complete remission had 2-year disease-free survival (DFS) of 48% and 13%, respectively.[51] However, outcomes of patients with advanced-stage disease were worse, with 2-year DFS of 1% and 7% for patients with AML and ALL, respectively.[51] Studies investigating haploBMT in children had very similar results; findings of high NRM (37% at 5 years of follow-up) and poor outcomes of patients with ALL who were not in complete remission were particularly similar to the situation in adult patients.[52,53] Two small studies by North American groups detected poor outcomes, such as high NRM, when using the TCD– haploBMT approach to treat patients with advanced-stage haematological malignancies.[54,55] Another American study, in which antithymocyte globulin and other monoclonal antibodies were used to remove T cells for TCD–haploBMT in patients with acute leukaemia, also showed high NRM of 51% after 5-years of follow-up.[56]

Refinements to the TCD–haploBMT platform

Unacceptably high NRM was seen in studies using the TCD–haploBMT approach described in the previous section (Table 1); therefore, several groups have pursued refinements of this platform. The Tübingen and Memphis groups introduced $CD3^-/CD19^-$-cell selection rather than $CD34^+$-cell selection in order to produce a graft containing other $CD34^-$ cells (such as NK cells, monocytes, dendritic cells and other myeloid cells), which might enable better immune recovery without leading to GVHD.[57] This approach was used in combination with reduced-intensity conditioning (RIC) in an attempt to reduce treatment-related toxicity.[58] In

one study, all but one of 29 patients had successful engraftment; however, grade II–IV acute GVHD occurred in 48% of patients, and eight patients (28%) had NRM, with seven deaths caused by infections and one by GVHD.[58] A paediatric study evaluating transplantation using $CD3^-/CD19^-$-cell selection after myeloablative conditioning showed successful engraftment in 88% of 46 patients, grade II–IV acute GVHD in 20%, grade III–IV acute GVHD in 7%, chronic GVHD in 21%, and NRM in 20% after 5 years of follow-up.[59] In this study, relapse occurred in 63% of patients after 2 years of follow-up, although 43% of the patients who underwent transplantation had active disease at the time of treatment.[59] Overall, the use of $CD3^-/CD19^-$-cell selection might slightly reduce NRM, but seems to carry a substantially greater risk of GVHD compared with $CD34^+$-cell selection.

A very narrow post-transplantation T-cell repertoire exists in patients treated with TCD–haploBMT, owing to limited post-transplantation thymic activity, particularly in adults receiving myeloablative conditioning. This limited T-cell repertoire could potentially contribute to the high susceptibility to viral and other infections observed in patients who have received TCD-haploBMT. As a result of this high susceptibility to infection, research has focused on infusing grafts that contain selected T cells that might provide an overall favourable immune reconstitution without substantially increasing the risk of GVHD. Adding back low numbers of alloreactivity-depleted or IL-10-anergized T cells, which could improve immune reconstitution without inciting much GVHD, is a straightforward and promising approach.[60,61]

A second approach has focused on restricting TCD to $\alpha\beta^+$ T cells. $\gamma\delta^+$ T cells have been shown to mediate viral-specific responses to both cytomegalovirus and Epstein-Barr virus,[62–64] which both cause substantial post-transplantation morbidity and mortality. Furthermore, $\gamma\delta^+$ T cells have antitumour activity,[62,63,65] while potentially also having a lower risk of initiating GVHD than $\alpha\beta^+$ T cells.[66,67] Following successful clinical-scale depletion of $\alpha\beta^+$ but not $\gamma\delta^+$ T cells,[68] the results of a small study of 23 paediatric patients with nonmalignant disorders were published in 2014.[69] This study used combined depletion of $\alpha\beta^+$ T cells and B cells, and detected similar levels of GVHD compared with those of patients who received $CD34^+$-cell-selected TCD–haploBMT, but with encouragingly low NRM of 9.3%.[69]

Another approach by the Perugia group has involved infusing immunomagnetically selected regulatory T (T_{REG}) cells 4 days before transplantation and conventional T cells on the same day as the TCD allograft.[70,71] In the first study of this approach, 26 of 28 patients had successful engraftment.[70] Only two patients developed grade II–IV acute GVHD, and both received the highest T-cell doses in the study cohort; no chronic GVHD was observed. T-cell reconstitution was markedly improved, including expanded T-cell repertoires and improved pathogen-specific responses. No patients developed cytomegalovirus-associated disease. Unfortunately, NRM occurred in 13 of 26 engrafted patients (50%), eight of whom died following infection.[70] An updated report of data from an expanded cohort of patients showed similar results and suggested that, in both mouse and human models, the infusion of T_{REG} cells did not seem to affect graft-versus-tumour (GVT) immunity.[71] Indeed, relapse rates in patients treated in this study, using add-back of low numbers of T_{REG} cells and conventional T cells, were strikingly lower than those in historical controls. Nevertheless,

problems, such as high NRM and deaths from infections, remained despite laboratory evidence of improved immune reconstitution.

A fourth promising approach to refining TCD– haploBMT involves the infusion of viral-specific cytotoxic T-cell lines for the prevention or treatment of viral infections.[72] These T cells expand *in vivo* following infusion, seem to exert antiviral effects without causing GVHD, and might also have antitumour activity.[72,73] A final refinement has involved infusing donor lymphocytes expressing suicide genes that could be activated if GVHD developed.[74,75] In a study of 50 patients, use of this treatment strategy markedly accelerated immune reconstitution.[74] When GVHD did occur, it could be effectively abated by induction of the suicide gene; however, NRM still occurred in 40% of patients.[74]

The GIAC protocol

Biological and preclinical data

A T-cell-replete (TCR)–haploBMT protocol has been developed that involves four main components, prompting the acronym ‘GIAC:’ ‘G’CSF-stimulation of the donor; ‘I’ntensified immunosuppression through post-transplantation CsA, mycophenolate mofetil (MMF), and short-course methotrexate; ‘A’ntithymocyte globulin added to conditioning to help prevent GVHD and aid engraftment; and ‘C’ombination of PBSC and bone-marrow allografts (Figure 2 and Box 2). The development of this strategy benefited from growing experience with antithymocyte globulin,[76] the emergence of pharmacological agents, such as MMF, with which to control GVHD, and the introduction of GCSF-stimulated PBSC allografts. After the development of GCSF-stimulated PBSC allografts, investigators were perplexed as to why rates of acute GVHD were not markedly higher despite the infusion of substantially (up to 10-fold) greater numbers of T cells than would typically be present in a bone-marrow allograft.[77] At that time, T-helper type 1 (T_H1) cell differentiation was believed to promote GVHD, whereas T-helper type 2 (T_H2) cell differentiation was thought to be protective,[77] although further investigations have demonstrated GVHD to be a much more complex process.[78] T cells mobilized from the bone marrow into the blood under the influence of GCSF were less proliferative, had reduced production of T_H1 cytokines, and had increased production of the T_H2 cytokine IL-4, resulting in improved survival and reduced GVHD in murine alloBMT models.[77,79] Exposure to GCSF also resulted in mobilization of dendritic cells, which promoted skewing of T cells towards a T_H2 phenotype.[80] Furthermore, GCSF-stimulation accentuated the IL-10-mediated suppression of alloantigen-induced T-cell proliferation by $CD14^+$ antigen-presenting cells, owing in part to markedly higher monocyte to T-cell ratios.[81,82] Additionally, GCSF-stimulation decreased expression of both the CD86 co-stimulatory molecule by $CD14^+$ cells and the CD28 responsive complex by $CD4^+$ T cells.[82,83] Such immunological effects on T-cell hyporesponsiveness and polarization could be maintained when mixing GCSF-stimulated PBSC and bone-marrow allografts.[84] Administration of GCSF post-transplantation, to aid haematological recovery, potentiated this skewing towards a T_H2 phenotype at the cost of delayed recovery of normal T_H1 responses to pathogens.[85]

Clinical outcomes

Building on this preclinical work, pilot studies investigating a TCR-haploBMT approach were commenced in Beijing, China, at the Air Force General Hospital and the Peking University.[19,86,87] Although both studies used similar GIAC protocol-type transplantation platforms, the Air Force General Hospital group used only bone-marrow allografts whereas the Peking University group used a combination of bone marrow and PBSCs. In a large study published in 2006 by the Peking University group, engraftment was achieved in all 171 patients who underwent transplantation.[87] The cumulative incidences of grade II–IV and grade III–IV acute GVHD at 100 days of follow-up were 55% and 23%, respectively. The cumulative incidences of chronic GVHD and extensive chronic GVHD after 2 years of follow-up were 74% and 47%, respectively. The 2-year probabilities of NRM, relapse and DFS were 20%, 12% and 68% for patients with standard-risk disease and 31%, 39% and 42% for patients with high-risk disease, respectively.[87] Overall, these studies found that haploBMT using the GIAC protocol might enable complete engraftment, acceptable NRM and favourable DFS after TCR–haploBMT, but is associated with high rates of severe acute GVHD and chronic GVHD (Table 1). A paediatric study by the Peking University group limited to patients ≤14 years of age revealed outcomes similar to the initial studies, in which the median patient ages were 15–23 years.[88]

Several groups have attempted to reduce the relatively high rates of GVHD associated with use of the GIAC protocol. A Korean study used a modification of this platform with RIC and only GCSF-mobilized PBSC allografts, and demonstrated reduced rates of grade II–IV acute (20%) and chronic (34%) GVHD.[89] The Air Force General Hospital group also modified its original protocol, which used TBI-based conditioning and only GCSF-primed bone marrow, by adding basiliximab for further GVHD prophylaxis.[90,91] This approach resulted in a markedly reduced rate of grade II–IV acute GVHD of 11%. Chronic GVHD was still seen in most patients, although it was primarily limited in severity. A consortium of Italian investigators used this same modified GIAC approach and had encouraging results in terms of GVHD (grade II–IV and III–IV acute GVHD of 24% and 5%, respectively, and chronic GVHD of 6%), but 1-year NRM was 36%.[92]

Retrospective comparative studies have shown that haploBMT using the GIAC protocol confers similar results to those seen with HLA-matched-sibling donor alloBMT. In a comparison study conducted by the Peking University group,[19] patients receiving haploBMT were treated according to the GIAC protocol (Box 2), while patients receiving HLA-matched-sibling donor alloBMT were treated with a platform similar to the GIAC protocol, except that it employed a lower dose of cytosine arabinoside, omitted ATG and, in a subset of patients, used either bone-marrow or PBSC allografts. Primary engraftment was universal in both cohorts and, in fact, occurred three days earlier in those who received haploBMT (median time to neutrophil engraftment of 12 days versus 15 days). Patients receiving haploBMT had a higher risk of grade II–IV acute GVHD (40% versus 32%; RR 1.57 in multivariate analyses; $P = 0.024$); however, the incidence of chronic GVHD within 2 years of transplantation was similar (55% versus 56%). Two-year NRM was higher after haploBMT (22% versus 14%), as was relapse (18% versus 13%), but neither of these differences was statistically significant. Adjusted 2-year overall survival rates were nearly

identical between the two groups (72% versus 71%).[19] Two other Chinese research groups have reported very similar results from retrospective studies comparing outcomes of patients who received haploBMT with those of patients who received HLA-matched-sibling or HLA-matched-unrelated alloBMT.[20,93] In addition, a prospective, multicentre study of haploBMT (n = 231) versus HLA-matched-sibling alloBMT (n = 219) using biological randomization based on donor availability was published in May 2015, and confirmed the similar outcomes between these two groups.[94]

Survival outcomes of patients with acute leukaemia who received haploBMT using the GIAC protocol have been particularly encouraging. In fact, results from one study even suggested that patients with very-high-risk acute leukaemias had better outcomes than patients receiving HLA-matched-sibling alloBMT, owing primarily to a much lower incidence of relapse in the haploBMT group (26% versus 49%; P = 0.008).[95] In 2009, the Peking University research group reported the results of a study of 250 consecutive patients with acute leukaemia, of whom 108 had AML and 142 had ALL.[96] Survival outcomes of patients with AML were particularly favourable, with 3-year overall survival rates of 73% and 56% reported for standard-risk and high-risk groups, respectively. Similarly excellent outcomes were seen in a second study of haploBMT using the GIAC protocol in adult patients with AML in first complete remission.[94] By contrast, outcomes of patients with high-risk ALL after this treatment appear poor, owing to very high rates of NRM (51% after 3 years of follow up) and relapse (49% after 3 years of follow up).[96] Patients with standard-risk ALL, however, had an encouraging 3-year overall survival of 60%.[96] Two other studies published within the past year have confirmed excellent survival of patients with ALL in first complete remission treated with haploBMT using the GIAC protocol.[97,98] Another study retrospectively investigated adult patients with ALL who had high-risk disease (defined differently than in the aforementioned studies) and were in first complete remission, but lacked an HLA-matched-sibling or HLA-matched-unrelated donor;[99] consolidation therapy with either 2 years of chemotherapy (n = 104) or haploBMT (n = 79) was chosen by the patients; those who received haploBMT had markedly better 3-year DFS (64% versus 21%), overall survival (72% versus 27%), and cumulative incidence of relapse (19% versus 61%) than those in the chemotherapy group.[99] In multivariate analyses, treatment with haploBMT was the only factor associated with reduced risk of relapse and improved overall survival.

In these studies of haploBMT using the GIAC protocol, the extent of HLA disparity did not affect overall survival.[18,20,87] However, the findings of a later study suggested that HLA-B-mismatch was associated with higher acute GVHD and NRM as well as worse DFS and overall survival,[100] although the Peking University group's most-recent analysis, published in 2014, did not confirm this finding.[18] In an analysis of the effects of donor characteristics on patient outcomes, this group found that the lowest NRM and best overall survival were seen when using allografts from younger, male donors.[18] A reduced frequency of acute GVHD was seen when using as the donor the patient's child or an HLA-haploidentical relative who was mismatched for non-inherited maternal antigens (NIMA) (Figure 1); however, use of either of these donor types was not associated with any significant improvement in patient survival outcomes. By contrast, use of maternal donors was

associated with higher rates of acute and chronic GVHD and worse overall survival. Overall, the authors suggested that a NIMA-mismatched male child was the best possible donor for haploBMT using the GIAC protocol, and that the use of older mothers or non-inherited paternal antigen-mismatched donors "should probably be avoided."[18]

Post-transplantation cyclophosphamide

Biological and preclinical data

As one of the oldest chemotherapeutic agents, the effects of cyclophosphamide on immunological tolerance have been studied since the early 1960s.[101] In 1963, high-dose cyclophosphamide was found to be effective in prolonging murine skin allograft survival only when given shortly after allograft placement or up to the 4th post-transplant day, with the optimal effectiveness of this treatment being at 2 days post-transplantation.[102] This work was continued by a number of other investigators, but was most fully explored by a Kyushu University group, whose results were published over a series of 13 related manuscripts from 1984–1987, with related mechanistic studies continuing into the mid-1990s.[103,104] By administering donor spleen cells followed 48–72 hours later by cyclophosphamide, long-lasting tolerance to skin allografts was established. Three primary mechanisms of PTCy-induced tolerance were delineated in this model: firstly, direct elimination of host T cells responding to donor antigens in the periphery; secondly, intrathymic clonal deletion of donor-reactive host T cells; and thirdly, generation of tolerogen-specific host suppressor T cells.[105] Suppressor T cells were found to inhibit responses to both major and minor histocompatibility antigens through active suppression, but not clonal deletion, of alloreactive T cells.[106] Induction of tolerance by PTCy was disrupted by the administration of CsA or corticosteroids before adoptive cell transfer and cyclophosphamide treatment,[107,108] but was not affected by the administration of GCSF starting the day after PTCy treatment.[109]

Subsequently, the PTCy approach was extended to alloBMT. In major histocompatibility complex (MHC)-mismatched mouse models, treatment with PTCy reduced the dose of radiation required to induce reliable engraftment[110–112] and also prevented GVHD and prolonged survival.[113] Graft failure could be further reduced by the use of antilymphocyte globulin,[114] and radiation in the conditioning regimen could be replaced entirely by fludarabine, although high levels of donor chimerism required at least 100 cGy of TBI.[113] The resultant mixed chimeras were tolerant to both donor and host, but maintained reactivity against third-party alloantigens in mixed lymphocyte culture.[111,113,114] This tolerogenic effect allowed skin and heart allografts from the MHC-mismatched donor strain to survive, whereas MHC-disparate third-party grafts were rejected.[111,114]

Parallel to the effects seen on host T cells in skin allograft models, in mouse alloBMT models, the tolerogenic effects of PTCy were exerted through elimination of alloreactive donor T cells.[115] Donor T cells exposed to host antigens on day 0 were largely depleted, whereas non-alloreactive donor T cells, which divided more slowly in a lymphopenic environment, were relatively spared.[115] However, destruction of alloreactive donor T cells was necessary but not sufficient for PTCy-induced tolerance: in mouse models of MHC-matched alloBMT in which donor CD4^{+} T cells promote GVHD, as well as in xenograft

models, donor T_{REG} cells were necessary to prevent lethal GVHD after PTCy treatment,[116,117] an effect consistent with the results from skin allograft models. Donor T_{REG} cells in both mouse and human models of alloBMT were resistant to PTCy-induced cytotoxicity owing to increased expression of aldehyde dehydrogenase, the enzyme primarily responsible for *in vivo* detoxification of cyclophosphamide,[118] upon alogeneic stimulation in a lymphopenic environment.[116,117]

PTCy is the most commonly used approach to selective alloreactive T-cell depletion, although *ex vivo* approaches also have been explored. These strategies include using mixed lymphocyte cultures to eliminate allo-activated cells that either express the activation marker CD25 or retain a dye that becomes highly cytotoxic upon activation with visible light.[15,119,120] This latter photodepletion approach also spares T_{REG} cells and has shown promise in early reports from ongoing clinical studies.[16,121]

Clinical outcomes

In the preclinical studies described in the previous section, PTCy was only reported to effectively induce tolerance when administered at high doses.[115,122] Coupled with the finding that high-dose cyclophosphamide was not toxic to haematopoietic stem cells owing to their high aldehyde dehydrogenase expression, and supportive clinical studies showing that high-dose cyclophosphamide could be safely administered to patients with autoimmunity without requiring stem-cell rescue,[118] the use of high-dose PTCy for GVHD prophylaxis was explored clinically. In a phase I study of 13 patients at the Johns Hopkins Hospital (JHH), the findings of which were published in 2002, patients received 50 mg/kg PTCy 3 days after receiving TCR–haploBMT using RIC with fludarabine, cyclophosphamide (cohort 2 only) and low-dose (200 cGy) TBI.[123] For additional GVHD prophylaxis, MMF and tacrolimus were administered the day after patients received PTCy (post-transplantation day 4) and continued for at least 30 days. Among the 10 patients in the second cohort, eight had successful engraftment, and six developed grade II–IV acute GVHD. Six of these 10 patients, five of whom had active disease at haploBMT, were alive after a median follow-up duration of 284 days.

A phase I/II study of 68 patients at two institutions sought to improve upon the previously described JHH regimen by further reducing the incidences of GVHD and graft failure.[124] The 28 patients treated on this study at the FHCRC received TCR–haploBMT in line with the protocol described previously[121] except that tacrolimus was continued until 180 days post-transplantation; the remaining 40 patients treated at JHH also received a second dose of PTCy on the 4^{th} day post-transplantation (Figure 2 and Box 2).[124] Nine of the 68 patients (13%) had graft failure, although, owing to the low-intensity conditioning used, all but one experienced rapid autologous neutrophil recovery at a median of 15 days post-transplantation. Engrafted patients achieved complete or near-complete donor chimerism by 1–2 months post-transplantation. Grades II–IV and III–IV acute GVHD occurred in 34% and 6% of patients, respectively. The incidence of extensive chronic GVHD was low in both cohorts, but was significantly lower in patients who received two doses versus one dose of PTCy (5% versus 25%, respectively; $P = 0.05$). NRM was 15% after 1 year of follow-up.[124] Consistent with preclinical data suggesting sparing of non-alloreactive T cells,[115,125] no

patients had cytomegalovirus disease; only two died from fungal infections (one had graft failure).[124] Longer follow up of expanded cohorts treated in line with the JHH protocol confirmed low rates of NRM, acute GVHD and chronic GVHD.[126,127] The extent of HLA disparity had no negative effects on acute GVHD or progression-free survival (PFS).[17] The relatively high rate of relapse (46%) observed in this study in part reflected the advanced disease state of patients who received transplants: a disease-risk-stratified analysis of 372 patients treated at JHH (Table 1) showed that survival outcomes were comparable with patients receiving HLA-matched alloBMT.[127]

Nevertheless, in an effort to reduce relapse rates, the effects of intensifying the conditioning phase of the PTCy– haploBMT protocol were investigated. In two studies,[128,129] myeloablative conditioning was associated with similar rates of acute GVHD and slightly higher, but still favourable, rates of chronic GVHD (26% and 35%, respectively), similar NRM (18% and 10%, respectively), and lower rates of relapse (22% and 40%, respectively). The first of these two studies also spaced the dosing schedule of PTCy to be administered on post-transplantation days 3 and 5, and started MMF and CsA treatment before PTCy.[128] Unlike in preclinical studies,[107] tolerance was still induced when CsA was started before PTCy, as evidenced by low rates of grade II–IV acute GVHD (12%) and chronic GVHD (26% in this study).[128] Another study published this year, [130] which used TBI-based myeloablative conditioning with PBSCs for haploBMT, showed an excellent survival rate (78%), with low rates of NRM (3%) and relapse (24% for all patients and 0% for patients with low-risk or intermediate-risk disease) after 2 years of follow up, albeit with higher rates of acute (23% grade III–IV) and chronic (56% overall and 22% moderate or severe) GVHD. An alternative ‘two-step’ approach to myeloablative PTCy– haploBMT has involved TBI-based conditioning, a fixed peripheral blood T-cell dose on pretransplantation day 6, cyclophosphamide at 60 mg/kg per day on pretransplantation days 3 and 2, and a $CD34^{+}$-selected PBSC allograft on day 0.[131,132] This procedure mimicked the timing of the standard PTCy platform except that stem cells were spared exposure to cyclophosphamide. Results of patients who were in remission at the time of haploBMT according to this protocol have been encouraging: grade III–IV acute (4%) and chronic (21%) GVHD rates were low, NRM was only 3.6%, and relapse-related mortality was 19%, leading to 2-year DFS and overall survival of 74% and 77%, respectively.[132]

Several groups have explored the use of PBSCs rather than bone marrow for PTCy–haploBMT in an attempt to further improve engraftment and reduce relapse.[129,130,133,134] However, the effects of this substitution are currently unclear, particularly as heterogeneity between studies makes a definitive assessment of the effects on relapse challenging. Graft failure rates seem to be similar or, at most, only slightly improved by the use of PBSCs. The substitution of PBSCs for bone marrow would be expected to produce higher rates of chronic GVHD,[135] although it is not obvious if this is indeed true for PTCy-based protocols. Three studies investigating the replacement of bone-marrow with PBSCs have shown higher but still favourable rates of chronic GVHD,[129,130,132] whereas two others have reported rates of chronic GVHD similar to those seen after bone-marrow PTCy–haploBMT.[133,134] In these studies, the rates of grade III–IV acute GVHD and NRM were not consistently higher than previously seen in patients who received bone-marrow PTCy–haploBMT.

PTCy–haploBMT has generally been well tolerated, although a few potential complications are of particular note. Fevers characteristically occur within the first few days post-transplantation, particularly when using PBSCs.[129,131,136] These fevers can become quite severe, are generally culture-negative, and are thought to be cytokine-mediated and related to uncontrolled allo-reactivity; therefore, they tend to abate within hours to days of cyclophosphamide administration. Haemorrhagic cystitis occurs not infrequently after receiving PTCy– haploBMT, but generally is of limited severity, and is regularly attributable to polyomavirus (predominantly BK virus) infection.[129,137] Graft rejection remains a potential complication of haploBMT with any approach, and is usually related to donor HLA-specific antibodies (DSA) being present pretransplantation in the recipient.[138,139] In patients with detectable DSAs to all potential HLA-haploidentical donors, desensitization procedures can reduce DSA titres such that haploBMT can be successfully performed.[138,140] Notably, Epstein–Barr virus-related post-transplantation lymphoproliferative disease within the first year post-transplantation was not seen among 785 patients treated with PTCy,[141] and no increase in donor-derived malignancies was detected.[142]

Comparative studies

In retrospective comparisons, the PTCy–haploBMT approach seems to produce similar outcomes to those seen after HLA-matched-sibling or HLA-matched-unrelated alloBMT;[21–23,127,143] the experience of these procedures at the Northside Hospital in Atlanta, GA, USA, revealed that NRM and chronic GVHD were actually lowest in patients treated with haploBMT, and acute GVHD and survival outcomes were similar.[21] Investigators from the San Martino Hospital in Genoa, Italy, compared their outcomes of PTCy–haploBMT with four other donor types (HLA-matched-sibling, HLA-matched-unrelated, HLA-mismatched-unrelated, and umbilical cord blood) among 459 consecutive alloBMTs performed from 2006–2012.[22] Although patients undergoing haploBMT were the most likely to have advanced-stage disease at alloBMT, DFS and overall survival were similar for all groups, and were in fact highest for patients who received haploBMT; NRM, grade II–IV acute GHVD, and chronic GVHD rates were lowest with haploBMT, and relapse rates were similar. The rate of immune reconstitution after haploBMT was second only to that for HLA-matched-sibling alloBMT.[22] Investigators at the MD Anderson Cancer Center reported similar rates of engraftment, immune reconstitution, GVHD, and survival after haploBMT compared with HLA-matched-sibling or HLA-matched-unrelated alloBMT.[23] Registry data published in 2015 showed equivalent survival outcomes between HLA-matched-unrelated alloBMT and PTCy–haploBMT for patients with AML, while the rates of acute and chronic GVHD were lower after PTCy– haploBMT.[144] Promising results have been achieved using RIC–PTCy–haploBMT in patients with peripheral T-cell lymphoma and non-Hodgkin lymphoma compared with HLA-matched alloBMT.[145,146] Finally, the FHCRC consortium and JHH group found that patients with Hodgkin lymphoma who underwent PTCy–haploBMT had superior PFS compared with patients who received HLA-matched-sibling or HLA-matched-unrelated alloBMT.[147] Similarly excellent results in patients with Hodgkin lymphoma who were treated with PTCy–haploBMT have been reported by other groups.[148,149] Overall, these studies suggest that PTCy–haploBMT

provides similar outcomes to alloBMT using HLA-matched-sibling or HLA-matched-unrelated donors when not using PTCy.

Finally, the PTCy–haploBMT approach has been retrospectively compared with other alternative donor strategies. In a retrospective study of 65 adult patients receiving haploBMT with either TCD or PTCy, survival was significantly better after PTCy;[150] disease progression was similar between the patient groups, therefore, this difference was largely a result of markedly lower NRM after PTCy (16% versus 42% at 1 year) with a lower risk of viral (2-fold lower) and fungal (5-fold lower) infections. T-cell reconstitution was more rapid and incidence of chronic GVHD (7% versus 18%) was lower following PTCy in this study.[150] Two parallel prospective BMT Clinical Trials Network (CTN) studies assessed RIC with PTCy–haploBMT or umbilical cord blood transplantation (UCBT).[151] After a median follow-up duration of 1-year, almost all parameters (engraftment, grade III–IV acute GVHD, chronic GVHD, NRM and grade 3–4 toxicities) favoured PTCy– haploBMT; however, the relapse rate was lower after UCBT, leading to similar PFS between the two cohorts.[151] Longer-term follow-up data showed similar trends for 3-year outcomes between UCBT and PTCy–haploBMT (NRM 28% versus 8%, relapse 36% versus 58%, PFS 36% versus 35%, overall survival 39% versus 54% for UCBT and haploBMT, respectively).[152] Findings of another retrospective study, which compared UCBT and PTCy– haploBMT, showed markedly faster platelet engraftment, lower rates of acute and chronic GVHD, a lower relapse rate and better PFS for patients who received PTCy– haploBMT.[153] UCB and PTCy–haploBMT are currently being compared in an ongoing randomized phase III trial (NCT01597778).[154]

PTCy in other transplant settings

Given the success in facilitating haploBMT, PTCy has subsequently been applied to other transplantation settings. After myeloablative conditioning and HLA-matched-related or HLA-matched-unrelated bone-marrow transplantation, PTCy has proven to be effective as single-agent GVHD prophylaxis.[155–157] Among 209 patients with leukaemia who received transplants at JHH from 2004–2011, rates of grade II–IV acute GVHD, grade III–IV acute GVHD and chronic GVHD were 45%, 11% and 13%, respectively, and the 3-year NRM was 17%.[156] Similarly encouraging results were seen in a multi-institutional study using this approach with a different myeloablative conditioning regimen.[157] This strategy is currently being compared with TCD and calcineurin-inhibitor-based GVHD prophylaxis in a three-arm randomized phase III study (NCT02345850).[158]

Nevertheless, PTCy might be insufficient as single-agent GVHD prophylaxis after HLA-matched alloBMT using PBSCs and/or RIC. Two small studies using a RIC–PBSC approach showed higher rates of grade III–IV acute GVHD and NRM.[159,160] A larger study of 49 patients using either bone marrow or PBSCs for RIC– HLA-matched alloBMT, found GVHD rates for patient who received PTCy that were relatively similar to those previously reported using myeloablative conditioning.[161] However, 2-year NRM was high at 39%, which in part might reflect the use of ATG in the treatment of 65% of HLA-matched-unrelated patients (NRM: 25% without ATG versus 40% with ATG). At the JHH, the standard approach has been to use PTCy in combination with MMF and tacrolimus for

patients receiving RIC or PBSCs for HLA-matched alloBMT, analogous to the haploBMT platform; this approach is one of three GHVD prophylactic arms for RIC–HLA-matched alloBMT currently being investigated in an ongoing randomized phase II study (NCT02208037).[162] Alternatively, rapamycin might be substituted for MMF and tacrolimus in RIC–PBSC– HLA-matched alloBMT with favourable rates of GVHD and NRM.[163]

Lastly, PTCy has shown promise in facilitating solid-organ transplantation. One group reported an approach to combined kidney/bone-marrow transplantation in which fludarabine/cyclophosphamide conditioning treatment was given before renal transplantation and column-selected PBSCs were given the day after renal transplantation.[164–166] MMF and tacrolimus were started 2 days before kidney transplantation, and PTCy was given at 50 mg/kg 3 days post-transplantation. According to the latest report,[166] 12 of 19 patients achieved functional tolerance, as demonstrated by successful cessation of all immunosuppression without graft rejection; tolerance induction seemed to be dependent on achieving sustained donor chimerism.[165]

Conclusions

Within the past two decades, HLA-haploidentical alloBMT has undergone a renaissance in terms of what can be achieved with this technique. This renewal is a result of the development of novel strategies intended to overcome the intense bidirectional alloreactivity, which had previously resulted in unacceptably high rates of graft failure and severe GVHD. HaploBMT can now be performed safely and indeed might result in outcomes similar to those reported using other donor types. Other challenges unique to partially HLA-mismatched alloBMT have been discovered, such as a pattern of relapse marked by loss of the unshared HLA molecules by malignant cells.[167] Each strategy for haploBMT discussed in this Review has its own advantages and disadvantages (Table 2). Therefore, investigators worldwide are developing ways to refine each approach and improve the outcomes of patients treated in each paradigm: reducing NRM and improving immune reconstitution is an active area of investigation for TCD; reducing GVHD, particularly chronic GVHD, is a major priority for researchers attempting to improve the GIAC protocol; and in PTCy–haploBMT, research efforts are focused on reducing relapse rates. Ultimately, the determination of the optimal approach to performing haploBMT will require direct comparisons in prospective randomized studies.

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Box 1

The HLA complex and HLA-haploidentical transplantation

The HLA is the human form of the major histocompatibility complex, which encodes proteins responsible for cell surface antigen presentation. Encoded by a group of closely linked genes on chromosome 6, two main classes of HLA proteins are known to exist: class I (A, B and C) and class II (DP, DQ and DR). HLA class I genes are constitutively expressed by most cell types, and the expressed proteins associate with β2-microglobulin to form the complete HLA class I molecule. HLA class I molecules present intracellular peptides that are processed in proteasomes and thus direct $CD8^+$ cytotoxic T-cells towards the elimination of infected cells or cells expressing other aberrant peptides. HLA class II genes are constitutively expressed on haematopoietic cells involved in antigen presentation. An HLA class II molecule is a heterodimer composed of separately encoded α and β chains. HLA class II molecules present peptides derived from the fusion of endocytic vesicles with lysosomes and thus direct $CD4^+$ T cells towards recognizing the presence of extracellular pathogens.

HLA molecules of one or both classes are expressed on virtually all cells. Thus, HLA antigens are abundant and elicit a robust immune response. HLA molecules are, therefore, a major determinant of the graft-versus-host response as host cell expression of HLA molecules not present in the donor elicits a strong non-self immune response by the graft within the host. This strong alloreactivity can also occur in the opposite direction, mediating a host-versus-graft response that can ultimately result in graft rejection. HaploBMT is the extreme form of this problem wherein only one of the two HLA haplotypes is shared and thus the unshared haplotype encodes allogeneic HLA molecules that strongly activate the immune system. Consequently, use of haploBMT has historically been associated with high rates of graft failure (the end result of host-versus-graft immunity) and severe graft-versus-host disease.

Abbreviations: haploBMT, human leukocyte antigen-haploidentical allogeneic blood or bone-marrow transplantation; HLA, human leukocyte antigen.

Box 2

Commonly used HLA-haploidentical alloBMT platforms

Myeloablative conditioning and T-cell depletion with 'megadose' CD34$^+$ cell allografts[50]

- TBI (8 Gy) on pretransplantation day 9
- Thiotepa (5 mg/kg/day) on pretransplantation days 8 and 7
- Fludarabine (40 mg/m^2/day) on pretransplantation days 7 to 3
- Rabbit antithymocyte globulin (5 or 6 mg/kg/day) on pretransplantation days 5 to 2
- CD34$^+$ selected PBSC allograft on day 0

Myeloablative conditioning and *in vivo* T-cell modulation using the GIAC protocol[19,87,96]

- Cytarabine (4 g/m^2/day) on pretransplantation days 10 and 9
- MMF from pretransplantation day 9 to post-transplantation day 60
- Ciclosporin-A from pretransplantation day 9 to post-transplantation day 180–300
- Busulfan (oral, 4 mg/kg/day; IV, 3.2 mg/kg/day) on pretransplantation days 8, 7 and 6
- Cyclophosphamide (1.8 g/m^2/day) on pretransplantation days 5 and 4
- Rabbit antithymocyte globulin (1.5 or 2.5 mg/kg/day) on pretransplantation days 5 to 2
- Semustine (250 mg/m^2) on pretransplantation day 3
- GCSF-stimulated T-cell-replete PBSC and bone-marrow allografts on day 0
- Methotrexate (15 mg/m^2) on post-transplantation day 1
- Methotrexate (10 mg/m^2/day) on post-transplantation days 3, 6 and 11

Reduced-intensity conditioning with high-dose, post-transplantation cyclophosphamide[124]

- Cyclophosphamide (14.5 mg/kg/day) on pretransplantation days 6 and 5
- Fludarabine (30 mg/m^2/day) on pretransplantation days 6 to 2
- TBI (2 Gy) on pretransplantation day 1
- T-cell-replete bone-marrow allograft on day 0
- Cyclophosphamide (50 mg/kg/day) on post-transplantation days 3 and 4
- MMF on post-transplantation days 5 to 35

- Tacrolimus on post-transplantation days 5 to 180

Abbreviations: alloBMT, allogeneic blood or bone-marrow transplantation; GCSF, granulocyte colony-stimulating factor; MMF, mycophenolate mofetil; PBSC, peripheral-blood stem cell; TBI, total body irradiation.

Box 3

Partially matched familial donors versus HLA-haploidentical donors

Strictly speaking, the term HLA-haploidentical donor refers to the situation in which the donor and recipient share a single inherited identical copy of chromosome 6 (containing the HLA loci). Therefore, all HLA-haploidentical donors must be related donors to ensure that the shared haplotype is indeed identical at all loci, whether tested or untested. The other copy of chromosome 6, while not identical, can be matched at a variable number of class I and II HLA loci (Figure 1). Thus, an HLA-haploidentical donor can be more than 'half-matched' based on the presence of partial matching at the unshared chromosome, resulting from chance or distant shared ancestry. By contrast, some individuals use the term HLA-haploidentical selectively to refer to the situation in which the donor and recipient share only the inherited identical chromosome while the unshared allele is completely HLA-mismatched ('half-matched'). However, the term HLA-haploidentical throughout this Review will refer to the strict definition. Even though the terms 'HLA-haploidentical donor' and 'partially matched familial donor' are used largely synonymously, HLA-haploidentical donors represent a subgroup of partially matched familial donors. Theoretically, two relatives could have completely dissimilar versions of one copy of chromosome 6 and have partial matching of the other copy (see 'Brother 2' in Figure 1b). While this uncommon situation could be termed a partially matched family member, it would not represent an HLA-haploidentical family member.

Abbreviation: HLA, human leukocyte antigen.

Box 4

Acute versus chronic GVHD

GVHD comes in two main types: acute and chronic. Historically, these were defined based on the time at which the clinical manifestations began: whether it was less than (acute) or more than (chronic) 100 days post-transplantation. Subsequent research and clinical experience determined that acute and chronic GVHD are actually distinct clinicopathological entities, probably with differing pathophysiology and are not bound by the timing of occurrence post-transplantation.

Typically, acute GVHD is considered to involve the skin, liver and gastrointestinal tract. Each of the three scored organs is staged based on the severity of symptoms within that organ. Individual organ scores are then combined to create an overall acute GVHD grade.[26] By definition, grade I acute GVHD is limited to skin-only involvement affecting less than 50% of the body surface area; when not progressing to higher grade disease, grade I acute GVHD is generally self-limited and does not require treatment other than perhaps topical steroids. Grade II acute GVHD is typically considered clinically significant, but does not always require treatment and generally is not directly life-threatening. Grade III–IV acute GVHD is considered severe, can be immediately life-threatening, and almost always requires systemic immunosuppressive therapy. Death can ensue from direct organ toxicity, infection from bacterial translocation across disturbed epithelial barriers or impaired immunity from systemic immunosuppression, or systemic manifestations, such as dehydration or electrolyte disturbances.

By contrast, chronic GVHD can affect virtually any organ. Defining the presence of chronic GVHD and the extent of its involvement is complex, involving organ-specific ‘diagnostic’ and ‘distinctive’ criteria.[168] Chronic GVHD is often a more insidious and persistent form of GVHD that frequently requires longer-term immunosuppressive therapy and can cause substantial morbidity and functional impairment in patients. Furthermore, chronic GVHD is the leading cause of late nonrelapse mortality in patients treated with allogeneic blood or bone-marrow transplantation.[169]

Abbreviation: GVHD, graft-versus-host disease.

Key points

- HLA-haploidentical allogeneic blood or bone-marrow transplantation (haploBMT) has historically been associated with poor outcomes, owing to high rates of graft failure and graft-versus-host disease (GVHD)
- Several transplantation platforms have been developed that successfully overcome these historical barriers to haploBMT; three main approaches have been used extensively to conduct haploBMT procedures in patients
- T-cell depletion with ‘megadose’ $CD34^+$ cells results in exceptionally low rates of GVHD, but is associated with poor T-cell function and thus high nonrelapse mortality (NRM), predominantly owing to infection
- The GIAC protocol, which involves *in vivo* modulation of T-cell-replete allografts, produces essentially universal engraftment with limited relapse and favourable survival, albeit with high rates of GVHD, particularly chronic GVHD
- Use of high-dose, post-transplantation cyclophosphamide after T-cell-replete allografting results in low rates of GVHD and NRM and favourable immune reconstitution, with somewhat higher rates of relapse, particularly after reduced-intensity conditioning
- No standard-of-care currently exists, as no completed prospective randomized studies have, thus far, compared any of these haploBMT approaches with each other or with transplantation approaches using other donor types

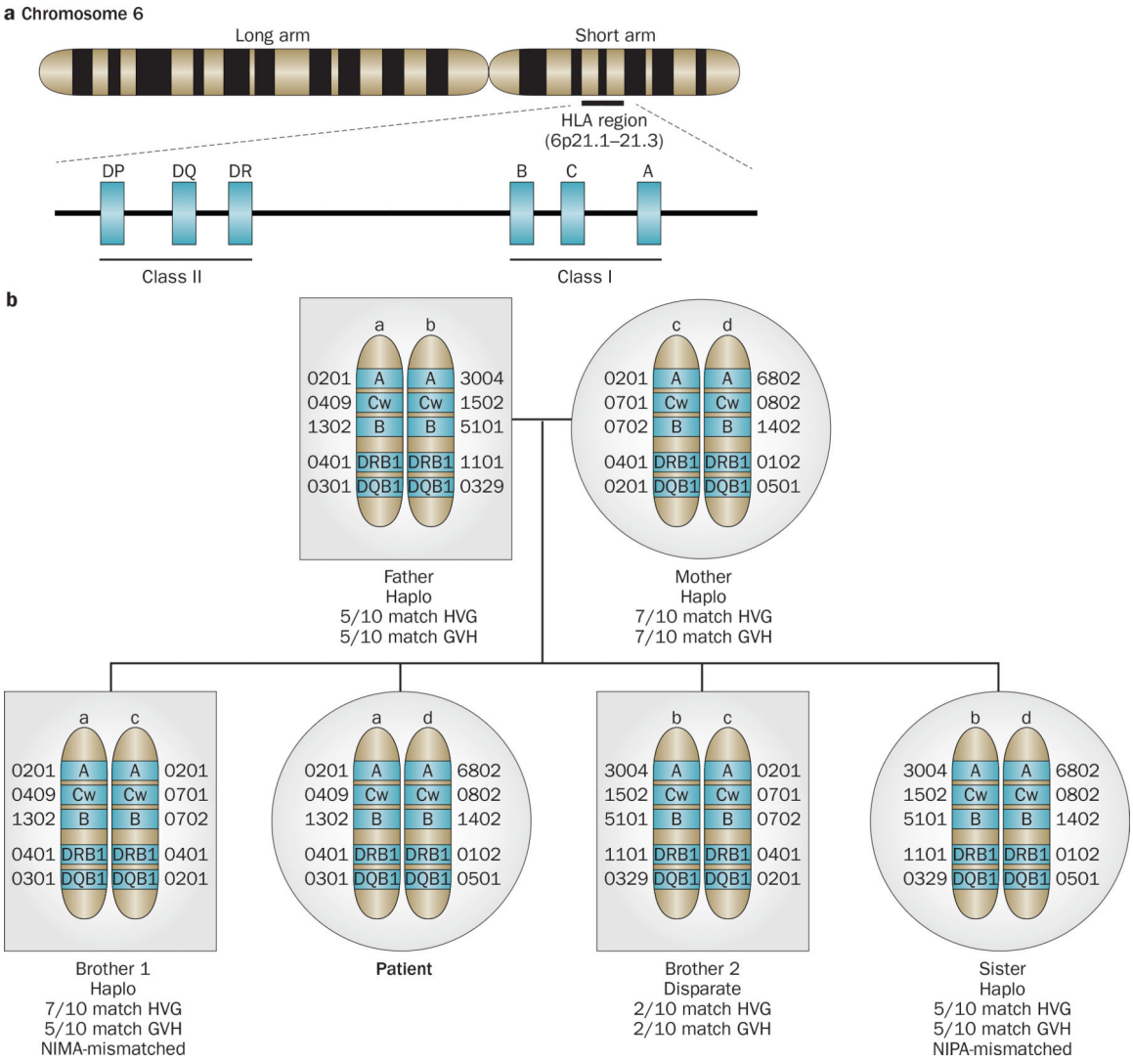

Figure 1.

HLA-haploidentical donors. **a** | Located on the short arm of chromosome 6, the HLA region contains the genes for class I and class II histocompatibility molecules, which are commonly tested as clinically relevant transplantation antigens. However, the HLA region is genetically complex and includes other class I and class II genes in addition to genes not involved in histocompatibility (not shown). **b** | An example pedigree is shown. The patient in this pedigree does not have an HLA-matched sibling, although she has four HLA-haploidentical family members. Each individual haplotype is denoted by a lower-case letter above it. Note that the term HLA-haploidentical simply denotes the presence of one shared haplotype and one unshared haplotype between the patient and her potential donors; a 'haplo' donor can be more than 'half-matched' if there are common alleles on the unshared haplotypes. HVG and GVH indicate the degree of matching in the host-versus-graft and graft-versus-host directions, respectively. Mismatching at non-inherited maternal or non-inherited paternal antigens, which also might affect the relative antigenicity of the donor:recipient pair, are indicated for the 'haplo' siblings. Abbreviations: GVH, graft-versus-host; HLA, human

leukocyte antigen; HVG, host-versus-graft; NIMA, non-inherited maternal antigens; NIPA, non-inherited paternal antigens.

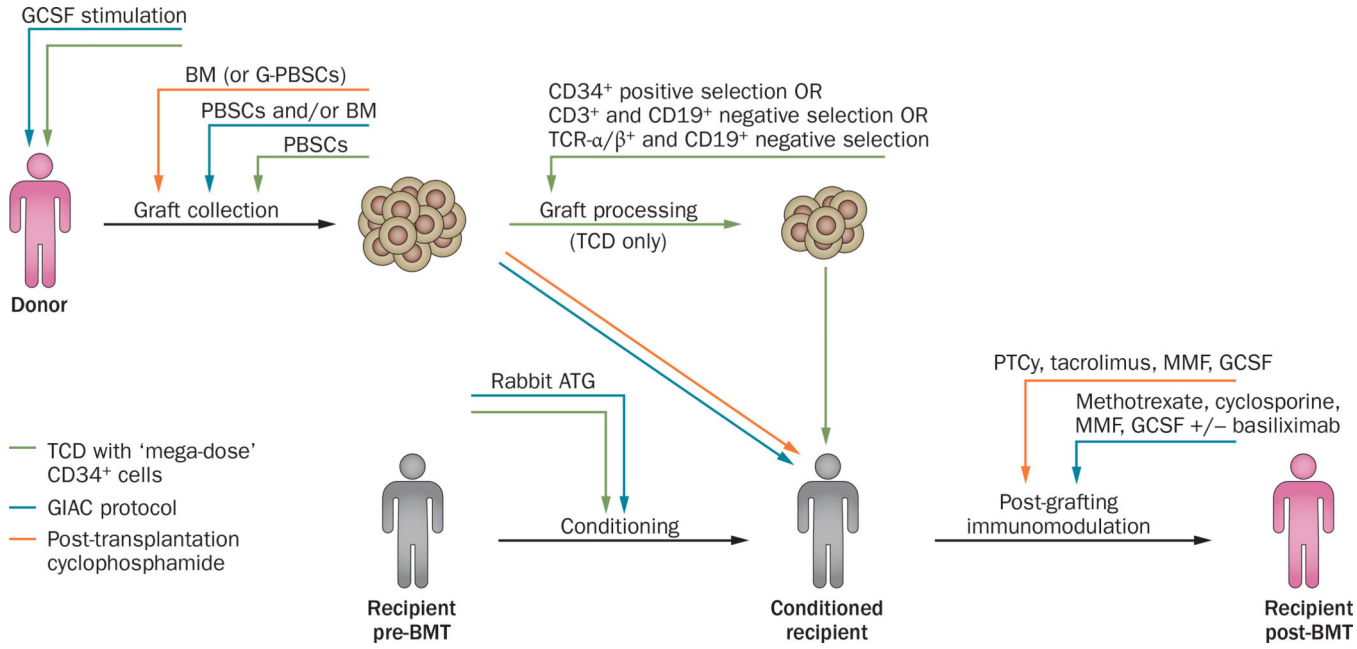

Figure 2.

Components of each transplantation platform. Interventions on the donor or recipient that are required for each transplantation platform are shown at each stage of the transplantation procedure. Abbreviations: ATG, antithymocyte globulin; BM, bone-marrow; BMT, blood or bone-marrow transplantation; GCSF, granulocyte colony-stimulating factor; G-PBSCs, granulocyte colony-stimulating factor-mobilized peripheral-blood stem cells; MMF, mycophenolate mofetil; PBSCs, peripheral-blood stem cells; PTCy, post-transplantation cyclophosphamide; TCD, T-cell depletion; TCR, T-cell receptor.

Table 1

Selected studies of three haploBMT approaches for treating haematological malignancies

Period of study, median follow-up (y), *n*, median age (y)	Diseases				Graft failure	GVHD			NRM (due to Infection)	Relapse	PFS/ DFS	OS
	AML or MDS	ALL	Lymph	Other		Acute II–IV	Acute III–IV	Chronic				
T-cell depletion with 'megadose' CD34$^+$ cells												
1993–1994[48] 0.6, *n* = 17, 23	5	9	0	3	6%	6%	6%	NR	53 (35)%	12%	35%	35%
1995–1997[49] 1.5, *n* = 43, 22	20	23	0	0	5%	0%	0%	0%	40 (26)%	30%	28%	NR
1995–2003[52] 4.1, *n* = 63, 9	12	32	4	15	17%	7%	0%	13%	29 (17)%	∼40%	41%	∼50%
1999–2004[50] 1.8, *n* = 104, 33	67	37	0	0	9%	8%	2%	5%	37 (26)%	25%	39%	40%
1995–2004[51] 3.9 (AML) and 2.4 (ALL), *n* = 147, 37 (AML) and 21 (ALL)	86 0	0 61	0 0	0 0	9%*	5% 18%	2% 11%	10% 19%	52 (30)% 48 (30)%	21% 27%	29% 23%	NR NR
1995–2004[53] NR, *n* = 102, 9	0	102	0	0	13%	22%	9%	17%	37 (22)%	36%	27%	29%
2003–2007[58] 0.7, *n* = 29, 42	16	7	3	3	3%	48%	14%	10%	28 (24)%	NR	35%	35%
2004–2012[59] 4.3, *n* = 46, 11	20	26	0	0	13%	27%	7%	21%	20 (2)%	63%	25%	37%
2008–2012[71]‡ 3.8, *n* = 43, 40	33	10	0	0	5%	15%	NR	2%	40 (20)%	5%	56%	NR
GIAC protocol												
1999–2000[86]§ 1.8, *n* = 15, 15	2	10	0	3	0%	33%	20%	100%	33 (13)%	7%	60%	60%
2000–2002[91]‖ 2.6, *n* = 38, 18	10	16	0	12	0%	11%	NR	89%	32 (18)%	NR	NR	53%
2000–2005[87]¶ 1.9, *n* = 171, 23	58	66	0	47	0%	55%	23%	74%	23 (12)%	19%	65%	65%
2002–2006[88] 3.0, *n* = 42, 10–14	12	24	0	6	0%	57%	14%	57%	20 (10)%	∼20% SR, 37% HR	57%	64%
2003–2005[20] NR, *n* = 56, 28	14	19	0	23	4%	27%	NR	23%	13 (5)%	22%	68%	70%
2001–2007[96]# 3.0, *n* = 250, 25	108	142	0	0	0%	46%	13%	54%	26 (17)%	18%	56%	59%
2004–2009[89]** 2.2, *n* = 83, 40	67	16	0	0	0%	20%	7%	34%	18 (6)%	∼30% CR, 79% AD	∼55% CR, 9% AD	∼50% CR, 9% AD
2005–2010[92]‖ 1.5, *n* = 80, 37	48	15	7	10	7%	24%	5%	17%	36 (20)%	28%	38%	45%
2008–2013[93]‡‡ 2.2, *n* = 99, 25	39	50	3	7	0%	42%	17%	41%	30 (16)%	14%	58%	61%
2010–2013[94] 2.6, *n* = 231, 28	231	0	0	0	0%	36%	10%	42%	13 (NR)%	15%	74%	79%

Period of study, median follow-up (y), *n*, median age (y)	Diseases				Graft failure	GVHD			NRM (due to Infection)	Relapse	PFS/ DFS	OS
	AML or MDS	ALL	Lymph	Other		Acute II–IV	Acute III–IV	Chronic				
Post-transplantation cyclophosphamide[§§]												
2002–2012[127]|||| 4.1, *n* = 372, 55	107	24	212	29	8%	32%	4%	13%	14 (8)%	46%	40%	50%
2005–2010[21] 3.0, *n* = 53, 46	21	10	18	4	2%	30%	11%	38%	7 (NR)%	33%	60%	64%
2006–2009[131]¶¶ 3.3, *n* = 27, 52	18	4	3	2	8%	59%	7%	16%	22 (11)%	32%	NR	48%
2006–2012[22] 1.6, *n* = 92, 45	49[##]		25	18	NR	14%	4%	15%	18 (10)%	35%	43%	52%
2008–2010[151] 1.0, *n* = 50, 48	22	6	19	3	2%	32%	0%	13%	7 (5)%	45%	48%	62%
2009–2011[150] 0.9, *n* = 32, 45	16	4	5	7	6%	20%	5%	7%	16 (9)%	34%	50%	64%
2009–2012[149] 1.7, *n* = 49, 45	0	0	49	0	4%	26%	NR	5%	16 (14)%	19%	63%	71%
2009–2013[133] 1.4, *n* = 55, 49	21	2	25	7	4%	53%	8%	18%	23 (19)%	28%	48%	51%
NR[132]¶¶ 2.6, *n* = 28, 47	16	10	2	0	0%	39%	4%	22%	4 (0)%	21%	74%	77%
2012–2014[130] 2.0, *n* = 30, 46	17	6	2	5	0%	43%	23%	56%	3 (0)%	24%	73%	78%

Where an explicit cumulative incidence or survival probability by competing risk analysis was not provided in a manuscript, a simple percentage was substituted where possible. Where an explicit percentage was not reported but was shown in a figure, then an approximate percentage is reported.

[*] Engraftment was reported for both cohorts (patients with AML and patients with ALL) together.

[‡] Infusions of regulatory and conventional T cells were added to the TCD-haploBMT platform.

[§] This initial pilot study used an approach similar to the GIAC protocol except that the conditioning was different and it used only BM allografts. This study was used as a comparator group in reference 91.

[||] Differs from the GIAC protocol in terms of the conditioning, the use of only BM allografts, and the addition of basiliximab.

[¶] Includes an expanded cohort of reference 19, so is listed instead of reference 19.

[#] 117 patients were also reported in reference 87, but had longer follow-up in this study.

[**] GIAC protocol was modified in terms of RIC, the use of PBSC allografts only, and the omission of MMF.

[‡‡] GIAC protocol was modified in terms of the use of only PBSC allografts and longer durations of MMF (100 days) and CsA (9 months).

[§§] PTCy studies all have in common the use of PTCy for GVHD prophylaxis after haploBMT. The conditioning used and the source of stem cells (peripheral blood versus bone marrow) vary between (and sometimes within) studies.

[||||] Includes an expanded cohort of patients reported in reference 124, so is listed instead of reference 124. Infectious deaths were not reported in this manuscript but had been reported for a slightly smaller cohort of 210 patients (reference 126). Consequently, the percentage from reference 126 is substituted here.

[¶¶] Involves a 'two-step' approach to PTCy–haploBMT in which, after conditioning, a fixed-dose of peripheral blood T-cells is given, then PTCy is administered 3 and 4 days later, and finally a $CD34^{+}$-selected PBSC allograft is given.

[##] Indicates patients had either AML, MDS or ALL.

Abbreviations: AD, active disease at haploBMT; AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; BM, bone-marrow; CsA, ciclosporin-A; CR, complete remission at haploBMT; DFS, disease-free survival; GVHD, graft-versus-host disease; haploBMT, human leukocyte antigen-haploidentical allogeneic blood or bone-marrow transplantation; HR, high-risk disease; Lymph, lymphoma; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; *n*, number of patients transplanted; NR, not reported; NRM, nonrelapse mortality; PBSC, peripheral blood stem cells; PFS, progression-free survival; PTCy, post-transplantation cyclophosphamide; RIC, reduced-intensity conditioning; SR, standard-risk disease; TCD, T-cell depletion; y, years.

Table 2

Relative advantages and disadvantages of each approach to haploBMT

Clinical outcome	T-cell depletion	GIAC protocol	PTCy
Engraftment	2–**3**	**1**	**2**–3
Acute GVHD	**1**	**3**	**2**
Chronic GVHD	**1**–2	**3**	1–**2**
Infection/deaths from infection	**3**	**2**	**1**
Nonrelapse mortality	**3**	**2**	**1**
Relapse	**2**–3	**1**	2–**3**

1 indicates most favourable; 2, intermediate; 3, least favourable. When more definitive ratings are unclear, a range is shown with the probable rating indicated in bold. Ratings take into account the findings of the available published studies (Table 1), but are unable to account for many factors that influence outcomes, such as differences between studies in patient characteristics or the malignant disease types, features or pretransplantation remission status.

Abbreviations: GVHD, graft-versus-host disease; haploBMT, human leukocyte antigen-haploidentical allogeneic blood or bone-marrow transplantation; PTCy, post-transplantation cyclophosphamide.