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Hematopoietic SCT from partially HLA-mismatched (HLA-haploidentical) related donors

HJ Symons[1] and **EJ Fuchs**[2]

1*Department of Oncology, Division of Pediatric Oncology, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, USA*

2*Department of Oncology, Cancer Immunology and Hematologic Malignancies, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, USA*

Abstract

Hematopoietic SCT from a partially HLA-mismatched (HLA-haploidentical) first-degree relative offers the benefits of rapid and near universal donor availability but also the risks that result from traversing the HLA barrier; namely, graft failure, severe GVHD and prolonged immunodeficiency. Improvements over the last 10 years in conditioning regimens, graft engineering and pharmacological immuno-prophylaxis of GVHD have substantially reduced the morbidity and mortality of HLA-haploidentical SCT. Highly immunosuppressive but nonmyeloablative conditioning extends the availability of HLA-haploidentical SCT to elderly hematologic malignancy patients lacking HLA-matched donors and permits recovery of autologous hematopoiesis in the event of graft failure. Current regimens for HLA-haploidentical SCT are associated with a 2-year non-relapse mortality of 20 ± 5%, relapse of 35 ± 15% and overall survival of 50 ± 20%. Major developmental areas include harnessing natural killer cell alloreactivity to reduce the risk of disease relapse and improving immune reconstitution by delayed infusions of lymphocytes selectively depleted of alloreactive cells. Hematologic malignancy patients who lack suitably matched related or unrelated donors can now be treated with HLA-haploidentical related donor or unrelated umbilical cord blood SCT. Future clinical trials will assess the relative risks and benefits of these two graft sources.

Keywords

allo-SCT; alternative donors; natural killer cells; conditioning regimens; GVHD; immune reconstitution

Introduction

Allo-SCT, following either myeloablative or reduced intensity conditioning, is a potentially curative therapy for a variety of hematologic malignancies and non-hematologic disorders. Of all the potential sources of allografts, transplantation of stem cells from an HLA-matched sibling has generally produced the best overall and progression-free survivals.[1] Unfortunately, only about one-third of candidates for allo-SCT have HLA-matched siblings.[2] For patients who lack HLA-matched siblings, there are three alternative sources of stem cells for allo-SCT: (1) volunteer unrelated donors (VUDs); (2) umbilical cord blood and (3) partially HLA-mismatched, or HLA-haploidentical, related donors. Next to HLA-matched related donors,

Correspondence: Dr EJ Fuchs, Cancer Immunology and Hematologic Malignancies, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, 488 Cancer Research Building, 1650 Orleans Street, Baltimore, MD 21231, USA. E-mail: E-mail: fuchsep@jhmi.edu.

phenotypically matched VUDs are the most widely sought for allo-SCT.[3] However, the chance of finding an HLA-matched VUD varies significantly depending upon the racial and ethnic background of the recipient, ranging from over 60–70% in Caucasians, to about 10–20% for ethnic minorities.[4] The search for an HLA-matched VUD is also hindered by the amount of time it takes from search initiation to donor identification.[5,6] In contrast, a partially HLA-compatible first-degree relative can be identified and mobilized immediately for transplantation in nearly all situations. This is because a patient shares exactly one HLA haplotype with each biological parent or child, and each sibling of the patient has a 50% likelihood of sharing one HLA haplotype while being variably mismatched for HLA genes of the other haplotype. Thus, when a patient lacks an HLA-identical sibling, the treating physician must balance the risks that the patient's disease will progress or health will deteriorate while searching for a VUD versus the risk of crossing HLA barriers with the use of an HLA-haploidentical donor. The aim of this article is to review the history and recent progress of allo-SCT using HLA-haploidentical donors. Included in this review will be a discussion of efforts to improve the outcome of HLA-haploidentical SCT and a comparison of the relative advantages and disadvantages of partially HLA-mismatched related donor vs unrelated umbilical cord blood (UCB) SCT.

Evolution of HLA-haploidentical SCT

Table 1 lists some of the largest published studies of HLA-haploidentical SCT after either myeloablative or nonmyelo-ablative conditioning. The table illustrates the substantial progress that has been made in improving the safety, efficacy and utility of the procedure for patients with hematologic malignancies. Much of the progress in HLA-haploidentical SCT can be attributed to advances in supportive care such as monitoring and preemptive therapy against CMV[13] and EBV-related lymphoproliferative dis-ease[14] and improved detection and treatment of invasive fungal infections.[15]

Haploidentical SCT after myeloablative conditioning

The earliest studies of T-cell-replete HLA-haploidentical SCT for hematologic malignancies were characterized by high risks of graft rejection, GVHD, and treatment-related mortality.[1,16–19] Outcomes were significantly worse after HLA-haploidentical related donor as compared to HLA-matched sibling SCT, and increasing HLA disparity in the host-vs-graft and graft-vs-host directions were associated with increasing risks of graft failure[16,20] and GVHD,[20,21] respectively. However, overall survival was similar after HLA-matched vs 1 HLA Ag-mismatched SCT for patients with acute leukemia in remission.[18] The unacceptably high incidence of severe acute GVHD after haploidentical SCT motivated early trials of graft T-cell depletion (TCD). Encouraging results were obtained for patients with SCID,[22] but patients with leukemia experienced a high incidence of fatal graft rejection, up to or exceeding 30%.[7] Although patients who receive T-cell-depleted HLA-haplo-identical SCT have a reduced risk of acute and chronic GVHD compared to recipients of T-cell-replete grafts, the incidence of graft failure is increased and there is no improvement in leukemia-free survival[20] because of a high mortality from infection,[8] EBV-related lymphoproliferative disease[23] and possibly an increased risk of relapse.[24]

Megadose SCT: a turning point in HLA-haploidentical SCT

A solution to the problem of graft failure after T-cell-depleted allo-SCT was provided by Reisner *et al.*,[25] who found that graft rejection could be obviated by administering an extremely high dose or 'megadose' of MHC-incompatible stem cells. 'Megadose' SCTs in humans, piloted by Aversa *et al.*[26] in Perugia, Italy, initially consisted of G-CSF-mobilized PBSC and BM cells, both depleted of T-cells *ex vivo* by soybean agglutination and E-rosetting and a conditioning regimen, including TBI, CY, thiotepa and antithymocyte globulin (ATG), with

no post transplant immunosuppression. The Perugia group subsequently modified this regimen extensively, with fludarabine replacing CY in the TBI-based conditioning regimen in an attempt to reduce the conditioning regimen toxicity without jeopardizing its immunosuppressive effect.[27] Other advances included implementation of a $CD34^+$ cell selection device that provides a 4.5 log TCD and the elimination of G-CSF administration after transplantation.[9] This cytokine impairs DC production of IL-12, leading to abnormalities in Ag-presenting function and T-cell activation.[28] Over the past decade, the Perugia group has demonstrated that full HLA-haplotype mismatched transplants can be successful in patients with acute leukemia in first or second CR when a megadose of stem cells, typically > 10^7 $CD34^+$ cells per kilogram of recipient body weight, is infused after an immunomyeloablative conditioning regimen. However, the profound depletion of host and donor T cells that was required to reduce GVHD and graft rejection was accompanied by significant infectious morbidity and mortality and a prolonged time to immune reconstitution. Early results showed a non-relapse mortality (NRM) rate of 40%,[27] with infection the leading cause of death. Somewhat improved immune reconstitution and fewer deaths secondary to infection occurred when G-CSF was eliminated from the regimen.

Other approaches using myeloablative conditioning and high-dose $CD34^+$ cell-selected grafts described similarly favorable engraftment and GVHD rates, but unfortunately, recurrent malignancy and problems with infectious-related deaths were reported. In a Canadian multicenter study, all 11 study patients engrafted without GVHD but 10 of 11 patients died from leukemic relapse or infection.[29] Waller *et al.*[30] reported a 93% mortality rate in patients who received T-cell-depleted, $CD34^+$ -enriched HLA-haploidentical SCT after an ATG-based regimen, with most deaths a result of infection or relapse. In a retrospective analysis from Japan, severe infections occurred in 20 of 32 patients receiving CD34-selected PBSCs from 2–3 HLA-antigen-mismatched related donors.[31] Seventeen of 32 patients (53%) died from treatment-related causes, including 10 (31%) from infection, and 9 patients died from complications of progressive disease. These results suggest that transplantation of highly purified $CD34^+$ PBSCs from haploidentical donors is associated with a low incidence of GVHD but an increased risk of disease progression or fatal infection. Recently, methods of depleting $CD3^+$ T cells and $CD19^+$ B cells from megadose PBSC collections have been developed.[32] CD3/CD19 depleted grafts contain not only $CD34^+$ stem cells but also $CD34^-$ progenitors, natural killer (NK) cells, DCs and graft facilitating cells, all of which may enhance immune reconstitution after HLA-haploidentical SCT. Preliminary results of HLA-haploidentical SCT using CD3/CD19-depleted grafts are encouraging in this regard.

Blood vs marrow from G-CSF-primed donors

Treatment of BM donors with G-CSF before donation increases marrow $CD34^+$ and CFU-GM cells, reduces total lymphocytes and reverses the $CD4^+$ /$CD8^+$ T-cell ratio. To enhance engraftment by increasing the dose of transplanted HSCs, 15 patients with high-risk leukemia received myeloablative conditioning with cytarabine, Cy, and 1000 cGy TBI, G-CSF-primed BM from haploidentical donors, and GVHD prophylaxis with rabbit ATG (5 mg/kg/day on days −4 to −1), CsA, MTX and mycophenolate mofetil.[33] All 15 patients had prompt trilineage hematopoietic engraftment, the cumulative incidence of GVHD was 33%, and nine of 15 patients were alive at a median follow-up of 22 months (range 13–35 months) at the time of reporting. Based upon these results, Lu *et al.*[10] at Peking University in Beijing, China compared the outcomes of 293 patients with leukemia receiving HLA-matched sibling (n = 158) or HLA-haploidentical related grafts (n = 135) from G-CSF-primed donors. Patients undergoing haploidentical SCT were conditioned with cytarabine, oral BU, CY and methyl-CCNU, received G-CSF-primed BM on day 0 (n = 134) and/or G-CSF-primed PB on day 1 (n = 131) and GVHD prophylaxis with ATG 2.5 mg/kg/day on days −4 to −1, CsA, MTX and mycophenolate mofetil. All but two haploidentical SCT patients had sustained engraftment of

donor neutrophils. The cumulative incidences of acute grades II–IV, grades III–IV and chronic GVHD in recipients of matched vs mismatched SCT were 32 vs 40% ($P = 0.13$), 11 vs 16% (no P-value provided) and 56 vs 55% ($P = 0.90$). Mismatched patients had a higher incidence of CMV antigenemia (65 vs 39%; $P < 0.001$) and hemorrhagic cystitis (35 vs 13%; $P < 0.001$) but not of CMV disease. Two-year rates of relapse and NRM were 13 vs 18% ($P = 0.40$) and 14 vs 22% ($P = 0.10$) for recipients of matched vs mismatched transplants, respectively. The 2-year probabilities of overall survival were 72 vs 71% ($P = 0.72$) and of leukemia-free survival were 71 vs 64% in the matched and mismatched cohorts, respectively. In a follow-up report of 157 consecutive recipients of G-CSF-primed BM and PB from haploidentical related donors, recipients of $CD3^+$ T-cell doses higher than the median (1.77×10^8/kg) had a significantly lower NRM, better leukemia-free survival and better overall survival.[34] The Beijing results are extremely encouraging and this regimen for haploidentical SCT needs to be evaluated at other centers. Novel aspects of the regimen that may contribute to the low rates of graft failure and GVHD may be the use of low-dose rabbit ATG,[35] the use of G-CSF-mobilized BM and PB[36,37] and the combination of CSP, MTX and mycophenolate mofetil.

Haploidentical SCT after nonmyeloablative conditioning

In an effort to reduce the regimen-related mortality while retaining a graft-vs-tumor effect, several recent clinical trials have evaluated the efficacy of nonmyeloablative conditioning for HLA-haploidentical SCT. Clinical trials at the Massachusetts General Hospital have been performed using nonmyeloablative conditioning with $CY^{+/-}$ fludarabine, *in vivo* TCD, pretransplant thymic irradiation and most recently, *ex vivo* TCD.[38] The rationale for this approach, pioneered in mouse models by Sykes *et al.*,[39,40] has included (1) the reduction of regimen-related toxicities with nonmyeloablative conditioning, (2) prevention of GVHD with *in vivo* and *ex vivo* TCD and (3) the capture of an optimal graft-vs-tumor effect with the use of delayed DLI, when clinically indicated. Their current protocol includes CY, fludarabine, MEDI-507 (a MoAb against the CD2 Ag on T cells) and thymic irradiation, which has resulted in a high incidence of mixed chimerism without early GVHD and the potential for conversion of T-cell chimerism with manageable or no GVHD. Recurrent malignancy and late infections have been the main reasons for treatment failure with this approach.[41]

Rizzieri *et al.*[11] at Duke University developed a nonmyeloablative conditioning regimen incorporating fludarabine, CY and alemtuzumab for 49 hematologic malignancy patients receiving PBSCs from HLA-haploidentical donors. Mycophenolate mofetil, with ($n = 25$) or without CsA ($n = 24$), was used for post transplantation GVHD prophylaxis. A total of seven patients (14%) experienced either primary or secondary graft failure, and the incidences of acute grades II–IV and chronic GVHD were 16 and 14%, respectively. Fifteen patients (31%) died of causes unrelated to disease progression. Twenty-five percent of patients experienced a severe infection, reactivation of CMV occurred in 86% and CMV disease developed in 14%. Overall survival of patients 1 year after transplantation was 31%. Absence of GVHD was associated with improved recovery of $CD4^+$ and $CD8^+$ T cells and $CD56^+$ NK cells following transplantation.

Selective allodepletion using CY-induced immunologic tolerance

Luznik *et al.*[12] exploited the protocol of drug-induced immunological tolerance, first described in 1959 by Schwartz and Dameshek,[42] to achieve selective *in vivo* depletion of alloreactive T cells after nonmyeloablative HLA-haploidentical BMT. In this protocol, *in vivo* exposure to antigen induces the proliferation of Ag-specific lymphocytes, which are then killed by the timely administration of a drug that is selectively toxic to proliferating over resting cells. Studies in mice established that high-dose, post transplantation CY inhibits both graft rejection and GVHD after either MHC-matched or -mismatched SCT.[43–46] Based upon these studies, 68 patients with hematologic malignancies ($n = 67$) or paroxysmal nocturnal hemoglobinuria

($n = 1$) received CY 50 mg/kg on day 3 ($n = 28$) or days 3 and 4 ($n = 40$) after nonmyeloablative conditioning and transplantation of T-cell-replete BM from HLA-haploidentical related donors.[12] Graft failure occurred in 9 of 66 (13%) evaluable patients, and was fatal in one. The cumulative incidences of acute grades II–IV and grades III–IV GVHD were 34 and 6%, respectively, and of chronic GVHD was 22%. Serious infections were relatively infrequent: there were no cases of CMV disease and only five cases of invasive fungal infection, two of which were fatal. NRM and relapse at 1 year after transplantation were 15 and 51%, respectively. Actuarial overall and EFS at 2 years after transplantation were 36 and 26%, respectively. These results suggest that post transplantation CY induces selective allodepletion *in vivo*, inhibiting fatal graft rejection and severe GVHD, while sparing functional immunity to infection.

Methods to reduce GVHD and improve immune reconstitution after HLA-haploidentical SCT

As discussed above, nonselective depletion of grafted T cells significantly reduces the incidence and severity of GVHD after partially HLA-mismatched related donor SCT but also increases the risk of graft failure and fatal opportunistic infection from prolonged immune compromise. Aside from selecting donors with the least degree of HLA mismatch with the patient, a number of additional strategies have been envisioned or used to reduce the risk of GVHD without causing profound immune compromise. These strategies include: (1) selection of donors based upon the principle of tolerance to non-inherited maternal Ags, or NIMA; (2) selective depletion of alloreactive T cells from the graft; (3) reconstitution of T-cell-depleted grafts with T cells that protect against infection but do not cause GVHD; or (4) adding cells that suppress GVHD to T-cell-replete grafts.

Selection of donors tolerant to non-inherited maternal antigens

Exposure of the developing fetus to maternal cells, which occurs during pregnancy,[47] can lead to either immunity or tolerance of non-inherited maternal HLA Ags (NIMA) and subsequently have an effect on transplant outcome. Two separate studies have demonstrated that approximately 50% of individuals with antibodies against a large number of HLA Ags do not have antibodies against NIMA.[48,49] Reactivity against non-inherited paternal antigens (NIPA) is significantly higher. Siblings who are HLA-haploidentical to each other share either the paternal or the maternal HLA haplotype. When siblings share the paternal HLA haplotype, they are mismatched for both inherited and non-inherited maternal antigens (NIMAs). Thus, HLA typing of both of the patient's parents is required to assign parental haplotypes for determining whether a sibling is mismatched for NIMA or for NIPA. It has been speculated that there should be less GVHD and less graft rejection with NIMA- rather than with NIPA-mismatched transplantations. Because graft failure and GVHD affect the outcome of HLA-haploidentical SCT, NRM and overall survival might also differ between NIMA- and NIPA-mismatched transplants. To date, several population studies have provided evidence in favor of the presence of the tolerogenic 'NIMA' effect. Such evidence includes low rates of aGVHD in T-cell-replete, HLA-haploidentical SCT from a NIMA-mismatched sibling,[50,51] as well as in unmanipulated marrow transplantation from fully HLA-haploidentical mothers using standard preparative regimens combined with peritransplantation ATG,[52] and a significantly lower risk of cGVHD in recipients of non-T-cell depleted maternal transplants vs paternal transplants.[50] Several recent studies[53–56] have also demonstrated sustained remissions of chemorefractory hematologic malignancies with acceptable rates of GVHD after T-cell-replete, HLA-haploidentical SCT from microchimeric NIMA-mismatched family members. Further studies are required to evaluate more precisely the effects of NIMA- or NIPA-specific allotolerance and to identify genetic factors associated with GVHD, NRM and relapse-free survival in a given NIMA-mismatched donor–recipient pair.

Selective graft allodepletion

Another strategy to reduce GVHD after HLA-haploidentical SCT is to induce tolerance, or 'anergy,' in host-reactive T cells by exposing the graft *ex vivo* to host alloantigens ('signal 1'), while simultaneously blocking the delivery of T-cell costimulatory signals ('signal 2').[57] Guinan *et al.*[57–59] conducted two pilot trials of myeloablative, HLA-haploidentical SCT in which donor marrow was incubated with irradiated recipient mononuclear cells in the presence of CTLA-4-Ig, a fusion molecule that blocks interaction of the T-cell costimulatory receptor CD28 with its ligands, B7-1 and B7-2, on APCs ($n = 19$) or with a combination of MoAbs against B7-1 and B7-2 ($n = 5$). GVHD developed in only 9 of 21 evaluable patients (four grade II, four grade III, one grade IV) and eight patients were alive at a median of 8 years after transplantation. *Ex vivo* tolerance induction with the combination of antibodies against B7-1 and B7-2 resulted in a 99% reduction in T cells capable of proliferating to host Ags with no significant loss of reactivity to third-party alloantigens, viral Ags or the WT-1 tumor Ag.[60,61] These *in vitro* results correlated with the low incidence of late viral infections or of opportunistic infections requiring admission.[58] However, there were 12 early deaths due to bacterial or fungal infection and/or regimen-related toxicity. The investigators are currently studying the effects of administering allo-anergized T cells after CD34-selected haploidentical SCT, to determine the optimal dose for augmenting immune reconstitution without causing GVHD.[62]

Graft TCD followed by infusion of allodepleted lymphocytes

An alternative to selectively depleting the stem cell graft of alloreactive lymphocytes is to administer a TCD stem cell graft followed by delayed infusion of mature lymphocytes selectively depleted of alloreactive cells. Selective allodepletion has been achieved by activating donor lymphocytes *ex vivo* with host APCs, followed by targeted removal based upon differential expression of surface activation markers, proliferation or retention of photoactive dyes. Methods of alloreactive cell elimination include treatment with immunotoxins,[63,64] immunomagnetic separation,[65–69] activation of suicide genes,[70,71] activation-induced cell death,[72] flow cytometric sorting[73,74] or photodynamic purging.[75,76] To achieve selective allodepletion, the group at Hôpital Necker in Paris, France, cocultured donor and irradiated host lymphocytes *ex vivo*, followed by addition of a ricin A-chain-coupled MoAb against CD25, the α-chain of the IL-2 receptor.[77] This procedure results in a > 2 log depletion of host-reactive cells, while sparing reactivity to viral and bacterial Ags as well as third-party alloantigens.[63] Allodepleted lymphocytes in doses ranging from 1 to 8×10^5 cells per kg were infused into 15 patients from 15 to 47 days after myeloablative conditioning and transplantation of CD34-selected stem cell grafts from HLA-haploidentical donors. Grades I and II acute GVHD occurred in four patients, correlating with antihost residual proliferation above 1% in a mixed lymphocyte reaction, and limited chronic GVHD in one. Compared to controls, recipients of allodepleted T cells had a faster recovery of $CD4^+$ and $CD8^+$ T cells, and infections from EBV, CMV and adenovirus were eliminated following infusion. At the time of reporting, 8 of 15 patients were alive and well at a median of 24 months of follow-up. A trial currently enrolling patients uses immunomagnetic depletion of $CD25^+$ cells from HLA-haploidentical donor lymphocyte infusions (DLI), which are then given to improve immune reconstitution after HLA-haploidentical SCT.[78] Two recipients of 3×10^5 allodepleted T cells per kg achieved > 200 $CD3^+$ $CD4^+$ T cells per μl blood as early as 60 days after SCT, and one developed grade II GVHD.

Amrolia *et al.*[80] conducted a clinical trial of infusing allodepleted lymphocytes, using the ricin A chain-conjugated anti-CD25 moAb, into 16 recipients of T-cell-depleted haploidentical SCT.[79] Eight patients received a dose of 10^4 T cells per kg, whereas another eight patients received 10^5 T cells per kg. Recipients of the higher dose demonstrated improved T-cell recovery resulting from expansion of the effector memory population without evidence of new

T-cell generation in the thymus. *In vitro* T-cell responses to CMV- and EBV-associated Ags were detected as early as 2–4 months after transplantation in four of six recipients of the higher T-cell dose but not until 6–12 months after transplantation among recipients of the lower T-cell dose. Acute and chronic GVHD occurred in only two patients each. More recently, the same group has found that immunomagnetic depletion of alloactivated lymphocytes expressing CD25 and/or CD71 is more effective at reducing alloreactivity than strategies based on depleting only $CD25^+$ T cells. This double depletion strategy may facilitate infusion of larger doses of T cells to promote immune reconstitution while avoiding GVHD.

A potentially promising strategy for enhancing immune reconstitution and preserving GVL after TCD haploidentical SCT arises from the observation that effector memory ($CD44^+$ $CD62L^-$) $CD4^+$ T cells do not cause GVHD following their transfer into irradiated MHC-mismatched recipients.[81–84] Their inability to cause GVHD stands in contrast to their ability to mediate GVL effects[83,85] as well as protection from infection. These results suggest a strategy of augmenting immune reconstitution and GVL by infusing effector memory T cells into recipients of TCD haploidentical SCT.

Adding T cells that suppress GVHD to T-cell replete grafts

MSCs and $CD4^+$ $CD25^+$ *foxp3*$^+$ regulatory T cells are two types of cells that can inhibit T-cell responses to alloantigens and so could be infused with or after T-cell-replete grafts to inhibit GVHD after haploidentical SCT. MSCs are BM stromal cells that can differentiate into other cells derived from mesoderm, including chondrocytes, tenocytes and myoblasts.[86] MSCs can be immunosuppressive, inhibiting the proliferation of human T cells stimulated by irradiated allogeneic PBMCs.[87–91] Third-party MSCs have been cotransplanted with stem cell grafts to suppress GVHD after HLA-matched sibling SCT[92] and to treat established GVHD after HLA-matched or mismatched donor SCT.[93] In contrast to their effects on T-cell responses to alloantigens, MSCs have little effect on established T-cell responses to EBV and CMV.[94]

Regulatory T cells have an established role in the maintenance of self-tolerance and the prevention of autoimmunity.[95] Addition of large numbers of donor regulatory T cells to stem cell grafts suppresses the development of acute GVHD after MHC-mismatched allogeneic SCT in mice without impairing GVL activity.[96–99] Successful translation of these findings to haploidentical SCT in humans requires protocols to expand regulatory T cells *ex vivo* to sufficient numbers to suppress alloreactivity *in vivo*.

Strategies to decrease relapse after haploidentical SCT

NK cells

HLA-haploidentical transplants have the potential to trigger beneficial donor-vs-recipient NK cell-mediated alloreactions. NK cells express activating and inhibitory receptors, termed killer immunoglobulin-like receptors (KIRs), that stimulate or inhibit NK cell cytotoxicity, respectively. The nomenclature for the KIRs describes the number of extracellular immunoglobulin-like domains (2D or 3D) and the length of the cytoplasmic tail (L for long, S for short). The KIR family (Figure 1) includes inhibitory receptors, or iKIRs, for polymorphic determinants of HLA-A (KIR3DL2), HLA-B (KIR3DL1) and HLA-C (KIR2DL1, KIR2DL2 and KIR2DL3).[100] In HLA-haploidentical SCT, the potential for NK cell alloreactivity in the graft-vs-host direction exists when the recipient's cells lack expression of an HLA allele that is required to deliver an inhibitory signal through a donor iKIR. Figure 2 depicts several models described in the literature to predict the potential for NK cell alloreactivity in the setting of SCT. For instance, the 'ligand–ligand' or 'ligand incompatibility' model (Figure 2a) incorporates information from high resolution HLA typing and predicts donor NK cell alloreactivity when a known HLA ligand for an iKIR is present on donor but not on recipient

cells. In contrast, the 'receptor-ligand' model (Figure 2b) predicts donor NK cell alloreactivity when an HLA ligand is absent on recipient cells and the corresponding iKIR is expressed by the donor, as determined by genotyping of iKIRs or by flow cytometry for surface expression of the iKIR. In studies by the Perugia group, KIR ligand incompatibility (HLA ligand present in the donor but absent in the recipient; Figure 2a) reduced the risk of relapse in 57 AML patients while improving engraftment and protecting against GVHD.[104] Their updated analysis of greater than 90 HLA-haploidentical transplants for high-risk AML showed that transplantation from NK alloreactive donors was associated with control of AML relapse and improved EFS, with a greater than 65% EFS of AML patients transplanted in remission from NK alloreactive donors and a 30% EFS of chemoresistant AML patients. This was compared to an EFS of 18% in AML patients transplanted from non-NK alloreactive HLA-haploidentical donors.[105] The 'receptor-ligand' model (Figure 2b) was better able to predict relapse in pediatric AML and ALL patients than the ligand incompatibility model.[101] Still, utilizing a third method where genotyping of inhibitory KIR was performed (Figure 2d), patients with KIR gene mismatches (that is, KIR gene present in the donor but absent in the recipient, or vice versa) had a higher incidence of GVHD than those without mismatches.[102] Among patients receiving nonmyeloablative haploidentical SCT with high-dose post transplantation CY, inhibitory KIR gene mismatches between donor and recipient were associated with improved overall survival and EFS.[106]

Activating KIRs also deserve evaluation in HLA-haploidentical transplantation. Activating KIRs exhibit allelic polymorphisms in specific genes and extensive variation in gene number and content, which lead to heterogeneity within the general population and within diverse ethnic groups. In some studies, transplantation from donors carrying activating KIR genes was associated with improved control of leukemia relapse after HLA-identical transplantation,[107] and improved survival after unrelated donor transplantation.[108] Other reports have shown that transplantation from donors carrying activating KIR genes adversely affected transplantation outcomes after partially TCD HLA-haploidentical transplants, mainly through an increased risk of GVHD.[109] Conversely, it has been shown that transplantation from donors carrying activating KIR genes (group B haplotype) did not cause GVHD but was surprisingly associated with less infectious mortality and better survival.[105]

Since non-transformed tissues generally do not over-express ligands for activating receptors on NK cells,[110,111] NK cell adoptive immunotherapy has the potential to induce GVL effects without causing GVHD.[104,112] There are several strategies available to enhance the antitumor effects of NK cells in the context of HLA-haploidentical SCT. Since each patient has on average five HLA-haploidentical first-degree relatives who are eligible to donate stem cells (HJS and EJF, unpublished observations), donors could be selected on the basis of optimal NK cell alloreactivity, as determined by models (Figure 2) or by *in vitro* assays. Chemotherapy can enhance the antitumor efficacy of subsequent NK cell infusions through multiple mechanisms, including the induction on tumor cell expression of stress ligands for NK cell-activating receptors,[113] sensitization of tumor cells to NK cell-induced apoptosis[114–116] or enhancement of the survival of adoptively transferred NK cells through lymphopenia-induced cytokines.[117] Finally, therapeutic MoAbs, such as rituximab, may enhance the tumoricidal activity of NK cells by engaging activating Fc receptors, such as $Fc_{\gamma}RIII$ (CD16).[118] More study is clearly needed to define the optimal conditions and strategies for enhancing the antitumor effect of NK cells in the context of HLA-haploidentical SCT.

Donor T-cell infusions

The published literature on DLI after HLA-haploidentical SCT is scanty. In a study from Israel, 28 patients received prophylactic ($n = 6$) or therapeutic DLI ($n = 22$) in doses ranging from 100 to 1.5×10^9 T cells per kg.[119] Of the six patients receiving prophylactic DLI, three patients

remain in remission, one relapsed and two died of GVHD. CR was achieved in only four of the 22 recipients of therapeutic DLI, and only one remains in CR. The group in Beijing administered G-CSF-primed DLI prophylactically to 29 patients[120] and therapeutically to 20 patients.[121] Two-year EFSs were 37.3 vs 40% of recipients of prophylactic vs therapeutic DLI, respectively. Severe GVHD occurred in six patients in each group. Further studies to define dose–response relationships for both GVHD and antitumor efficacy are clearly required before DLI can be routinely recommended for the prevention or treatment of relapse after HLA-haploidentical SCT.

It is worth noting that the presence of T cells in allogeneic stem cell grafts affects NK cell reconstitution and function after unrelated[122] and HLA-haploidentical related donor SCT.[123] Cross talk between T cells, NK cells and DCs occurs at the interface of innate and adaptive immunity,[124] and these complicated interactions are only beginning to be explored in the context of allo-SCT. Both T cell and NK cell adoptive immunotherapies will benefit from an improved understanding of these cellular interactions. The ready availability of the original transplant donor for repeated lymphocyte donations is a distinct advantage of HLA-haploidentical related over unrelated donor SCT.

Unrelated donor UCB vs haploidentical related donor SCT

Patients who lack suitably HLA-matched related or unrelated donors have a choice between two sources of alternative donor stem cells: unrelated UCB or HLA-haploidentical related stem cells. Is there any *a priori* or empirical basis for choosing between these two alternatives?

Unrelated UCB has an established track record in the treatment of hematologic malignancies of children. A retrospective analysis compared the outcomes of unrelated UCB transplantation (UCBT; $n = 503$) vs eight of eight HLA allele (HLA-A, -B, -C and -DRB1)-matched unrelated donor marrow transplantation ($n = 116$) for children under the age of 16 with leukemia.[125] Typing of the UCB grafts was performed at low resolution (antigen level) for HLA-A and -B and at high resolution (allele level) for HLA-DRB1, and results for 1 HLA locus mismatch grafts were analyzed according to cell dose ($> 3.0 \times 10^7$ nucleated cells per kg vs $\leq 3.0 \times 10^7$ nucleated cells per kg). The results in Table 2 show that, at the very least, HLA-matched and high-dose, single locus-mismatched UCB grafts produce overall and leukemia-free survivals that are at least as good as is seen after 8/8 allele-matched unrelated BMT. Leukemia-free survival after 1 or 2 HLA locus-mismatched UCBT was not significantly worse than after HLA-matched unrelated donor SCT. These results establish 4–6/6 HLA Ag-matched UCB as a viable alternative to the use of HLA-matched unrelated donor BM for the transplantation of children with acute leukemia. Further, the results suggest that HLA-matched UCB is the new ‘gold standard’ among alternative graft sources for allo-SCT in childhood leukemia.

There are not enough data at present to make statistically valid comparisons of the outcomes of HLA-haploidentical related vs HLA-matched unrelated donor SCT in the treatment of childhood leukemia. Therefore, HLA-haploidentical related donor SCT for childhood leukemia should only be conducted in the context of carefully designed clinical trials.

For adult patients, cell dose is a major limitation in the use of UCBT. Most single UCB units simply do not contain enough hematopoietic stem cells to guarantee reliable engraftment in older adults. In the first series of US adults receiving UCBT, median UCB graft cell dose was 10-fold lower than among recipients of HLA-matched or mismatched marrow (0.22 vs 2.4 and 2.2×10^8 cells per kg, respectively), sustained neutrophil engraftment occurred in < 70% of UCBT recipients, and NRM occurred in 95 of 150 patients, many due to infection within the first 100 days after transplantation.[126] HLA matching is also a significant limitation of UCBT. In an analysis of 1511 recipients of single cord blood units from the New York Blood Center National Cord Blood Program, the degree of HLA mismatch was found to correlate adversely

with engraftment, GVHD, relapse, treatment-related mortality and overall survival.[127] Although cell dose did not affect the outcome of fully HLA-matched UCB transplants, a twofold increase in the cell dose was required to overcome differences in treatment-related mortality and survival for 2 vs 1 HLA Ag-mismatched grafts. These findings are significant because the likelihood of finding a single cord blood unit that is mismatched for at most 1 HLA Ag and that contains $> 3 \times 10^7$ nucleated cells per kg for an adult recipient is low.

Recently, adult transplantation protocols incorporating the infusion of two UCB units, each containing $\geq 1.5 \times 10^7$ nucleated cells per kg, have been a major step toward overcoming the limitations of inadequate cell dose in individual units. Double unit UBCT after myeloablative conditioning was associated with improved engraftment and lower NRM compared to historical controls receiving a single unit, and 1-year disease-free survival among 23 patients was 57%.[128] In that study, the median total nucleated cell dose was 4.8×10^7/kg, and 13 patients received at least one unit that was matched to the patient at 5–6/6 HLA Ags. Two recent studies have demonstrated the feasibility of double unit UCBT after nonmyeloablative conditioning in adults.[129,130] Among 110 patients studied by Brunstein *et al.*,[130] 93 (85%) required two units to achieve the target nucleated cell dose of 3×10^7/kg. Fifty-three (57%) of these patients received at least one unit matched for 5–6/6 HLA Ags, whereas the remainder received two units matched to the patient and each other at 4/6 HLA Ags. Among the total group of patients, neutrophil engraftment occurred in 92%, treatment-related mortality was 19% at 180 days and 26% at 3 years, and overall and EFSs at 3 years after transplantation were 45 and 38%, respectively. Importantly, receipt of double UCBT was associated with favorable EFS.

The encouraging preliminary results of nonmyeloablative alternative donor SCT may at last provide the means to offer a therapeutic graft-vs-tumor effect to a major portion of the hematologic malignancies universe; that is, elderly patients who lack HLA-matched siblings. To address this issue of donor availability, the US Blood and Marrow Transplant Clinical Trials Network (BMT CTN) is sponsoring parallel multicenter phase II trials of double unit UCB vs HLA-haploidentical marrow transplantation after nonmyeloablative conditioning for leukemia or lymphoma. Patients between the age of 21 and 70 with a diagnosis of acute leukemia or Burkitt's lymphoma in CR, Hodgkin's or large cell lymphoma in chemosensitive relapse, or multiply-relapsed follicular or marginal zone lymphoma are potentially eligible for either trial if autologous or HLA-matched allogeneic SCT is not a feasible option. The treatment schemas for the HLA-haploidentical related (BMT CTN 0603) and double unit UCB (BMT CTN 0604) trials are shown in Figure 3. The primary objective of each trial is to estimate the survival of patients 6 months after transplantation. The 6 month survival of patients receiving VUD SCT after nonmyeloablative conditioning is approximately 60%;[131] thus, comparable survival rates after UCB or haploidentical related donor SCT would justify further testing of either or both graft sources in the nonmyeloablative SCT setting.

In summary, UCB transplantation is an acceptable therapy for children with leukemia who lack an HLA-matched sibling donor. In light of the availability of 4–6/6 HLA-matched unrelated cord blood units, haploidentical SCT in children should only be performed in the context of clinical trials. In adults, unrelated double cord blood or haploidentical related donor SCT is a reasonable therapeutic option for patients who lack an HLA-matched sibling or an 8/8 HLA allele-matched unrelated donor. Nonmyeloablative SCT using UCB or haploidentical marrow can provide long-term disease-free survival for hematologic malignancy patients who are ineligible for intensive conditioning and who lack an HLA-matched donor. The relative merits of these two graft sources will be evaluated in multicenter clinical trials.

Conclusions

HLA-haploidentical related donor SCT has come a long way in the last 20 years. The problems of excessive graft rejection and severe GVHD have been addressed by transplanting megadoses of T-cell-depleted stem cells into intensively conditioned recipients or by selective allodepletion techniques. Nonmyeloablative conditioning safeguards against the possibility of fatal graft rejection and has extended the application of haploidentical SCT to older or more infirm patients and to those who have failed a prior autologous SCT procedure. The main developmental challenges for the future are to enhance immune reconstitution and to prevent relapse after haploidentical SCT. The respective contributions of UCB vs haploidentical related donor SCT for adult patients lacking HLA-matched donors need to be defined. Both of these graft sources offer the advantages of rapid and easy availability for nearly all patients in need of transplantation. Going forward, no patient should be denied access to hematopoietic SCT for lack of an available donor.

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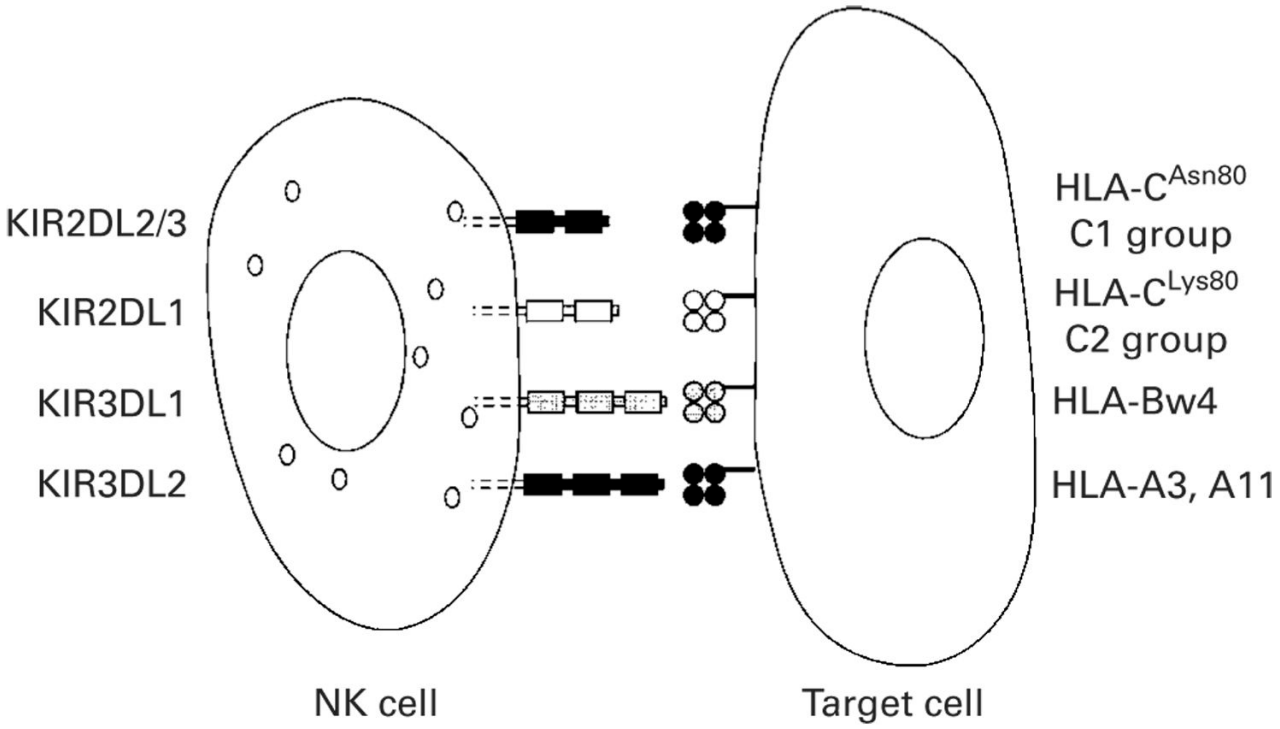

Figure 1.
Interactions between inhibitory killer immunoglobulin-like receptors (iKIRs) and their HLA ligands of relevance to natural killer (NK) cell alloreactivity after allo-SCT. For convenience, a single NK cell expressing four distinct iKIRs is shown. Each NK cell need only express one molecular species of iKIR for functional maturation to occur. High resolution HLA typing is required to determine whether specific alleles of HLA-B and HLA-Cw are ligands of specific iKIRs. Group 2 HLA-C alleles (C2; for example, -Cw2, -Cw4, -Cw5 and -Cw6) are the ligands for KIR2DL1, whereas group 1 HLA-C alleles (C1; for example, -Cw1, -Cw3, -Cw7, -Cw8) are the ligands for KIR2DL2 and KIR2DL3. High resolution typing of HLA-B and -Cw loci are incorporated into the ligand incompatibility, receptor-ligand and missing ligand models of NK cell alloreactivity (Figure 2). Interactions between KIR3DL2 and HLA-A3 or -A11 are generally not considered in these models.

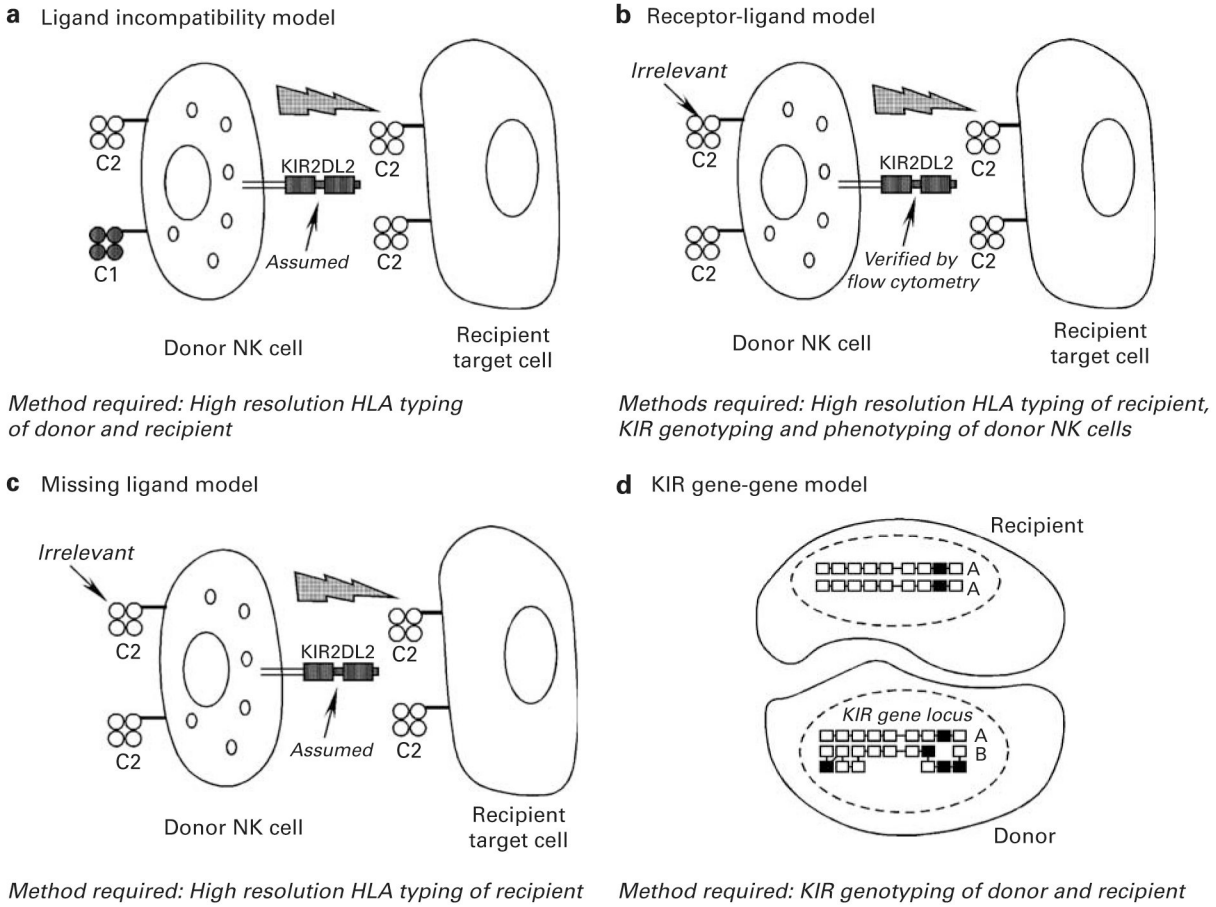

Figure 2.

Models of natural killer (NK) cell alloreactivity after allo-SCT. Models of NK cell alloreactivity incorporate some or all of the following information: (1) high resolution HLA typing of donor and recipient;[103] (2) genotyping of the killer immunoglobulin-like receptor (KIR) locus by PCR of genomic DNA using sequence-specific oligonucleotide probes (SSP) [102] and (3) phenotyping of KIR expression by flow cytometry using commercially available antibodies.[101] (**a**) The ligand incompatibility model predicts NK cell alloreactivity in the graft-vs-host direction (depicted) when the recipient lacks expression of an HLA ligand for inhibitory KIR, in this case a member of the HLA-C1 group, that is present in the donor. The presence of functional donor NK cells expressing KIR2DL2, the receptor for molecules of the HLA-C1 group, is assumed in this model. (**b**) The receptor-ligand model predicts NK cell alloreactivity in the graft-vs-host direction when the recipient lacks an HLA ligand for donor inhibitory KIR, whose presence is verified by KIR genotyping and flow cytometry of donor NK cells. The HLA type of donor cells is irrelevant to this model. (**c**) The missing ligand model predicts NK cell alloreactivity in the graft-vs-host direction when recipient cells lacks expression of at least one of the HLA ligands (C1, C2 or -Bw4) for inhibitory KIR. (**d**) The KIR gene–gene model predicts NK alloreactivity when the donor and recipient are mismatched for KIR gene content. Inhibitory KIR genes are shown as unshaded boxes, whereas black boxes represent activating KIR genes. In the example shown, the recipients KIR genotype is said to be ‘included’ in the donor’s KIR genotype.[103]

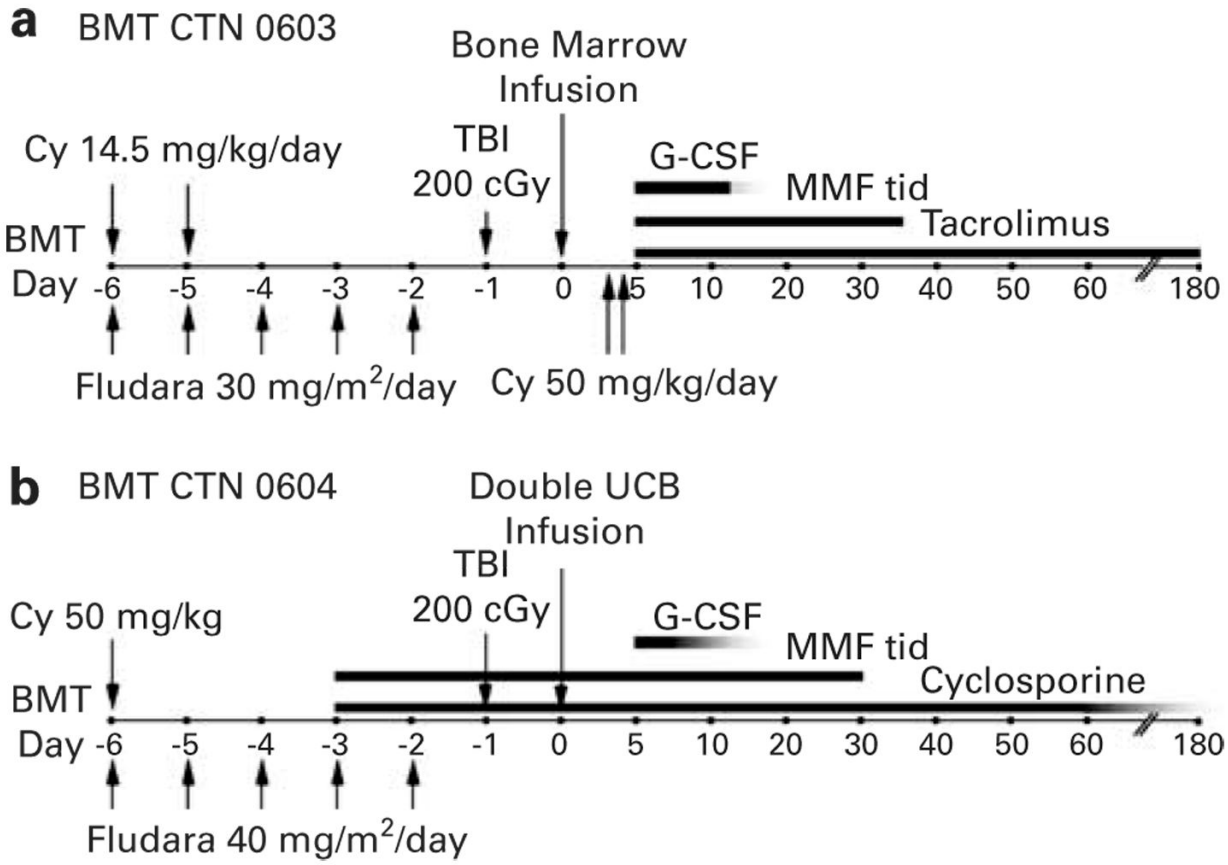

Figure 3.

Treatment schemata for Blood and Marrow Transplant Clinical Trials Network (BMT CTN) multicenter clinical trials of nonmyeloablative conditioning and transplantation of (**a**) partially HLA-mismatched (haploidentical) BM (BMT CTN 0603) or (**b**) double unit unrelated umbilical cord blood (UBC) (BMT CTN 0604) for adults with leukemia or lymphoma.

Table 1

Selected published studies of HLA-haploidentical SCT

Authors	N	Median age (years)	T depletion	Graft failure (%)	GVHD (%)			NRM (%)*	Relapse (%)*	Overall survival (%)*	EFS (%)*
					Acute II–IV	Acute III and IV	Chronic				
Myeloablative conditioning											
Szydlo *et al.*[1]	340	25	*Ex vivo* (49%)								
1 Ag MM				9	44	27	52	50–57	28–65	—	15–36
2 Ag MM				16	56	36 *a*	60	55–67	37–45	—	20–25
O'Reilly *et al.*[7]	52		*Ex vivo*	30 *c*	9	3		>50 51 *b*		20	
Mehta *et al.*[8]	201	23	In (71%)+ *ex vivo*	2	13	—	15		31	18	19
Aversa *et al.*[9]	104	33	*Ex vivo*	9	8	—	7	37	25	39	—
Lu *et al.*[10]	135	24	*In vivo*	1	40	16	55	22	18	71 64 *d*	
Nonmyeloablative conditioning											
Rizzieri *et al.*[11]	49	48	*In vivo*	14	16	—	14	31	—	31	—
Luznik *et al.*[12]	68	48 *e*	*In vivo*	13	34	6	22	15 *f*	51	36	26

Boxes illustrate salient results:

a effect of HLA mismatch on severe GVHD after myeloablative, T-cell-replete BMT

b excessive NRM in early trials of haploidentical SCT

c increased risk of graft failure with *ex vivo* graft TCD without intensive immunosuppressive conditioning

d improved outcome of myeloablative SCT using *in vivo* TCD

e,*f* nonmyeloablative conditioning permits transplantation of older patients with reduced treatment-related mortality. See text for details of the studies.

* Data on NRM (non-relapse mortality) are for 1–2 years after transplantation. Data on relapse, overall survival and EFS are for 1–2 years (all studies except Lu *et al.*[10]) or for 5 years after transplantation (Lu *et al.*). 1 Ag MM = 1 HLA Ag mismatch.

Table 2

Clinical outcomes of unrelated adult marrow vs cord blood transplantation for leukemia in children

Graft	TRM (%)	Relapse (%)	LFS (%)	OS (%)
BM, allele matched at HLA-A, -B, -C, -DRB1	19	41	38	45
CB, A, B, antigen-matched, DRB1 allele matched	6	34	60	63
CB, 1-locus mismatched, high cell dose	29	31	41	45
CB, 1-locus mismatched, low cell dose	43	21	37	36
CB, 2-loci mismatched any cell dose	49	20	33	33

Abbreviations: CB = cord blood; LFS = leukemia-free survival; OS = overall survival; TRM = treatment-related mortality. Low cell dose, $\leq 3.0 \times 10^7$ nucleated cells per kg; high cell dose, $> 3.0 \times 10^7$ nucleated cells per kg. From Eapen *et al.*[125]