



Molly Pomerance. *Gourd Party*. Oil on canvas, 24" × 30".

Preliminary studies indicate that the use of alternative donors can provide reliable engraftment, although graft-vs-host disease remains a concern.

Nonmyeloablative Allogeneic Stem Cell Transplantation Using Alternative Donors

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Background: *The reduced intensity of nonmyeloablative stem cell transplant (NMSCT) has enabled older patients to benefit from allogeneic therapy. Identification of suitable donors remains an obstacle. The use of alternative donors for stem cell therapy is essential to ensure broad applicability of allogeneic therapy.*

Methods: *Clinical results using alternative hematopoietic stem cell donors are reviewed, including matched unrelated donors, partially matched family member donors, and unrelated partially matched umbilical cord blood.*

Results: *The successful use of NMSCT in the treatment of hematologic and nonhematologic diseases has increased the number of patients capable of receiving allogeneic therapy. However, the stem cell donor pool remains limited due to the infrequent number of patients with matched siblings.*

Conclusions: *The use of alternative donor stem cell sources can expand the number of patients able to receive allogeneic therapy.*

Introduction

Retrospective analyses of allogeneic myeloablative therapy consistently note increased age and multiple comorbidities as major risk factors for toxicities due to conditioning regimens, as well as for graft-vs-host disease (GVHD).^{1,6} The reduced intensity of nonmyeloablative stem cell transplant (NMSCT) has enabled older patients to experience the benefits of allogeneic immunotherapy or graft-vs-malignancy effect. Several studies have shown the benefit of allogeneic immunotherapy.^{1,5}

Mixed chimeras were first shown in laboratory dogs receiving marrow grafts from leukocyte antigen-identical littermates. Various chemotherapy regimens were used.^{7,8} Cynomolgus monkeys were later treated with anti-T-lym-

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Table 1. — Doses of Total Body Irradiation Needed for Engraftment of Leukocyte Antigen-Identical Marrow in the Absence of Postgrafting Immunosuppression in Laboratory Dogs

Total Body Irradiation Dose (cGy)	No. of Dogs Studied	Sustained Engraftment (%)	Autologous Recovery (%)
920—immunosuppressive	21	95	0
800	5	80	0
700	5	60	0
600	23	52	17
450—myeloablative and supralethal	39	41	36

* Delivered at 7 cGy/min.
From Thomas ED, Blume KG, Forman SJ. *Hematopoietic Cell Transplantation*. 2nd ed. Malden, Mass: Blackwell Science; 1999. Reprinted with permission.

phocyte globulin (ATG) and total body irradiation (TBI) followed by posttransplant immunosuppression. Marrow donors were major histocompatibility complex (MHC)-mismatched, and donor-type chimerism was noted in up to 57.8%.⁹⁻¹¹ Later studies noted that decreases in TBI dose still allowed sustained engraftment (Table 1).¹² Yu et al¹³ showed that varying the posttransplant immunomodulatory regimen affected the rate of allogeneic engraftment (Table 2). Additionally, working with the mice model, Stewart et al¹⁴ showed that cell dose, while not a factor with ablative therapy, correlates with improved engraftment when low doses of irradiation are given (Figure).

Initial trials of NMSCT in patients receiving human leukocyte antigen (HLA)-matched stem cells have been encouraging (Table 3).¹⁵⁻¹⁹ Although preparative regimens varied among trials, most used matched sibling donors having greater than 80% engraftment and less than 20% transplant-related mortality in older, refractory, and high-risk patients.¹⁵⁻¹⁸ The risk of GVHD remains high (30% to 50%), although the use of T-cell depletion has shown improvement in the incidence of GVHD.¹⁸ Alemtuzumab concentrations in patients receiving NMSCT appear to be in excess of the required dose to kill infused CD52⁺ cells when used in reduced-intensity regimens (usually a total dose of 100 mg).¹⁸ The levels of alemtuzumab in the reduced-intensity regimens were lympholytic for approximately 56 days posttransplant, or 26

days longer than the myeloablative conditioning regimens. The role of alemtuzumab in NMSCT at the current dosing regimen needs to be further delineated.²⁰ In our experience, T-cell-depleted nonmyeloablative transplant using HLA-matched sibling hematopoietic stem cell donors results in less than 10% risk of GVHD with greater than 95% engraftment.^{18,21-26}

The chance that a patient requiring allogeneic stem cell transplantation will have an HLA-compatible sibling is less than 30%.²⁷⁻²⁹ Identification of alternative donors for stem cell transplantation remains essential to ensure broad applicability of the technological advance of NMSCT. In this review, we discuss clinical results using alternative hematopoietic stem cell donors: matched unrelated

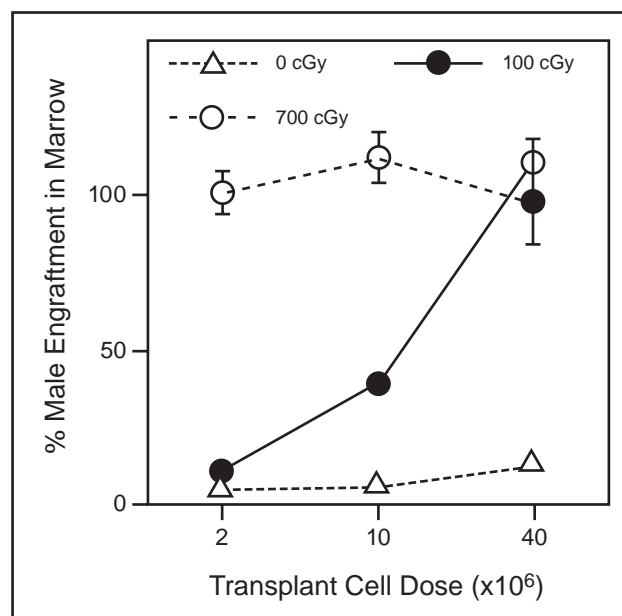
Table 2. — Engraftment Results Using 450 cGy Total Body Irradiation at 7 cGy per Minute and Marrow Grafts From Leukocyte Antigen-Identical Canine Littermates

Postgrafting Immunosuppression	No. of Dogs With Sustained Engraftment/ No. of Dogs Studied
No	6 / 17
Cyclosporine*	7 / 7
Prednisone**	0 / 5

} P=.01

Cyclosporine = 15 mg/kg p.o. b.i.d. on days -1 to 35.
Prednisone = 12.5 mg/kg p.o. b.i.d. on days -5 to -3 with subsequent taper through day 32.

From Thomas ED, Blume KG, Forman SJ. *Hematopoietic Cell Transplantation*. 2nd ed. Malden, Mass: Blackwell Science; 1999. Reprinted with permission.



Cell dose and engraftment in minimally irradiated recipients. Male marrow cells in doses of 2, 10, 40 million cells were infused into female recipients treated with 0, 100, and 700 cGy, and the percentage of engraftment was determined after 2 months. The results show that donor cell readout in hosts after transplant is related to cell dose and irradiation dose to recipient animals. Cell doses as low as 10 million may yield up to 40% in the readout in animals receiving 100 cGy. Experimental points include 5 mice per cell level; where standard errors are not apparent, they fall within the symbol. From Stewart FM, Zhong S, Wu J, et al. Lymphohematopoietic engraftment in minimally myeloablated hosts. *Blood*. 1998;91:3681-3687. Copyright American Society of Hematology, used with permission.

Table 3. — Initial Trials of Histocompatible Antigen-Matched Sibling Nonmyeloablative Stem Cell Transplantation

	Slavin et al ¹⁵	Giralt et al ¹⁶	Khoury et al ¹⁷	Kottaridis et al ¹⁸	Mohty et al ¹⁹
No. of Patients	26 ^a	15 ^b	15	36 ^a	101
Preparative Regimen	Fludarabine, ATG, and busulfan	<u>Arm 1:</u> fludarabine, idarubicin, and cytarabine or melphalan <u>Arm 2:</u> 2-chloro-deoxyadenosine and cytarabine	<u>Arm 1:</u> fludarabine and cyclophosphamide <u>Arm 2:</u> fludarabine, cisplatin, and cytarabine	Alemtuzumab, fludarabine, and melphalan	Fludarabine, busulfan, and ATG
Engraftment	100%	40% (68% complete) full-donor chimerism)	73%	100%	100% ^f (18 of 31)
GVHD Grade 2–4	9	2	5 ^c	2	36
GVHD Grade 3–4	6	0	1	0	17
Transplant-Related Mortality	0	1	1	4 ^d	18
ATG = anti-T-lymphocyte globulin ^a One patient was grafted with A and C locus mismatches with positive mixed lymphocyte reaction in the direction of host-vs-donor. ^b Two patients had one-antigen-mismatched donors. ^c Two patients developed acute GVHD grade 2 following donor leukocyte infusion. ^d At a median follow-up of 9 months (range 3 to 29 months), 33 of 44 total patients were alive in complete remission or without evidence of disease progression. ^e Total number of patients = 44 (8 matched unrelated donors). ^f Engraftment was defined as sustained absolute neutrophil count of $>0.5 \times 10^9/L$; 6 patients failed to achieve a sustained platelet count of $>20 \times 10^9/L$.					

donors, partially matched family member donors, and unrelated partially matched umbilical cord blood.

Matched Unrelated Donors

Major obstacles to successful transplantation with matched unrelated donors (MUDs) include GVHD (54% to 77% of patients) and graft rejection (5% to 8% of patients). In addition, severe infections can limit the success of this therapy.^{28,30-33} It has been demonstrated that GVHD can be abrogated by T-cell depletion of donor cells (Table 4).^{18,22-26}

The formation of the National Marrow Donor Program (NMDP) and the need for allogeneic therapy in older patients promoted the use of this donor population in NMSCT. Initial clinical trials of MUDs^{15,30} were encouraging, although they included small numbers of mismatched unrelated donors (Table 5).^{18,22-25}

Maris et al²⁵ recently reported on 89 patients with MUD transplants, 16 of whom had HLA class I allele-level mismatches. Sustained donor engraftment occurred in 70 (79%) of 89 patients. This initial trial compared peripheral stem cells to bone marrow, and it noted trends toward improved durable engraftment with peripheral stem cells, higher donor T-cell chimerism, and longer progression-free and overall survival compared with marrow. Acute GVHD grade 2, 3, and 4 occurred in 42%, 8%, and 2%, respectively. T-cell chimerism of more than 50% was associated with improved overall survival and progression-free survival

and also with reduced nonrelapse mortality. The 1-year overall and progression-free survival rates were 52% and 38%, respectively.

A recently published trial by Niederwieser et al²⁴ used a nonmyeloablative conditioning regimen of fludarabine (30 mg/m² per day from days -4 to -2) and 200 cGy TBI on day 0. Patients received GVHD prophylaxis with cyclosporine and mycophenolate. Durable donor chimerism was achieved in 88% of patients. Acute GVHD grade 2–4 occurred in 63% of patients and was the primary cause of death in 9% of patients. The transplant-related mortality rate (100 days posttransplant) was 11%. At a median follow-up of 19 months, 35% of patients were alive, with 25% of patients in remission.

Nagler et al²³ used a reduced-intensity conditioning regimen of fludarabine (30 mg/m² daily for 6 days), busulfan (4 mg/kg daily for 2 days), and ATG (10 mg/kg daily for 4 days). This regimen depressed T-cell function and the T-cell compartment due to the presence of ATG. However, stem cells were not T-cell depleted. No graft rejection was observed, and 100% of patients achieved full donor chimerism. No patients developed toxicities higher than grade 2. At 36 months, overall survival and disease-free survival rates were 75% and 60%, respectively. These results suggest that stable engraftment with low-intensity conditioning, as well as active graft-vs-leukemia effects, can be achieved. However, 3 patients developed acute GVHD grade 3–4.

Bornhauser et al³⁴ investigated a similar regimen involving fludarabine (30 mg/m² per day for 5 days), busul-

fan (3.3 mg/kg per day for 2 days), and ATG (2.5 mg/kg per day for 4 days). This trial again showed a favorable response in patients with stable engraftment, with an actuarial disease-free survival rate of 64% at a median of 13 months. However, primary or secondary graft failure occurred in 21% of patients. The increased incidence of graft failure was possibly secondary to the use of the lower dosage of ATG.

Giralt et al²² reported a trial of 86 patients who had a variety of hematologic malignancies and were considered poor myeloablative candidates due to age or comorbidities. The majority of patients received a preparative regimen of fludarabine (25 mg/m² daily for 5 days) combined with melphalan at either 180 mg/m² (n = 66) or 140 mg/m² (n = 12). These patients received no T-cell deple-

tion, and 40 patients received MUD transplants. The median proportion of donor cells at 1 month in 75 patients was 100% (range 0% to 100%). Five patients had less than 50% donor cells (2 with graft rejection, 1 with mixed chimerism, and 2 persistent disease). At 3 months, 40 of 42 patients in remission had more than 90% donor cells. At 1 year, 17 patients remained in remission, and all had 100% donor engraftment. However, the incidence of acute GVHD grade 2-4 was 62%, and the incidence of grade 3-4 was 39% among patients receiving MUD transplants. Death due to acute GVHD occurred in 11 of 40 patients compared with 4 deaths in the matched related donor setting. The incidence of graft rejection in both trials was less than 10%. However, the risk of GVHD was unacceptably high in this patient population.^{13-15,17}

Table 4. — Trials of Matched Unrelated Donor Nonmyeloablative Transplantation

	Giralt et al ²²	Nagler et al ²³	Kottaridis et al ¹⁸	Niederwieser et al ²⁴	Maris et al ²⁵	Wong et al ²⁶
No. of Patients	86 ^a	16	44 ^c	52 ^d	89	29
Preparative Regimen	Arm 1: cladribine and melphalan (n = 8) and ATG Arm 2: fludarabine and melphalan (n = 78)	Fludarabine, busulfan, fludarabine, and melphalan	Alemtuzumab,	Fludarabine and TBI	Fludarabine and TBI	Fludarabine, melphalan, busulfan, and ATG
Engraftment	100% (in 75 pts at 1 month)	100% (15 pts had full donor chimerism)	100% (18 pts were full donor chimeras)	88%	87% ^e	86%
Grade 2-4 GVHD	34	7	2	33	46 ^f	12
Grade 3-4 GVHD	19	3	0	11	10 ^f	6
Transplant-Related Mortality	10 ^b	0	4	6	6 ^g	5
ATG = anti-T-lymphocyte globulin TBI = total body irradiation ^a Forty patients received 6 of 6 HLA matched unrelated donor stem cells. ^b Prior to day 100 of posttransplant, the cladribine combination had 7 toxic deaths, and the fludarabine combination had 3 toxic deaths. ^c Includes 36 patients with matched sibling donors. ^d Fifteen patients had mismatched unrelated donors and 37 patients had matched unrelated donors. ^e This represents the donor engraftment rate on day 28: 70 (79%) of 89 patients with sustained donor engraftment, 83 (93%) engrafted initially. ^f Only 46 patients were evaluable. ^g Six patients failed to recover cell counts after nonmyeloablative transplantation.						

Table 5. — Treatment Response in Matched Unrelated Donor Nonmyeloablative Transplantation

	Giralt et al ²²	Nagler et al ^{23 d}	Kottaridis et al ^{18 e}	Niederwieser et al ²⁴	Maris et al ²⁵
Survival	28% ^a	75%	75%	35%	52%
Alive With Disease Progression or Relapse	5% ^b	15%	16%	10%	14%
Mortality Rate	45% ^c	25%	18%	27% ^f	48% ^g
^a Overall probability of survival at 2 years. ^b Probability of disease progression or relapse. ^c Nonrelapse mortality at 2 years posttransplant. ^d Data at 36 months posttransplant. ^e Median follow-up of 9 months. ^f Mortality from disease progression: 11% transplant-related mortality, 65% overall mortality rate at the time of publication. ^g 32% due to underlying disease, and 16% nonrelapse causes at 1 year posttransplant.					

A recent trial in patients older than 55 years of age noted comparable results to those reported in younger patients with similarly advanced disease.²⁶ The results of 29 patients with a median age of 59 years revealed a probability of overall survival of 44%. Patients initially enrolled in the trial received fludarabine and 180 mg/m² of melphalan. However, the melphalan dose was reduced to 140 mg/m² due to toxicity. Eighteen patients received equine ATG (60 mg/kg in divided doses), 1 received cytarabine, and 5 received busulfan. Two patients had 1 HLA-DRB1 antigen mismatch with their donor, and 4 others had HLA-C and/or HLA-DQB1 mismatches. The initial full chimerism engraftment rate was 86%, and 3 patients had mixed chimerism. No patients surviving longer than 28 days had graft failure. Thirteen deaths occurred in the initial 100 days following transplant — 6 related to GVHD, 2 related to disease progression or relapse, and 5 related to regimen toxicity. The Kaplan-Meier survival curves estimated an impressive overall probability of survival at 1 year of 44%, similar to previous trials of reduced-intensity regimens. The use of multiple conditioning regimens is currently being investigated for both malignant and non-malignant illnesses.³⁵⁻³⁷

Numerous methods of T-cell depletion have been developed with similar outcomes in reducing GVHD in matched siblings. However, a rise in graft rejection and relapse rates has been noted in many reports.³⁸⁻⁴⁷ Symeonidis et al⁴⁸ reported a trial of 27 patients who received matched related *non-sibling* stem cells after reduced conditioning with varying regimens that included busulfan, cyclophosphamide, fludarabine, or TBI-based regimens. Seventeen patients received non-T-cell-depleted grafts and 10 received alemtuzumab T-cell-depleted bone mar-

row grafts. Overall, engraftments were successful in 26 (96%) patients; only 1 patient had late rejection. Acute GVHD was observed in 10 (37%) of the 27 patients (grade 2 in 3 patients, grade 3-4 in 7 patients), and 6 patients died. Six patients developed chronic GVHD. Regardless of the availability of matched related donors, the likelihood of extended family members who are phenotypically HLA-matched is small.

Kottaridis et al¹⁸ utilized alemtuzumab (CamPath-1H or anti-CD52 antibody) in MUD transplants. Patients received a reduced-intensity regimen consisting of alemtuzumab (20 mg per day on days -8 to -4), fludarabine (30 mg/m² daily on days -7 to -3), and melphalan (140 mg/m² on day -2). All patients who had chimerism studies performed were either mixed chimeras or full-donor chimeras (100% engraftment) at least 1 month posttransplantation. There were no cases of acute GVHD grade 3-4. Chakraverty et al⁴⁹ applied the same conditioning regimen in 27 patients who underwent transplantation using MUD stem cells. The primary graft failure rate was 4.5%, and chimerism studies in 34 patients noted that 85% attained initial full donor chimerism. Only 3 patients developed acute GVHD grade 3 or 4, and none developed chronic GVHD. Overall and progression-free survival rates at 1 year were 75.5%, and 61.5%, respectively.

Although it is too early to determine overall and long-term response rates to these treatment regimens, the initial results appear favorable. The results of initial trials have noted tolerance and successful engraftment, with some regimens also reporting acceptable rates of GVHD in the MUD setting (Table 6).^{21,48-50} Little is reported concerning functional immune recovery, antitumor effects, and rates of long-term disease control.

Table 6. — Trials of Haploidentical Sibling Nonmyeloablative Transplantation

	Symeonidis et al ⁴⁸	Koh et al ²¹	Sykes ⁵⁰	Chakraverty et al ⁴⁹
No. of Patients	27	29	5	20 ^h
Preparative Regimen	Various ^a	Alemtuzumab, fludarabine, and cyclophosphamide	Cyclophosphamide, thymic irradiation, and ATG	Fludarabine, melphalan, and alemtuzumab
Engraftment	96%	100% ^b	80% ^e	85% ⁱ
Grade 2-4 GVHD	10	3 ^c	5 ^f	3
Transplant-Related Mortality	22%	0% ^d	20% ^g	21% ⁱ
ATG = anti-T-lymphocyte globulin ^a Conditioning regimens included busulfan and cyclophosphamide (n = 16), fludarabine (n = 8), total body irradiation (n = 3), and alemtuzumab (n = 10). ^b Engraftment analysis at 4 weeks posttransplant demonstrated a median myeloid donor chimerism of 91.5% (range 1%-99%) and lymphoid donor chimerism of 94% (1%-99%). ^c Included only grade 3-4. ^d No patients died within the first 30 days of transplant. ^e Predominance of donor lymphoid tissue and varying degrees of myeloid chimerism. ^f Grade 4 GVHD in no patients, grade 3 GVHD in 3 patients, and grade 2 GVHD in 2 patients. ^g One patient died of pulmonary hemorrhage on day 12. ^h 47 total patients: 20 patients with 1-2 HLA antigen-mismatched unrelated donor transplants and 27 with MUDs. ⁱ Initial donor chimerism in 29 of 34 patients evaluated at 1 month. ^j 10 of 47 total patients died of peritransplant-related causes: infection (n = 5), idiopathic pneumonitis (n = 2), multiorgan failure (n = 2), and perforated duodenal ulcer (n = 1).				

Haploidentical Sibling Donors

The ability to use hematopoietic stem cells from partially matched family members has many advantages. First, the ability to use cells from a haplomatched first-degree relative means that the majority of patients will have a suitable stem cell source that is readily available. Second, the rapid progression from initial consultation to time of transplantation is beneficial for most patients considering this procedure — a point that is often facilitated through working with family members. Third, the ability to utilize stem cells from a family member provides a readily available source if additional donor cells are necessary for posttransplant therapy.

Initial trials of full haplotype mismatched transplants are small, yet the pilot trials suggest that partially matched family members appear to be acceptable donor sources for high-risk patients.^{41,51}

The most common problems related to haploidentical myeloablative stem cell transplant are intractable GVHD^{52,53} and graft rejection in transplants involving T-cell depletion of donor cells.⁵⁴⁻⁵⁶ Studies have shown a direct relationship between the degree of HLA incompatibility and the risk of GVHD.^{57,58} The greatest risk occurs in patients with 3 HLA-incompatible loci, with a risk as high as 80% noted in some reports. This risk has been reduced in the ablative transplant setting with the use of stringent T-cell depletion.^{51,59-62} Additionally, recent success with haploidentical transplantation is likely due in part to improved peritransplant medical care and new anti-infective agents. One clinical trial has indicated that early and late posttransplant survival for patients with high-risk hematologic malignancies receiving haploidentical donors is comparable to that observed after MUD transplantation.⁶³

The advent of T-cell depletion heightened the concern for graft failure in haploidentical stem cell transplantation, although the use of “megadoses” of stem cells has helped to alleviate some of this difficulty.^{51,59-62} The fundamental role of CD34⁺ cell-dose escalation in promoting engraftment across HLA barriers in the ablative setting was investigated in a pilot study of ablative therapy.⁶¹ Forty-three patients with high-risk acute leukemia received a full-haplotype mismatch allogeneic transplant. All patients achieved full donor type engraftment. Twelve of the 43 patients were alive and disease-free at 18 months. Forty percent of patients died of transplant-related mortality. None of the patients who were evaluable developed GVHD. The reduction in incidence of GVHD was additionally appealing, and it appears that the CD34⁺ cells are endowed with potent “veto activity” (the inhibition or reduction of host antidonor responses). The mechanisms of this “veto response” have not been clearly defined, although some cell populations appear to be the primary mediators of this tolerance mechanism.⁶⁴ However, similar methods of nonselective T-cell depletion in

the nonablative setting have resulted in both immediate and late graft failure.⁶⁵⁻⁷⁷ Conditioning regimens have appeared to influence the risk of graft rejection, with lower amounts of TBI resulting in higher failure rates.^{42,69,78} Sykes et al⁵⁰ published an initial trial involving 5 patients with refractory NHL who underwent NMSCT with haploidentical donors. The conditioning regimen consisted of cyclophosphamide, thymic irradiation, and ATG. Four of the 5 patients showed engraftment, with a predominance of donor lymphoid tissue and varying degrees of myeloid chimerism. Chakraverty et al⁴⁹ included 20 patients who were mismatched for HLA class I and/or class II alleles. Conditioning involved T-cell depletion (described above), yet 29 of the 34 patients achieved primary donor chimerism, and only 3 developed GVHD grade 3-4. At our center, 29 patients underwent haploidentical NMSCT from partially matched family members.²¹ We utilized a non-radiation-based fludarabine and cyclophosphamide regimen in combination with alemtuzumab in our preparative regimen and in the donated stem cells to provide T-cell depletion. Additional posttransplant prophylaxis included the use of mycophenolate for 45 days. Over 95% of the patients engrafted, most with >80% donor engraftment at 4 weeks posttransplant. Only 3 of the 29 patients had GVHD grade 3-4. Although the majority of patients attained a remission with this approach, infections such as viral reactivations remained a prime concern.

These encouraging data provide a foundation for future nonablative therapy to improve antitumor effects using partially matched family members.

Umbilical Cord Blood

Partially matched umbilical cord blood (UCB) offers a potential advantage over unrelated donors, with decreased risk of infection, reduced severity and incidence of GVHD, and ready availability of donor cells for transplantation.^{63,79-84} Also, UCB expands the use of NMSCT across racial boundaries, giving most patients the ability to locate a potential 4 of 6 HLA-matched or better donor. The use of UCB as a stem cell source is an attractive option, but graft rejection remains a concern.

Cord blood has been established as a source of hematopoietic stem cells for allogeneic transplantation in adults and children, with more than 500 myeloablative transplants performed over the last 15 years.^{63,79-86} Ablative therapy in adult patients has approximately a 90% chance of engraftment with a 15% rate of transplant-related mortality, mostly due to infection. However, the use of nonmyeloablative conditioning regimens and cord blood transplantation is not well established. The use of UCB usually involves a one- to two-log reduction in CD34⁺ cells standardly infused with nonmyeloablative matched sibling transplantation. The use of “megadose” stem cell

infusion is not feasible in this setting without ex vivo expansion of stem cells or the use of combinations of units. Both approaches are in the early stages of investigation. However, the unique cellular composition of cord blood progenitor stem cells may facilitate engraftment, as suggested by the lower-than-expected graft failure rates in ablative therapy.

We published the initial report of NMSCT using UCB donor cells in 2 adult patients receiving a preparative regimen of fludarabine, cyclophosphamide, and ATG.⁸⁷ Cyclosporine and prednisone were used as acute GVHD prophylaxis. Donor engraftment of >99% has persisted at 6 months posttransplantation. We subsequently reported on additional patients with similar results, though we have also noted instances of graft rejection.⁸⁸ Cord blood NMSCT has also been successfully performed using a conditioning regimen of fludarabine and TBI. Despite minimal conditioning, 2 of 3 evaluable patients showed stable engraftment with this approach.⁸⁹

Hwang et al⁹⁰ used alemtuzumab (15 mg per day for 4 days), fludarabine (30 mg/m² per day for 3 days), and 200 cGy of TBI on the day of cord blood infusion. Post-transplant immunosuppression involved cyclosporine, mycophenolate mofetil, and methotrexate. Four patients received transplantations with mismatched unrelated cord blood, 2 patients engrafted, and 2 had relapsed disease soon after transplantation. In all cases, the transplants were well tolerated without significant organ toxicity. Two patients developed mild GVHD grade 2, which was

controlled with corticosteroids. Interestingly, 1 patient received a combined cord blood unit (2 units of cord blood from separate donors) due to insufficient cell counts of either independent unit. Cairo et al⁹¹ reported on 7 patients who received UCB after reduced-intensity transplantation with varying conditioning regimens. Maximal UCB donor chimerism following transplantation ranged from 55% (1 patient) to 100% (4 patients). The incidence of acute GVHD and chronic GVHD was 20% and 10%, respectively. Shen et al⁹² published an initial trial of 4 patients with various solid tumors (liposarcoma, NHL, yolk sac sarcoma, neuroblastoma) using a myelosuppressive regimen of vincristine, doxorubicin, and cyclophosphamide. Two of the 4 patients showed partial donor cell engraftment, and none had signs of GVHD.

A recently published trial of UCB in 43 patients utilized 1-2 HLA antigen-mismatched UCB and contained a median of 3.7×10^7 nucleated cells per kilogram of recipient body weight.⁹³ The conditioning regimen in the first 21 patients used busulfan (8 mg/kg), fludarabine (200 mg/m²), and TBI (200 cGy) with a sustained engraftment rate of 76%. The 22 remaining patients received cyclophosphamide (50 mg/kg), fludarabine (200 mg/m²), and TBI (200 cGy) with a sustained engraftment rate of 94%. Most important was the 9% incidence of acute GVHD grade 3-4. However, the survival rate at 1 year was 39%.

These initial trials showed that there is a potential role for UCB in nonmyeloablative regimens. However, engraftment rates, response rates, and the risk of GVHD cannot

Table 7. — Trials of UCB Nonmyeloablative Stem Cell Transplantation

	Rizzieri et al ⁸⁸	McSweeney et al ⁸⁹	Hwang et al ⁹⁰	Cairo et al ⁹¹ c	Barker et al ⁹³
No. of Patients	4	4	4	7	43
Conditioning Regimen	Fludarabine, cyclophosphamide, and ATG	Fludarabine and TBI	Alemtuzumab, fludarabine, and TBI	Fludarabine, busulfan, and ATG	Arm 1: busulfan, fludarabine, and TBI (200 cGy) (21 patients) Arm 2: cyclophosphamide, fludarabine, and TBI
Engraftment	75% (>99% donor at 6 months)	75% ^a	75% ^b	100% (>95% in 6 patients) ^d	Arm 1: 76% Arm 2: 94%
GVHD	2 (grade 2 gut)	1 (grade 1)	2 (mild grade 2)	2 (grade 2-4)	4 (grade 3-4)
Cell Dose (nucleated cells $\times 10^7$ /kg)	2.56-6.5	.75-1.2	Unknown	1.6-9.5	1.6-6.0
Survival	50% (1 year)	75% (5 months maximum follow-up)	Unknown	65% ^e	39% (1 year)
ATG = anti-T-lymphocyte globulin TBI = total body irradiation ^a 50% engraftment at 5 months, all mixed chimeras (maximum donor engraftment 99% granulocytes in one patient). ^b Too early at time of report to comment on chimerism status. ^c All patients with at least mixed chimeras at 60 days (one patient with 55% donor cells who died of progressive disease at day 79 post transplant). ^d Probability of 1-year survival. ^e Final analysis of results included 10 patients, 2 with allogeneic peripheral blood stem cells and 1 with allogeneic bone marrow stem cells.					

clearly be defined from these limited reports.⁹⁴ It appears from numerous preliminary studies that NMSCT with UCB HLA-matched and -disparate donors is feasible and tolerable, with a majority of data showing a less than 10% incidence of graft failure and greater than 90% of patients achieving at least 50% mixed donor chimerism (Table 7).

Immune Reconstitution Using Alternative Donors

Immune reconstitution following NMSCT using alternative donors remains poorly defined. T-cell reconstitution following ablative allogeneic transplantation involves both thymic education of donor-derived precursors and peripheral expansion of mature T cells from the graft. T-cell recovery following UCB transplant appears to be primarily from peripheral expansion of adoptively transferred T cells early in recovery. Compared with children, adults have a further delay in T-cell reconstitution, which may be related to thymic function.⁹⁵ T-cell reconstitution following UCB transplantation tends to occur up to 18 months after ablative transplantation in adults,⁹⁵ and recovery of thymopoietic function appears to be dependent on an increase in thymic volume.^{96,97} Initial results of alemtuzumab-treated NMSCT using mismatched related donors have shown varying degrees of immune recovery among T-cell subsets. We have shown that in patients who do not have severe GVHD, functional immune recovery begins approximately 3 months posttransplant and is robust by 6 months following transplant, as measured by the FastImmune Cytokine System (Becton Dickinson and Co, San Jose, Calif) and immunoscope analyses.²¹ The unique nature of cord blood stem cells and the potential for killer immunoglobulin-like receptor mismatching with mismatched donors make functional immunity and antitumor evaluation an intriguing aspect to explore with nonmyeloablative alternative donor transplantation.

Future Directions

As demonstrated in the preliminary studies published to date, the use of alternative donors can provide reliable engraftment, although GVHD remains a concern. T-cell depletion may assist in amelioration of this problem but with unacceptably high rates of relapse or infectious complications. Not all forms of T-cell depletion are the same; however, preliminary experience suggests that a higher engraftment rate with a lower incidence of GVHD can be achieved with alemtuzumab compared with other therapies. The benefits of graft-vs-tumor effect balanced with GVHD remain to be defined with larger trials. Additionally, the reduced-intensity conditioning regimen that provides the best possible outcome is yet to be determined among hematologic and nonhematologic diseases.^{46,98-102}

Posttransplantation immune modulation may assist in augmenting the graft-vs-tumor response. Increasing the number of patients with viable donor cells would increase the possibility of receiving immune modulation. We and others are currently investigating methods of modifying the immune system using allogeneic antitumor vaccines and cytokines in the posttransplant setting. The goal would be to promote T-cytotoxic cells to generate an immune response toward tumor antigens.

Killer immunoglobulin-like receptor mismatches have also been shown to improve the risk of relapse and overall survival and may be important to examine in the use of alternative donors.¹⁰³ This appeared to be greater in patients with acute myeloid leukemia than in those with acute lymphocytic leukemia. The exploitation of natural killer cell alloreactivity may improve clinical outcomes, and having available donors with specific HLA mismatches would be a priority for patients with relapsed disease. The importance of specific HLA mismatches remains to be defined among patients with various diseases.

The safe use of alternative donors for hematopoietic stem cell transplantation, in conjunction with NMSCT, may increase the number of patients with malignant and nonmalignant diseases who might benefit from allogeneic immunotherapy. New roles for NMSCT have included patients with nonmalignant diseases such as sickle cell anemia. Additional diseases that will soon be investigated include rheumatologic disorders such as systemic lupus erythematosus, and neurologic disorders such as multiple sclerosis. Trials in these disorders are currently undergoing further investigation, though success with acceptable toxicity will need to be achieved with matched siblings before alternative donor trials can be a common consideration. The field has moved rapidly over the past 2 years, and new horizons continue to emerge.

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