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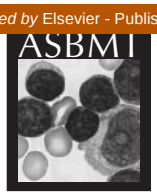

Marrow transplantation from unrelated donors for patients with severe aplastic anemia who have failed immunosuppressive therapy

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ABSTRACT

Allogeneic marrow transplantation offers curative therapy for patients with aplastic anemia. We analyzed retrospective results in 141 patients with severe aplastic anemia who received transplants between 1988 and 1995 from an unrelated volunteer donor identified through the National Marrow Donor Program (NMDP). All patients had failed one or more courses of immunosuppressive therapy. Of the patients, 121 (86%) received a radiation-containing conditioning regimen, and 20 (14%) were given chemotherapy only. Based on serologic human leukocyte antigen (HLA) typing (class I and II), 105 patients (74%) received HLA-matched marrow, and 36 (26%) received marrow mismatched for at least one HLA-A, -B, or -DR antigen. Allele-level (molecular) typing for HLA-DRB1 was available in 108 donor-recipient pairs; 77 patients received DRB1-matched and 31 DRB1-mismatched transplants. All but 13% of patients were given a cyclosporine-containing regimen for graft-vs.-host disease (GVHD) prophylaxis, and 45 patients (32%) received marrow that was T cell–depleted. Among 131 evaluable patients, 116 (89%) achieved sustained engraftment and 15 (11%) did not. Among patients with engraftment, acute GVHD of grades II–IV developed in 60 patients (52%) and extensive chronic GVHD in 24 patients at risk (31%). Currently, 51 patients (36%) are surviving at 11–94 months (median 36) after transplantation. All but five have Karnofsky scores ≥80. Patients who received a serologically matched transplant fared somewhat better than did patients given a serologically mismatched transplant (p = 0.03). Patients with donors matched by both serology and allele-level DRB1 typing had significantly better survival than DRB1-mismatched patients with 56 vs. 15% surviving at 3 years (p = 0.001). Outcome in patients transplanted within 3 years of diagnosis was superior to that among patients transplanted with greater delay. Major causes of death were graft failure, GVHD, and infections. These data suggest that unrelated marrow transplantation offers successful therapy for a proportion of patients who have failed immunosuppressive therapy.

KEY WORDS:

Aplastic anemia • Unrelated donor • DRB1 matching • Outcome

INTRODUCTION

Bone marrow transplantation from an HLA-identical sibling donor is effective therapy for patients with aplastic anemia. A 3-year actuarial survival of 92% was reported for

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39 patients conditioned with antithymocyte globulin (ATG) and cyclophosphamide and transplanted with marrow from an HLA-identical related donor [1]. A compatible family donor is available for <30% of patients, however; therefore, most patients receive nontransplant treatment, generally immunosuppressive therapy, as a first-line approach [2–5]. Frickhofen *et al.* [3] described a 70% response rate and 64% survival at 41 months in patients given ATG plus cyclosporine. Bacigalupo *et al.* [4] reported an 82% response rate and 92% survival at 34 months in 40 patients with aplastic anemia in whom treatment with ATG, cyclosporine, glucocorticoids, and granulocyte colony-stimulating factor (G-CSF) was initiated within 30 days of diagnosis. At other centers, however, response rates of 30–40%, either with single-agent ATG or a combination of ATG and cyclosporine, and actuarial 10-year survival in the range of 40% have been observed [2,5,6]. Patients who are unresponsive to immunosuppressive therapy or who have recurrent marrow failure after an initial response frequently are treated with repeated courses of immunosuppression or hematopoietic growth factors [7]. Patients who respond to immunosuppressive therapy generally do not achieve normal hematopoiesis [8]. Furthermore, as many as 15–45% of these patients eventually develop paroxysmal nocturnal hemoglobinuria, myelodysplastic syndrome, or leukemia [1,9–12].

The feasibility of marrow transplantation from alternative donors (relatives who are less than completely HLA matched or unrelated volunteer donors) has been explored. Results with partially matched related donors have been summarized in several recent publications [13–17]. Initial results with unrelated donors identified through the National Marrow Donor Program (NMDP) have been presented by Gajewski and Chattopadhyay [18] and Kernan *et al.* [19]. Here, we retrospectively analyzed results in 141 patients transplanted from unrelated donors recruited through the NMDP.

MATERIALS AND METHODS

Patients

The study population consisted of 154 patients with severe aplastic anemia (excluding patients with Fanconi's anemia) who received marrow transplants in the U.S. between 19 February 1988 and 10 May 1995. Baseline or follow-up reports were not available on 13 transplant recipients because the respective centers failed to complete data forms. Thus, data on 141 patients were available for analysis. Patient and transplant characteristics are summarized in Table 1. All patients had received one or several courses of immunosuppressive therapy consisting of a single agent or combination therapy (Table 2). The decision to proceed to transplant was exclusively that of the transplant center. Thus, the cohort under study includes patients who never showed any improvement of hematopoietic parameters, patients who did improve on immunosuppressive therapy and subsequently relapsed, and patients who had responded repeatedly to immunosuppression but never achieved a sustained response. Patients were transplanted at 36 transplant centers in the U.S., each of which carried out between one and 24 transplants. Three centers transplanted 24, 20, and 17 patients, respectively, accounting for 43% of all transplants.

Table 1. *Patient, donor, and transplant characteristics*

Characteristic	Value
Patient age (years)	0.9–47 (median 17.7)
Patient sex (male/female)	82 (58%)/59 (42%)
Donor sex (male/female)	81 (57%)/60 (43%)
Donor age (years)	21–53 (median 36)
Donor-patient sex combination*	
Female to female	26 (19%)
Female to male	33 (24%)
Male to female	33 (24%)
Male to male	48 (34%)
Disease duration (months)	3.3–162 (median 13)
Etiology	
Unknown/idiopathic	93/40 (66%/28%)
Hepatitis/viral	4 (3%)
PNH	2 (1%)
Other	2 (1%)
Blood cell count at transplant ($\times 10^9$/L)	
Leukocytes	0–11.7 (median 0)
Platelets	0–84 (median 0)
Donor-patient CMV status†	
Negative to negative	28 (24%)
Negative to positive	50 (42%)
Positive to negative	15 (13%)
Positive to positive	25 (21%)
Prior transfusions	141 (100%)
RBC‡	
Yes	37
No	10
Platelets‡	
Yes	40
No	7
Conditioning regimens	
TBI	1 (1%)
TBI + chemotherapy	93 (66%)
TBI + LFI + chemotherapy	7 (5%)
LFI + chemotherapy	20 (14%)
Chemotherapy	20 (14%)
Marrow cell dose $\times 10^8$/kg	0.03–13.6 (median 2.9)
GVHD prophylaxis§	
Methotrexate + cyclosporine	36 (25%)
Methotrexate + cyclosporine + other	35 (25%)
Cyclosporine	17 (12%)
Cyclosporine + other	40 (28%)
Methotrexate + other	4 (3%)
Other	6 (4%)
Data missing	3 (2%)
T-cell depletion	45 (32%)

For information on HLA matching, see Table 3. LFI, localized field irradiation; PNH, paroxysmal nocturnal hemoglobinuria; RBC, red blood cells; CMV cytomegalovirus.

**Unknown in one case.*

†Information incomplete on 26 transplants.

‡Detailed data available on the last 49 patients transplanted.

§Including 43 patients given T-cell depleted marrow.

Donor selection, collection of marrow, and processing

The selection of donors was based on HLA serotyping performed for HLA-A and -B and a combination of serotyping and genotyping for HLA-DRB1 according to standard procedures [20,21]. Donor characteristics are summarized in

Table 2. *Pretransplant therapy*

Modality*	No. patients
Any immunosuppression	**141**†
Glucocorticoids	**40**
ATG	**41**
Cyclosporine	**40**
Androgens	**10**
Growth factor‡	**13**

**Given alone or in combinations.*

†Specific information available only on the 49 most recently transplanted patients. Per patient, one to seven (median three) agents were used.

‡Interleukin-3, granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, stem cell factor.

Table 1. A mismatch of one HLA-A or -B antigen was characterized as minor if the mismatched antigen was in a cross-reactive group (CREG) and as major if not. High-resolution typing was performed retrospectively by several DNA-based methods, predominantly by sequence-specific oligonucleotide probes (SSOP) in 108 of 141 patients.

Marrows were collected at 56 different collection centers, each harvesting marrow on one to 11 donors. Marrow cells were harvested according to the policies of the NMDP [22], placed in sterile plastic bags, and brought to the transplant center by a courier.

Data collection

Data were collected in standardized forms provided by the NMDP and included pretransplant information, peritransplant events, and short-term and long-term follow-up. The median length of follow-up was 50 months (range 22–110). Data on survival are as of 1 March 1997.

Engraftment

The day of engraftment was defined as the first of 3 consecutive days on which the neutrophil count exceeded 500/mm^3. Thus, patients who did not have a neutrophil count of >500/mm^3 for 3 consecutive days at any time after transplantation were considered as having primary graft failure. Patients with initial engraftment in whom a severely hypocellular marrow or an absolute neutrophil count of <500/mm^3 recurred were considered to have secondary graft failure if they survived for at least 21 days. Patients who survived for <21 days were considered evaluable only if they had proven engraftment or developed GVHD before death.

GVHD

The stage of involvement of the skin, liver, and intestinal tract by acute GVHD was determined according to standard criteria [23,24]. An overall grade based on the sum of the individual stages was reported: 0 was assigned for a score of 0; I for scores of 1 to 2; II for scores of 3 to 4 (except in cases in which the skin, liver, or intestine alone was considered to have a score of 4, in which case a grade of III was assigned); and a grade of III–IV for scores of 5 or more [19]. In a few cases [12], the overall grade reported by physicians conflicted with the skin, liver, and gut involvement reported. In those cases, the overall grade was adjusted to be consistent with the organ-specific data. Chronic GVHD was assessed as limited (mild skin involvement only) or extensive (skin, liver, and intestinal tract involvement) [25].

Statistical analysis

Survival curves were estimated by the Kaplan-Meier product limit method [26]. In univariate analyses, the log-rank statistic [27] was determined. Cox proportional hazards regression [28] was used to assess the impact of the following factors on survival after transplant: recipient age, recipient cytomegalovirus (CMV) status, level of HLA matching (a disparity of 0 or ≥1 antigen by serotyping), donor age, donor-patient sex combination, T cell depletion, GVHD prophylactic drugs, total-body irradiation (TBI), and the interval between diagnosis and transplantation. Both univariate and multivariate Cox regressions were done. All p values reported are two sided.

Comparisons of proportions of patients engrafting were based on χ^2 statistics. The occurrence of grade II–IV acute GVHD was summarized using the cumulative incidence estimator [27]. Cumulative incidence methodology is necessary for this event because patients are subject to both ordinary censoring and a competing risk (transplant-related death).

RESULTS

Pretransplant therapy

All patients had received one or several cycles of immunosuppressive therapy consisting of a single agent or combinations of agents before transplantation. The actual agents used for therapy were available only on the last 49 patients transplanted (since the information became mandatory in the NMDP report forms) (Table 2). These patients received one to seven agents (median three); only three patients had been treated with a single agent (ATG). The interval from diagnosis to transplant was 3.3 to 162 months (median 13).

Identification of unrelated donors

Among the 141 patients, 105 (74%) received marrow from a donor phenotypically matched for serologically determined HLA-A, -B, and -DR antigens. The remaining 36 patients (26%) received a transplant from a donor who was a minor or major mismatch for at least one HLA class I (A or B) or class II (DR) antigen. Allele-level (molecular) typing for DRB1 was available in 108 donor/recipient pairs. Seventy-seven pairs, including 22 who were serologically mismatched for a class I antigen (minor in 12 and major in 10), were matched for DRB1, and 31 (including 26 who were serologically matched) were mismatched for DRB1 (Table 3). Thus, among the 108 pairs with molecular typing for DRB1, only 55 were matched for all antigens tested. Twenty-two were mismatched for at least one and three for at least two antigens.

The median time between initiation of the primary search for a donor and marrow transplantation was 5 months (range 0.5–78).

Table 3. *Correlation of serological (HLA-A, -B, -DR) and allele-level (molecular; DRB1) typing*

	Serologic HLA typing					
	Match	Mismatch				
Molecular typing for DRB1*	A + B + DR	A	B	A + B	DR	Total number of donor/recipient pairs
n	105	16	15	1	4	
DRB1 match	55	11†	11†	—	—	77
DRB1 mismatch	26	1	2	—	2	31
Not determined	24	4	2	1	2	33

**Available in 108 donor/recipient pairs.*

†Among these, 12 pairs differed for cross-reactive (CREG) antigens, and 10 showed major antigen differences.

Outcome

Transplant outcome is summarized in Table 4.

Ten patients died before day 21 without evidence of either engraftment or GVHD and were considered non-evaluable. Among the 131 evaluable patients, 116 (89%) achieved sustained engraftment at 9–71 days (median 18), and 15 patients (11%) experienced graft failure. In 13 patients, graft failure was primary, whereas two patients had transient evidence of engraftment but subsequently lost their graft. Among 97 evaluable patients transplanted from a serologically HLA-A, -B, -DR matched donor, 90 (93%) engrafted compared with 26 of 34 (76%) transplanted from a partially mismatched donor ($p = 0.01$). Among serologically matched transplant recipients for whom DRB1 typing was available, the incidence of graft failure was 6% with a DRB1-matched donor, and 14% with a DRB1-mismatched donor ($p = 0.26$).

Among 18 evaluable patients conditioned with a nonirradiation regimen, 16 (89%) engrafted compared with 100 of 113 (88%) conditioned with an irradiation-containing regimen ($p = 0.99$). Engraftment and graft failure by radiation dose are shown in Table 5. The incidence of graft failure was similar (10–16%) in patients who received cell doses in the first three quartiles and appeared markedly lower (3%) in patients receiving the highest cell doses ($>3.67\times10^8$/kg; fourth quartile). However, this relationship did not reach statistical significance ($p = 0.23$). Among patients transplanted with unmanipulated marrow, 94% achieved engraftment compared with 79% among those transplanted with T cell–depleted marrow ($p = 0.01$).

The cumulative incidence of grade II–IV acute GVHD among patients with sustained engraftment was 52% (Fig. 1). Among the 60 patients who developed GVHD requiring therapy, 43 (37% of evaluable patients) had GVHD of grade III–IV. The effects of conditioning regimen, marrow cell dose, GVHD prophylaxis, and histocompatibility are summarized in Table 5. The incidence of GVHD was highest in patients who received the largest number of donor cells ($p = 0.09$). Among patients transplanted from a serologically matched donor, those who were also matched at the allele level for DRB1 had a cumulative incidence of acute GVHD of 45% compared with 58% among patients transplanted from a donor who was DRB1 nonidentical ($p = 0.34$). Extensive chronic GVHD was reported in 24 of 77 patients (31%) surviving 90 days or longer (in three patients the chronic GVHD status was unknown).

At the time of last contact, 51 patients were surviving and 90 had died. The Kaplan-Meier estimates of the probability of surviving are 0.40 (confidence interval [CI] 0.32–0.48) at 1 year, 0.37 (CI 0.29–0.45) at 2 years, and 0.34 (CI 0.26–0.43) at 5 years (Fig. 2). Importantly, donor/recipient match by allele-level typing had a striking impact on survival (Fig. 3): patients given a transplant from a serologically matched donor who was also identical for DRB1 had a survival probability of approximately 56% at 2–3 years, compared with approximately 15% in patients transplanted from a nonidentical donor. Patients transplanted within 3 years of diagnosis had a higher probability of survival than patients transplanted later (Fig. 4) (see multivariable analysis). Among patients ≤20 years of age who were transplanted within 3 years of diagnosis from a donor who was matched by serologic and DRB1 typing, the probability of survival was 62%, and no deaths were observed after 1 year.

The Karnofsky performance status of surviving patients was 100 in 29 patients, 90 in 12 patients, 80 in five patients, and <80 in five patients.

Causes of death are summarized in Table 6. Graft failure, GVHD, and associated complications (infection, hemorrhage) were the major causes. Individual organ toxicity accounted for most of the remaining cases.

Cox regression analysis

Results of univariable and multivariable Cox regression analyses are summarized in Table 7. In both analyses, disease duration and HLA compatibility as determined by

Table 4. *Transplant outcome*

Event	No. patients/total patients (%)
Engraftment*	116/131 (89%)
Graft failure*	15/131 (11%)
Primary failure	13
Secondary failure	2
GVHD	
Acute (II–IV)	60/116 (52%)
Extensive chronic†	24/77 (31%)
Survival	51/141 (36%)

**Ten patients died without engraftment or GVHD before day 21 and were considered non-evaluable.*

†Among patients surviving to day 90.

Table 5. *Transplant variables and transplant outcomes*

Variable	n	Early deaths*	Graft failure		Acute GVHD (grade II–IV)‡	
			Total (secondary)	%	No. patients	Percent cumulative incidence
Conditioning regimen						
No irradiation	20	2	2 (0)	11	9	57
Irradiation (cGy)	121					
400–800	31	2	3 (1)	10	15	58
1200–1500	72	5	9 (0)	13	28	48
Unknown dose	18	1	1 (1)	6	8	50
Marrow cell dose (10^8/kg)						
<1.65	35	3	5 (1)	16	12	44
1.65–2.87	36	2	5 (1)	16	10	37
2.88–3.67	35	5	3 (0)	10	15	56
>3.67	35	0	1 (0)	3	23	68
GVHD prophylaxis†						
T-cell depletion	45	2	9 (1)	21	11	32
Other	95	8	5 (1)	6	49	60
Donor/patient HLA						
Serologic match	105	8	7 (1)	7	47	52
Serologic mismatch	36	2	8 (1)	24	13	50
Allele-level DRB1 typing§						
Identical	55	3	3 (1)	6	22	45
Nonidentical	26	4	3 (1)	14	11	58
Not completed	24	1	1 (0)	4	14	64

**Patients who died before day 21.*
†Unknown in one.
‡Among 116 patients with sustained engraftment.
§Among 105 serologically matched pairs.

serology were significant factors for survival. The impact of HLA matching was further emphasized by incorporating DRB1 typing results; the relative risk (RR) of death of a patient transplanted from a DRB1-mismatched compared with an -A, -B, -DRB1 matched donor was 2.7 (p = 0.001). The RR for the group of patients for whom DRB1 typing data were not available was 1.5 (p = 0.25). The relevance of allele-level typing for DRB1 was confirmed in a multivariable model controlling for patient and donor age, sex, CMV status, disease duration, conditioning regimen, and GVHD

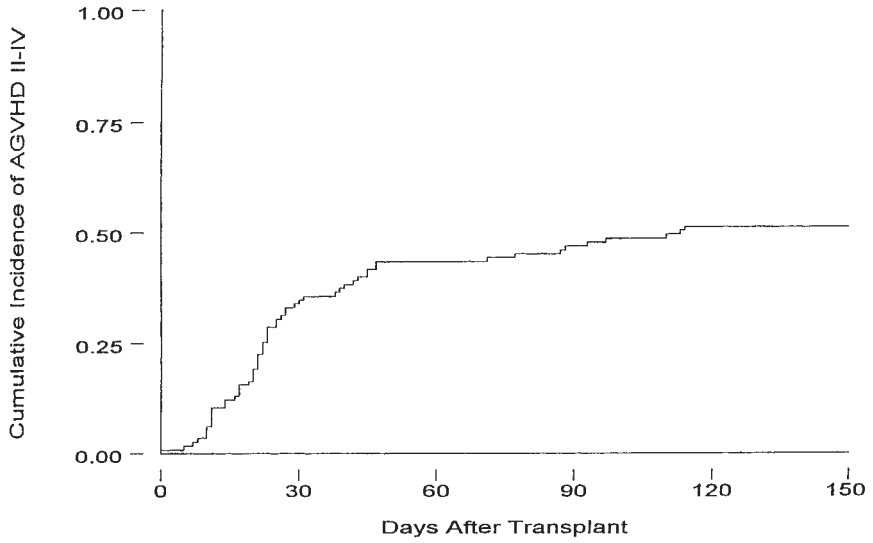

Figure 1. Incidence of acute GVHD
Cumulative incidence of grade II–IV acute graft-vs.-host disease in patients transplanted from an unrelated donor who achieved sustained engraftment.

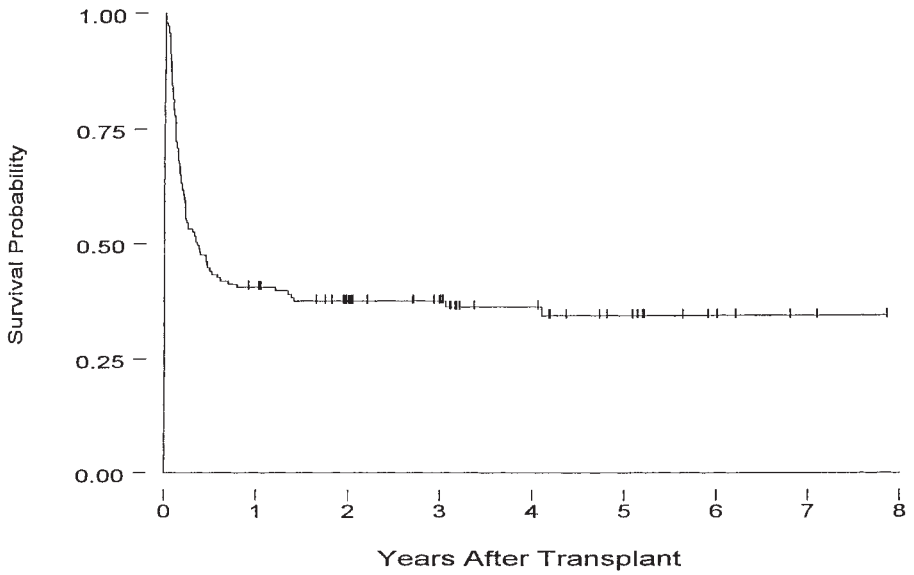

Figure 2. Overall survival

Probability of survival after transplantation from an unrelated donor after having failed immunosuppressive therapy. Tick marks indicate surviving patients.

prophylaxis: the RR for DRB1-mismatched vs. DRB1-matched transplants was 3.3 ($p = 0.001$). No other statistically significant factors were identified, although the data suggested that younger patients and patients transplanted from younger donors fared better. Information on other potential risk factors such as pretransplant immunosuppressive therapy and blood cell counts at transplantation was not sufficiently complete to allow for meaningful analysis.

DISCUSSION

Bone marrow transplantation offers curative therapy for severe aplastic anemia. With transplants from a genotypically HLA-matched sibling or phenotypically HLA-matched relative, as many as 90% of patients become long-term survivors [1]. A suitably matched related donor is available for less than one-third of otherwise eligible patients, however. The decision to perform a transplant from an "alternative" donor—an HLA-nonidentical relative [14–18] or an unrelated volunteer donor [29]—is difficult; although this procedure clearly has curative potential, it also carries a considerable risk of morbidity and mortality. For these reasons and because it takes on average at least 3 months from initiation of an unrelated donor search to transplant, patients with aplastic anemia who do not have an HLA-matched family donor generally receive immunosuppressive therapy as first-

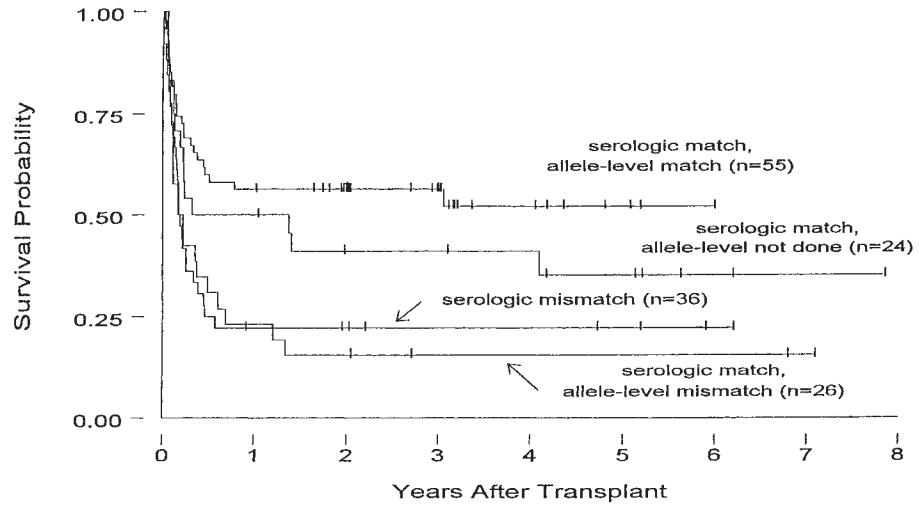

Figure 3. HLA matching and survival

Probability of survival by human leukocyte antigen matching on the basis of serological typing and high-resolution typing for DRB1.

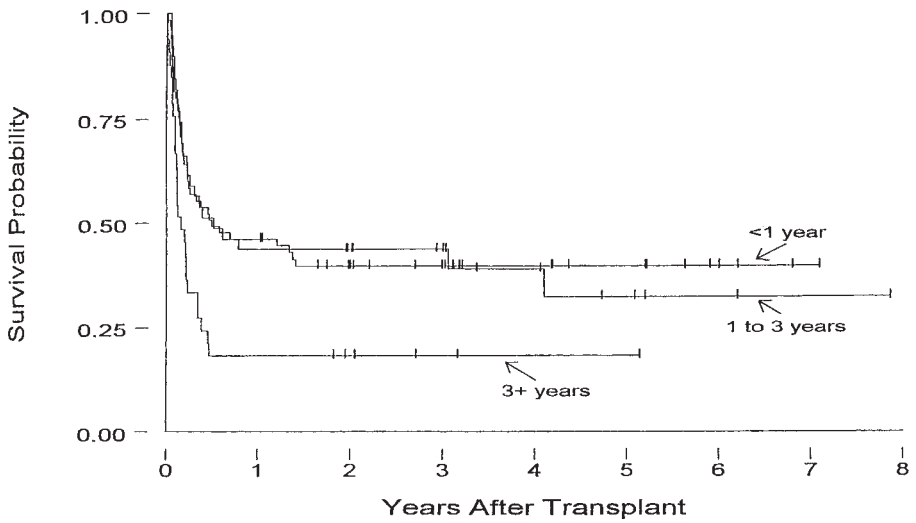

Figure 4. Effect of time from diagnosis on survival
Probability of survival by time interval from diagnosis to transplant.

line therapy [3–6]. Only if that approach fails are those patients considered for transplantation. Because of the long time from diagnosis to transplantation, patients generally have received multiple transfusions and are broadly sensitized and may suffer from iron overload. As a result of often profound neutropenia of long duration, patients are frequently infected [18,19,30]. They may also have been exposed to hepatitis B and C or other viruses. The objective of the present study was to assess the current status of unrelated transplantation for severe aplastic anemia, to identify potential risk factors for outcome, and to improve the database for the design of future studies.

As suggested by results in smaller cohorts of patients reported by individual centers [15–17], the major problems identified in the present analysis were failure of sustained engraftment, GVHD, infections, and organ toxicity, all contributing to lower survival than is seen with transplants from HLA-identical sibling donors. It is important to emphasize, however, that several parameters in the present population are distinctly different from those in HLA-identical sibling transplants. The disease duration in these unrelated transplant recipients (median 391 days) was four times longer than that among recipients of related transplants (median about 90 days), and the time from diagnosis did affect survival in the present study, although a strikingly lower survival was observed only in patients who were >3 years from diagnosis at the time of transplant. Exposure to transfusion products during the pretransplant interval presumably resulted in allosensitization and thereby increased the risk of graft rejection [18,30]. Because all patients had been transfused pretransplant, however, allosensitization per se could not be analyzed as a risk factor for transplant outcome.

The most significant risk factor recognized was HLA disparity between donor and recipient. A mismatch at the level of HLA serology significantly increased the risk of graft failure and decreased the probability of long-term survival. The difference in outcome was even more striking when allele-level typing for DRB1 was considered, although patient numbers were insufficient to show a significant impact on graft failure. Nevertheless, a survival of 56% (63% in a subset of patients younger than 20 years and transplanted within 3 years of diagnosis) in heavily pretreated patients given a transplant from an unrelated volunteer donor is encouraging. These data on DRB1 matching and outcome are in agreement with observations by Baxter-Lowe *et al.* [31] and Petersdorf *et al.* [32], who showed an increased incidence of acute GVHD and decreased probability of survival in patients with various diagnoses transplanted from an unrelated donor molecularly mismatched for a class II allele. Taken together, these data also suggest that young patients with aplastic anemia for whom a molecularly HLA-matched unrelated donor is identified should be considered for trans-

Table 6. *Causes of death*

Causes	Time posttransplant (days)	No. patients
Graft failure	18–79 (50)*	9
Acute GVHD	12–138 (62)	13
Infections		
Fungal	8–439 (39.5)	12
Viral	34–253 (86)	7
Other	42–513 (488)	3
Hemorrhage	3–504 (34.5)	12
Organ toxicity		
Pulmonary†	16–288 (51)	9
Hepatic	23–86 (62)	3
Cardiac	1, 2	2
Renal	7, 1494	2
Chronic GVHD	143–223 (167)	4
Malignancy	125–210 (136)	3‡
Other	16–84 (43)	10

**Range (median).*
†Includes five patients with acute respiratory distress syndrome (ARDS).
‡All three malignancies were posttransplant lymphoproliferative diseases.

Table 7. *Cox regression results*

	Univariate		Multivariate	
Factor	**Relative risk (of death)**	**p value**	**Relative risk (of death)**	**p value**
Patient age				
<20 years	**1.0**		**1.0**	
≤20 years	**1.3**	**0.24**	**1.5**	**0.09**
Donor age				
<35 years	**1.0**		**1.0**	
≥35 years	**1.4**	**0.11**	**1.4**	**0.14**
Donor sex → patient sex				
Female → female	**1.0**		**1.0**	
Female → male	**1.4**		**1.3**	
Male → female	**1.0**		**1.5**	
Male → male	**0.9**	**0.40***	**0.9**	**0.40***
Disease duration prior to BMT				
≤1.0 year	**1.0**		**1.0**	
1.0 to 3.0 years	**1.0**		**0.9**	
>3.0 years	**2.0**	**0.02***	**2.1**	**0.01***
Patient CMV status				
Negative	**1.0**		**1.0**	
Positive	**0.9**	**0.65**	**1.0**	**0.87**
Preparative regimen				
Chemotherapy only	**1.0**		**1.0**	
Regimen incorporating irradiation	**1.0**	**0.91**	**0.9**	**0.88**
GVHD prophylaxis				
Cyclosporine + methotrexate	**1.0**		**1.0**	
Other	**0.9**	**0.52**	**1.2**	**0.46***
T-cell depletion				
No	**1.0**		**1.0**	
Yes	**0.9**	**0.60**	**0.8**	**0.38**
HLA matching†				
Matched	**1.0**		**1.0**	
Mismatched	**1.7**	**0.03**	**2.0**	**0.006**

**The* p *value is for a likelihood ratio test for significance of entire set of factor levels in the Cox model.*
†*By serologic typing.*

plantation earlier in their course than is currently practiced. On the other hand, results in patients transplanted from a serologically or allele-level mismatched donor are probably not different from those in patients given supportive care only [33,34]. Further advances in management are required to improve results in these patients.

It is also important to note that only half of the patients included in the present analysis received GVHD prophylaxis with methotrexate plus cyclosporine, currently considered the standard regimen [35]; the remainder had been given single-agent prophylaxis, which is associated with a higher incidence of GVHD [36]. The more general use of the methotrexate plus cyclosporine combination or conceivably a combination of methotrexate plus FK506 (tacrolimus), which is under investigation in randomized trials in patients undergoing transplantation from related and unrelated donors [37], may reduce the incidence of GVHD.

T cell depletion of donor marrow, explored by many transplant teams as a means of preventing GVHD [13,16,29], was associated with a lower incidence of acute GVHD relative to patients given T cell–replete marrow (32 vs. 60%; $p < 0.01$) but also resulted in a higher incidence of graft failure (21 vs. 6%; $p = 0.01$) and did not improve survival (RR = 0.9; $p > 0.5$). Nevertheless, this approach remains attractive in patients with nonmalignant disorders in which no beneficial byproduct of GVHD in the form of a graft-vs.-tumor effect can be expected. The usual strategy to improve the probability of engraftment has been to increase the intensity of conditioning, often by incorporating irradiation into the regimen. In the present study, 85% of patients were prepared with an irradiation-containing regimen. Although other reports suggest that irradiation is effective in enhancing engraftment [18,38,39], this notion is not supported by the present analysis, in which four rejections occurred in 18 evaluable nonirradiated patients (11%) compared with 13 rejections in 113 evaluable patients (12%) given irradiation in one form or another. This is of interest because dose intensification (addition of irradiation) is associated with increased toxicity and often transplant-related mortality and, as a result, generally does not lead to improved survival [40]. Furthermore, intensive conditioning regimens are associated with a higher incidence of GVHD [40,41]. Thus, the goal must be to achieve engraftment, even of T cell–depleted marrow, with less intensive regimens to reduce regimen-related morbidity and mortality and thereby improve survival. On the basis of this rationale, an ongoing trial combines cyclophosphamide, ATG, and low-dose (3×200, 2×200, or 1×200 cGy) rather than conventional dose

(6×200 cGy) TBI. Preliminary results are encouraging in regard to both engraftment and survival [39].

Results of the present analysis also show that patients who achieve engraftment and survive the immediate posttransplant period usually do well subsequently. The current data suggest in particular that chronic GVHD may not be more frequent in recipients of unrelated transplants than in HLA-identical sibling transplantation [42]. Very few late deaths from infection or chronic GVHD were observed. These results are in agreement with reports from single-institution trials [14–17]. It is also of note that although three fatal posttransplant neoplasms occurred, no new hematologic disorders, as seen after immunosuppressive therapy [6,10,43], were observed.

Thus, the results of this analysis confirm that patients with aplastic anemia can be transplanted successfully from unrelated donors. Early morbidity and mortality are higher and survival lower than among matched related transplant recipients. Tolerance does develop eventually in these patients, however, allowing the return to a productive life. In particular, young patients with an HLA-matched donor who are transplanted early after diagnosis have an excellent prognosis. Further studies should be directed at selecting patients for earlier transplants, reducing the incidence of acute GVHD, and developing nontoxic regimens for patients who do not have a fully matched donor.

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REFERENCES

1 *Storb R, Etzioni R, Anasetti C, Appelbaum FR, Buckner CD, Bensinger W, Bryant E, Clift R, Deeg HJ, Doney K, Flowers M, Hansen J, Martin P, Pepe M, Sale G, Sanders J, Singer J, Sullivan KM, Thomas ED, Witherspoon RP:* Cyclophosphamide combined with antithymocyte globulin in preparation for allogeneic marrow transplants in patients with aplastic anemia. Blood 84:941, 1994.

2 *Doney K, Pepe M, Storb R, Bryant E, Anasetti C, Appelbaum FR, Buckner CD, Sanders J, Singer J, Sullivan K, Weiden P, Hansen JA:* Immunosuppressive therapy of aplastic anemia: results of a prospective, randomized trial of antithymocyte globulin (ATG), methylprednisolone and oxymetholone to ATG, very high-dose methylprednisolone and oxymetholone. Blood 79:2566, 1992.

3 *Frickhofen N, Kaltwasser JP, Schrezenmeier H, Raghavachar A, Vogt HG, Herrmann F, Freund M, Meusers P, Salama A, Heimpel H:* Treatment of aplastic anemia with antilymphocyte globulin and methylprednisolone with or without cyclosporine. N Engl J Med 324:1297, 1991.

4 *Bacigalupo A, Hows J, Gluckman E, Nissen C, Marsh J, Van Lint MT, Congiu M, de Planque MM, Ernst P, McCann S, Vitale V, Frickhofen N, Wursch A, Marmont AM, Gordon-Smith EC, for the EBMT Working Party on Severe Aplastic Anaemia*: Bone marrow transplantation (BMT) versus immunosuppression for the treatment of severe aplastic anaemia (SAA): a report of the EBMT SAA Working Party. Br J Haematol 70:177, 1988.

5 *Champlin R, Ho W, Gale RP:* Antithymocyte globulin treatment in patients with aplastic anemia: a prospective randomized trial. N Engl J Med 308:113, 1983.

6 *Doney K, Leisenring W, Storb R, Appelbaum FR, for the Seattle Bone Marrow Transplant Team:* Primary treatment of acquired aplastic anemia: outcomes with bone marrow transplantation and immunosuppressive therapy. Ann Intern Med 126:107, 1997.

7 *Champlin RE, Nimer SD, Ireland P, Oette DH, Golde DW:* Treatment of refractory aplastic anemia with recombinant human granulocyte-macrophage-colony-stimulating factor. Blood 73:694, 1989.

8 *Nissen C, de Planque MM, Wursch A, Moser Y, Carbonare VD, Speck B:* Testosterone reduces complement sensitivity of precursor cells in aplastic anaemia patients treated with antilymphocyte globulin. Br J Haematol 69:405, 1988.

9 *Tichelli A, Gratwohl A, Wursch A, Nissen C, Speck B:* Late haematological complications in severe aplastic anaemia. Br J Haematol 69:413, 1988.

10 *Gordon-Smith EC, Hows J, Bacigalupo A, Gluckman E, Van Lint MT, Congiu A, James DCO, Barrett AJ, de Planque MM, Siimes MA, Toivanen A, Ringden O, Marmont AM:* Bone marrow transplantation for severe aplastic anaemia (SAA) from donors other than HLA identical siblings: a report of the EBMT working party. Bone Marrow Transplant 2:100, 1987.

11 *Storb R:* Bone marrow transplantation for aplastic anemia. In: *Bone Marrow Transplantation*. SJ Forman, KG Blume, ED Thomas, Eds. Boston: Blackwell, 583, 1994.

12 *Young NS, Alter BP:* Pathophysiology II: immune suppression of hematopoiesis. In: *Aplastic Anemia: Acquired and Inherited*. Philadelphia: Saunders, 68, 1994.

13 *Wagner JL, Deeg HJ, Seidel K, Anasetti C, Doney K, Sanders J, Sullivan KM, Storb R:* Bone marrow transplantation for severe aplastic anemia from genotypically HLA-nonidentical relatives: an update of the Seattle experience. Transplantation 61:54, 1996.

14 *McCann SR, Bacigalupo A, Gluckman E, Hinterberger W, Hows J, Ljungman P, Marin P, Nissen C, van't Veer Kerthof E, Raghavachar A, Socié G, Frickhofen N, Locasciulli A, Schrezenmeier H:* Graft rejection and second bone marrow transplants for acquired aplastic anaemia: a report from the Aplastic Anaemia Working Party of the European Bone Marrow Transplant Group. Bone Marrow Transplant 13:233, 1994.

15 *Camitta B, Deeg HJ, Castro-Malaspina H, Ramsay NKC:* Unrelated or mismatched bone marrow transplants for aplastic anemia: experience

at four major centers. In: E Gluckman, L Coulombel (eds) *Ontogeny of Hematopoiesis Aplastic Anemia*. Montrouge, France: John Libbey Eurotext, 325, 1995.

16 *Hows JM, Yin JL, Marsh J, Swirsky D, Jones L, Apperley JF, James DCO, Smithers S, Batchelor JR, Goldman JM, Gordon-Smith EC:* Histocompatible unrelated volunteer donors compared with HLA nonidentical family donors in marrow transplantation for aplastic anemia and leukemia. Blood 68:1322, 1986.

17 *Gordon-Smith EC, Fairhead SM, Chipping PM, Hows J, James DCO, Dodi A, Batchelor JR:* Bone-marrow transplantation for severe aplastic anaemia using histocompatible unrelated volunteer donors. Br Med J 285:835, 1982.

18 *Gajewski JL, Chattopadhyay A:* Treatment of aplastic anemia with bone marrow transplants from closely matched unrelated donors. In: RE Champlin, RP Gale (eds) *New Strategies in Bone Marrow Transplantation*. New York: Wiley-Liss, 101, 1991.

19 *Kernan NA, Bartsch G, Ash RC, et al.:* Retrospective analysis of 462 unrelated marrow transplants facilitated by the National Marrow Donor Program (NMDP) for treatment of acquired and congenital disorders of the lymphohematopoietic system and congenital metabolic disorders. N Engl J Med 328:593, 1993.

20 *Petersdorf EW, Perkins H, Sekiguchi S, Baxter-Lowe LA, Goeken N, Holzberger G, Hsia S, Rosner G, Tonai R, Yang SY, Hansen JA:* Marrow transplantation. In: K Tsuji, M Aizawa, T Sasazuki (eds) *HLA 1991*. London: Oxford Science Publications, 812, 1992.

21 *Baxter-Lowe LA, Eckels D, Ash R, Casper J, Hunter JB, Gorski J:* HLA oligonucleotide genotyping refines selection of bone marrow transplant donors (Abstract). Blood 76 (Suppl. 1):527a, 1990.

22 *McCullough J, Hansen JA, Perkins H, Stroncek D, Bartsch G:* Establishment of the National Bone Marrow Donor Registry. In: RP Gale, RE Champlin (eds) *Bone Marrow Transplantation: Current Controversies, UCLA Symposia on Molecular and Cellular Biology, New Series*. New York: Liss, 641, 1989.

23 *Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, Lerner KG, Thomas ED:* Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation 18:295, 1974.

24 *Przepiorka D, Weisdorf D, Martin P, Klingemann H-G, Beatty P, Hows J, Thomas ED:* Consensus conference on acute GVHD grading. Bone Marrow Transplant 15:825, 1995.

25 *Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE, Hackman R, Tsoi MS, Storb R, Thomas ED:* Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med 69:204, 1980.

26 *Kaplan EL, Meier P:* Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457, 1958.

27 *Kalbfleisch JD, Prentice RL: The Statistical Analysis of Failure Time Data*. New York: Wiley & Sons, 1980.

28 *Cox DR:* Regression models and life-tables (with discussions), series B. J Royal Statist Soc 34:187, 1972.

29 *Margolis D, Camitta B, Pietryga D, Keever-Taylor C, Baxter-Lowe LA, Pierce K, Kupst MJ, French J, Truitt R, Lawton C, Murray K, Garbrecht F, Flomenberg N, Casper J:* Unrelated donor bone marrow transplantation to treat severe aplastic anaemia in children and young adults. Br J Haematol 94:65, 1996.

30 *Deeg HJ, Anasetti C, Petersdorf E, Storb R, Doney K, Hansen JA, Sanders J, Sullivan KM, Appelbaum FR:* Cyclophosphamide plus ATG conditioning is insufficient for sustained hematopoietic reconstitution in patients with severe aplastic anemia transplanted with marrow from HLA-A, B, DRB matched unrelated donors (Letter). Blood 83:3417, 1994.

31 *Baxter-Lowe LA, Kollman C, Awdeh Z, Beatty P, Begovich A, Confer D, Fernandez-Vina M, Flomenberg N, Kitajima D, Noreen H, Salazar M, Schmeckpeper B, Williams T, Yunis E, Hurley C:* Use of molecular HLA analysis to examine the relationship between class II HLA disparity and outcome of unrelated bone marrow transplants (Abstract). Blood 90:397a, 1997.

32 *Petersdorf EW, Longton GM, Anasetti C, Martin PJ, Mickelson EM, Smith AG, Hansen JA:* The significance of HLA-DRB1 matching on clinical outcome after HLA-A, B, DR identical unrelated donor marrow transplantation. Blood 86:1606, 1995.

33 *Paquette RL, Tebyani N, Frane M, Ireland P, Ho WG, Champlin RE, Nimer SD:* Long-term outcome of aplastic anemia in adults treated with antithymocyte globulin: comparison with bone marrow transplantation. Blood 85:283, 1995.

34 *Camitta BM, Thomas ED, Nathan DG, Gale RP, Kopecky KJ, Rappeport JM, Santos G, Gordon-Smith EC, Storb R:* A prospective study of androgens and bone marrow transplantation for treatment of severe aplastic anemia. Blood 53:504, 1979.

35 *Storb R, Deeg HJ, Pepe M, Appelbaum FR, Anasetti C, Beatty P, Bensinger W, Berenson R, Buckner CD, Clift R, Doney K, Longton G, Hansen J, Hill R, Loughran T, Jr, Martin P, Singer J, Sanders J, Stewart P, Sullivan K, Witherspoon R, Thomas ED:* Methotrexate and cyclosporine versus cyclosporine alone for prophylaxis of graft-versus-host disease in patients given HLA-identical marrow grafts for leukemia: long-term follow-up of a controlled trial. Blood 73:1729, 1989.

36 *Chao NJ, Deeg HJ:* In vivo prevention and treatment of GVHD. In: *Graft-vs.-Host Disease*, 2nd ed. JLM Ferrara, HJ Deeg, S Burakoff, Eds. New York: Marcel Dekker, 639, 1997.

37 *Ratanatharathorn V, Nash RA, Przepiorka D, Devine SM, Klein JL, Weisdorf D, Fay JW, Nademanee A, Antin JH, Christiansen NP, Van Der Jagt R, Herzig RH, Litzow MR, Wolff SN, Longo WL, Petersen FB, Karanes C, Avalos B, Storb R, Buell DN, Maher RM, Fitzsimmons WE, Wingard JR:* Phase III study comparing methotrexate and tacrolimus (Prograf, FK506) with methotrexate and cyclosporine for graft-versus-host-disease prophylaxis after HLA-identical sibling bone marrow transplantation. Blood 92:2303, 1998.

38 *Feig SA, Champlin R, Arenson E, Yale C, Ho W, Tesler A, Gale RP:* Improved survival following bone marrow transplantation for aplastic anaemia. Br J Haematol 54:509, 1983.

39 *Deeg HJ, Schoch G, Ramsay N, Harris R, Castro-Malaspina H, Collins R, Gajewski J, Territo M, Casper J, Mueller B, Anasetti C, King R:* Marrow transplantation from unrelated donors for patients with aplastic anemia (AA) who failed immunosuppressive therapy (Abstract). Blood 90:1765, 1997.

40 *Clift RA, Buckner CD, Appelbaum FR, Bearman SI, Petersen FB, Fisher LD, Anasetti C, Beatty P, Bensinger WI, Doney K, Hill R, McDonald G, Martin P, Sanders J, Singer J, Stewart P, Sullivan KM, Witherspoon R, Storb R, Hansen J, Thomas ED:* Allogeneic marrow transplantation in patients with acute myeloid leukemia in first remission: a randomized trial of two irradiation regimens. Blood 76:1867, 1990.

41 *Deeg HJ, Spitzer TR, Cottler-Fox M, Cahill R, Pickle LW:* Conditioning-related toxicity and acute graft-versus-host disease in patients given methotrexate/cyclosporine prophylaxis. Bone Marrow Transplant 7:193, 1991.

42 *Morton AJ, Anasetti C, Gooley T, Flowers ME, Deeg HJ, Hansen JA, Martin PJ, Sullivan KM:* Chronic graft-versus-host disease (GVHD) following unrelated donor transplantation (Abstract). Blood 90:590a, 1997.

43 *Socié G, Henry-Amar M, Bacigalupo A, Hows J, Tichelli A, Ljungman P, McCann SR, Frickhofen N, Van't Veer-Korthof E, Gluckman E, for the European Bone Marrow Transplantation-Severe Aplastic Anaemia Working Party:* Malignant tumors occurring after treatment of aplastic anemia. N Engl J Med 329:1152, 1993.