

Fludarabine, cyclophosphamide and anti-thymocyte globulin for alternative donor transplants in acquired severe aplastic anemia: a report from the EBMT-SAA Working Party

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Summary:

We have developed a reduced-intensity conditioning regimen for patients with severe aplastic anemia (SAA) undergoing alternative donor transplants, which includes fludarabine (120 mg/m²), cyclophosphamide (1200 mg/m²) and antithymocyte globulin (7.5 mg/kg). Graft-versus-host disease (GvHD) prophylaxis consisted of cyclosporine and methotrexate. We have enrolled 38 SAA patients in this trial: median age of 14 (3–37) years, transplanted from unrelated ($n=33$) or family mismatched ($n=5$) donors, with unmanipulated marrow ($n=36$) or peripheral blood ($n=2$). Seven patients (18%) had evidence of graft failure, 11% developed grade II–III acute GvHD and 27% developed chronic GvHD. The actuarial 2-year survival is 73%, with a median follow-up of 621 days. Younger patients (≤ 14 years) had a lower risk of rejection (5%) and improved actuarial survival (84%). Causes of death were infections ($n=3$), graft failure ($n=2$), Epstein–Barr virus lymphoma ($n=2$) and hemorrhage ($n=2$). In conclusion, the actuarial 2-year survival is encouraging in young SAA patients receiving a radiation-free conditioning regimen. The significant risk of graft failure in patients 15 years or older may require modification of the conditioning regimen in adults.

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In 1988, the European Group for Blood and Marrow Transplantation (EBMT) reported 46 patients with severe aplastic anemia (SAA) undergoing bone marrow transplantation (BMT) from mismatched family ($n=35$) or

unrelated donors (UD) ($n=11$),¹ with 26% surviving 16–84 months post-BMT. Favorable predictors were HLA identity and the combined use of cyclosporine (CsA) and methotrexate (MTX). Causes of death were rejection ($n=15$), graft-versus-host disease (GvHD) ($n=13$) and pneumonitis ($n=5$).¹ Other studies reported similar results.^{2–11}

Recently, there have been two important studies: one from the USA¹² and one from Japan.¹³ The first tested de-escalating doses of radiation, from 6 Gy down to 2 Gy. In all, 10 of 20 patients who received 6 or 4 Gy, and eight of 13 who received 2 Gy, are alive.¹² The Japanese study reported 154 SAA patients undergoing a UD transplant: 11% rejected, 20% experienced acute GvHD, 30% experienced chronic GvHD and 64% survive.¹³ Unfavorable factors for survival were older age (>20 years), conditioning without anti-thymocyte globulin (ATG) and a long (>3 years) interval from diagnosis to transplant. The Japanese study included a large number of patients who received low-dose radiation, and these had a significantly lower risk of rejection. Radiation, however, increases the risk of second tumors.¹⁴

For this reason, the SAA Working Party of the EBMT designed a radiation-free preparative regimen, modified from the original fludarabine (FLU) cyclophosphamide (CY) protocol, reported by the Houston group¹⁵ with the addition of ATG. We are now reporting the first 38 patients allografted with this regimen.

Materials and methods

Patients

Patients were grafted between 26.3.1998 and 25.5.2004 in 13 transplant centers reporting to the EBMT. All patients had failed one or more courses of immunosuppressive therapy, and all were transfusion dependent at the time of transplant. The protocol was approved by the Ethical Committee of the participating institutions and all patients gave informed consent. Clinical data of patients are outlined in Table 1.

Bone marrow donors

The donor was a UD in 33 or an HLA mismatched family member in five. Of the UD, 28 were reported as matched at

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the allelic level at A,B,DRB1, whereas five were reported to have mismatches at C and DQ ($n=3$), C and DQ ($n=1$) or DP alone ($n=1$). The five related donors (RD) were one antigen mismatched at class I ($n=3$) or two antigens at class I ($n=1$) or one antigen at class II ($n=2$).

Stem cell source

The stem cell source was unmanipulated bone marrow (BM) ($n=36$) or unmanipulated peripheral blood ($n=2$).

Transplant protocol

Patients received 30 mg/m² FLU on days -6, -5, -4 and -3, 300 mg/m² CY on days -6, -5, -4 and -3 and 3.75 mg/kg ATG (Thymoglobulin, Genzyme, USA) on days -6, -5, -4 and -3. On day 0, unmanipulated BM was infused. Prophylaxis and monitoring of viral and fungal infections and post transplant monitoring for CMV, Epstein-Barr virus (EBV) and *Aspergillus* were carried out as per the institutional protocols in the different centers.

Table 1 Clinical data of patients with acquired aplastic anemia

Number of patients	38
<i>Donor type</i>	
HLA mismatched family members	$n=5$
Unrelated donors	$n=33$
Age, median (range) (years)	14 (3-37)
Gender: male/female	19/19
<i>Stem cell source</i>	
Bone marrow	36
Peripheral blood	2
Nucleated cells infused ($\times 10^8$ /kg)	3.3 (1.3-10.0)
Interval from diagnosis to BMT (days)	590 (44-3000)
Acute GvHD, grade 0-I, II, III-IV	31, 2, 2
<i>Chronic GvHD</i>	
No/limited/extensive/not assessed	24/7/2/2
Alive	29
Follow-up, median (range) (days)	621 (183-2218)
Deceased	9
Follow-up, median (range) (days)	122 (6-737)
100-day mortality	8%

Table 2 Clinical data and outcome of seven patients with graft rejection or graft failure

Patient number	3	15	17	21	26	36	37
Age	23	7	24	37	15	15	23
Interval Dx-Tx (days)	1115	44	496	1665	482	92	585
Donor type	RD	UD	UD	UD	UD	UD	UD
Cells ($\times 10^8$ /kg)	10	8.4	4.5	1.8	4.5	?	1.6
Rejection type	Rej	NoGr	GF	Rej	Rej	Rej	GF
Comment	3Tx		Late	2Tx	2Tx	2TX	Late
Autologous recovery	No	Yes	No	No	Yes	No	Yes
Alive (A)/dead (D)	D	A	A	D	A	A	A
Follow-up (days)	269	985	838	165	570	204	224
Transfusion dependent	—	No	No	—	No	No	Yes

Dx-Tx = interval between diagnosis and transplant; RD = related donor; UD = unrelated donor; Tx = transplant; 3Tx = three transplants; 2Tx = two transplants; Rej = rejection; NoGr = primary nonengraftment; GF = graft failure.

Graft-versus-host disease prophylaxis

CsA was started on day -5 at a dose of 1 mg/kg/day either as a continuous intravenous infusion or as twice daily 2-h intravenous infusion, and then increased to 2 mg/kg/day starting on day -1. Intravenous CsA was continued until patients were able to have oral intake and then it was given orally at a dose of 6-10 mg/kg/day for at least 6 months. MTX was given at a dose of 10 mg/m² on day +1, and at a dose of 8 mg/m² on days +3, +6 and +11.

Graft failure

Graft failure was classified as follows: (1) primary non-engraftment (failure to reach a neutrophil count of 0.5×10^9 /l after transplant); (2) rejection (decrease in blood counts to less than 0.5×10^9 /l neutrophils, after achieving a neutrophil count of 0.5×10^9 /l); (3) late graft failure (decrease of blood counts beyond day +100 to less than 1×10^9 /l neutrophils and less than 30×10^9 /l platelets).

Statistical analysis

The Number Cruncher Statistical System (NCSS) package was used to analyze data. The probability of survival was estimated by the Kaplan-Meier method and the log-rank test (Mantel-Cox) was used to assess differences between survival curves.

Results

Engraftment, chimerism and graft failure

All patients were evaluable for engraftment. Median time to 0.5×10^9 neutrophils was day 17 (11-67) and median time to 30×10^9 /l platelets was day 23 (15-126). Data on 22 patients were available for chimerism studies: average minimum donor chimerism within day +100 was 90% (range 40-100) and average maximum donor chimerism within day +100 was 97% (83-100). Average donor chimerism beyond day +100 was 96% (17-100), with 20/22 patients having 100% donor chimerism. Overall, there were seven episodes of graft failure/rejection, which could be further classified as primary nonengraftment ($n=1$), engraftment followed by rejection ($n=4$) and engraftment followed by late graft failure ($n=2$) (Table 2).

Four patients received a second (in one case a third) transplant: two of these are alive – one with full donor chimerism, conditioned with 10 mg/kg thiotepa + 100 mg/kg CY (UPN 36) – one with autologous reconstitution, conditioned with 200 mg/kg CY alone (UPN 26). Two patients died after a second transplant, without sustained engraftment: one was conditioned with thiotepa and one with TBI. Of the seven patients who rejected, five remain alive, three with autologous reconstitution. In patients aged 14 years or less ($n=19$), there was one rejection (5%), whereas in 19 patients aged 15 years and over, there were six rejections (32%) ($P=0.03$). Patients rejecting had similar interval from diagnosis to BMT as patients not rejecting (both 585 days) and similar grafted cell dose ($4.5 \times 10^8/\text{kg}$ vs $3.5 \times 10^8/\text{kg}$, respectively).

Graft-versus-host disease, survival and causes of death

Acute GvHD II–III developed in 11% and chronic GvHD in 27% of evaluable patients. A total of 29 patients (76%)

survive with a median follow-up of 621 days and an actuarial 2-year survival of 73% (Figure 1): there was a trend for improved outcome for young patients (≤ 14 years) (84 vs 61%) (Figure 2) and early transplants (< 590 days) (85 vs 61%). The interval from diagnosis to BMT seemed to be more important in the older age group as compared to younger patients: the actuarial 2-year survival in patients > 14 years grafted within or beyond 590 days was 90 vs 37% ($P=0.07$) and 79 vs 90% in younger children ($P=0.5$). Nine patients died at a median of 122 days (6–737): causes of death were³ graft failure,² EBV-related lymphoma² and hemorrhage.²

Discussion

We have shown in this study that a radiation-free, low-intensity conditioning regimen can allow engraftment of unrelated or family mismatched marrow in young patients with acquired SAA. The overall survival is 73% at 2 years, with a low risk of GvHD and a relatively low risk of graft failure.

Graft failure has been of concern for many years in SAA patients undergoing an HLA identical BMT. In some centers, busulfan¹⁶ or low-dose TBI² has been added to CY to reduce graft failure; in other centers, ATG has been used successfully to reduce graft rejection.¹⁷ In the present series of alternative donor transplants, we have seen an overall graft failure rate of 18%, with a significant age effect: patients aged 15 years or over showed a graft failure rate of 32%, which is significantly higher when compared to younger patients (5%). One would have expected older patients to have a longer interval from diagnosis to transplant, due to one or more courses of immunosuppressive therapy, but this was not the case (595 vs 585 days, $P=0.5$), nor was the cell dose lower ($3.4/\text{kg}$ vs $3.4 \times 10^8/\text{kg}$, $P=0.6$). Therefore, the higher risk of graft failure in young adults over 15 years remains unclear. It is interesting to note, however, that a significant proportion of rejecting patients (5/7) remain alive, possibly due to the low intensity of the conditioning regimen, which allows one to wait for autologous recovery, or to organize a second transplant. For patients aged 15 years and over, we are currently exploring the addition of low-dose TBI (2 Gy) to the FLU-CY program, to test whether the graft failure rate can be reduced and the outcome improved. Another option would be increasing the dose of fludarabine and CY.

GvHD was not a problem in this series of patients: this may be due to the lack of myeloablative agents, and to the use of MTX + CsA post transplant, combined with rabbit ATG pre-transplant for GvHD prophylaxis. Rabbit ATG (Genzyme) was given at a dose of 3.75 mg/kg \times 4, for a total dose of 15 mg/kg, which can be considered a high dose,¹⁸ and is effective in preventing acute GvHD.¹⁹ The drawback is reactivation of EBV, reported in transplants using *ex vivo* or *in vivo* T-cell depletion,^{20,21} and possibly dependent on the total ATG dose given. The two patients who died of EBV lymphoma in this series (5%) confirm the significant risk of EBV-related disorders, when using high-dose ATG in the setting of a UD transplant. Weekly monitoring of EBV-DNA levels and early use of rituximab

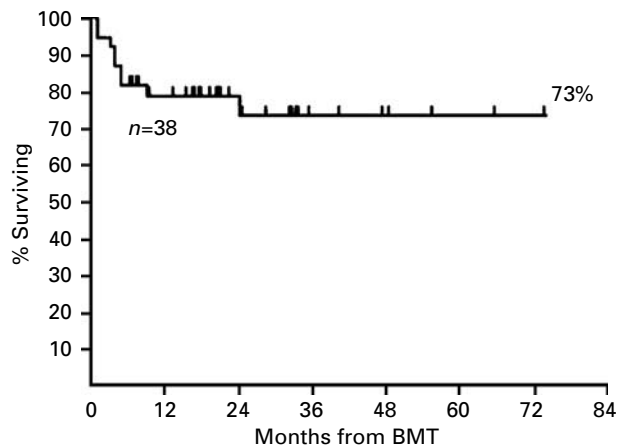


Figure 1 Actuarial survival of 38 patients with acquired SAA undergoing alternative donor transplants.

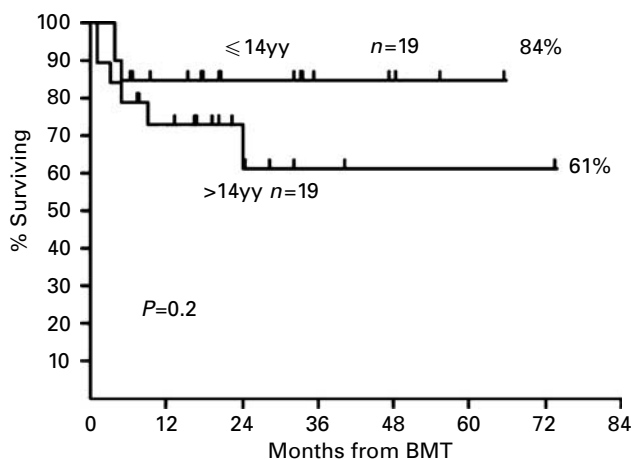


Figure 2 Actuarial survival of patients stratified according to age: there is a trend for improved outcome in patients aged ≤ 14 years (84%) as compared to patients aged > 14 years (61%).

for patients with over 1000 EBV copies²⁰ is recommended, if one is to maintain the 15 mg/kg ATG regimen.

Finally, should patients undergo a UD graft soon after diagnosis of acquired SAA, or should we give them a first course of immunosuppression, and proceed to transplant only in case of failure? Since identification of a suitable UD may take several months, a first course of ATG and CsA should be given, in keeping with current guidelines.²² In patients up to the age of 40 years, however, the activation of the UD search may start at the time of diagnosis: the hematologic response to ATG would then dictate whether the patient will proceed or not to transplantation.

In conclusion, this study confirms improved outcome in SAA patients undergoing alternative donor grafts: problems such as graft failure and infections remain, and need to be addressed by international cooperative programs.

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