Unrelated stem cell transplantation for severe acquired aplastic anemia: improved outcome in the era of high-resolution HLA matching between donor and recipient

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ABSTRACT

Background and Objectives

Severe acquired aplastic anemia (SAA) is a potentially fatal bone marrow failure syndrome occurring mainly in children and young adults. Immunosuppressive regimens and hematopoietic stem cell transplantation (HSCT) are the only two available curative treatments. Patients who lack an HLA-identical sibling donor may receive HSCT from an unrelated donor, a strategy historically associated with high mortality rates. Thus, for patients refractory to immunosuppressive regimens, the decision to transplant stem cells from unrelated donors is weighed against supportive care and often represents a dilemma for physicians. We aimed to determine whether outcome after unrelated HSCT has improved in recent years and, if so, to determine the factors responsible for the improvement.

Design and Methods

We analyzed the outcome of 89 patients (median age 17 years, range 0-52) with acquired SAA undergoing HSCT from an unrelated donor between 1989 and 2004. Cases were consecutively reported to the French Registry (SFGM-TC) by 25 centers.

Results

Patients transplanted during two successive time-periods (1989-1998 and 1999-2004) had different 5-year survival probabilities (±95% confidence interval): 29±7% and 50±7%, respectively ($p<0.01$). The main difference between the two cohorts concerned HLA matching between donors and recipients at the allelic level for the ten HLA-A, -B, -C, -DRB1 and -DQB1 antigens, which was more frequent in 1999-2004 than in the former period ($p=0.0004$). In multivariate analysis, the only two factors affecting survival were HLA allelic matching ($p<0.01$) and younger age of recipient (≤17 years, $p<0.0001$). Survival reached 78±11% at 5 years for the younger, fully HLA-matched patients.

Interpretation and Conclusions

Survival after unrelated HSCT for SAA has improved significantly over the past 15 years, mainly due to better HLA matching. Results for young patients who are fully HLA-matched at the allelic level with their donor are comparable to those observed after HSCT from a related donor.

Key words: aplastic anemia, volunteer donors, HLA matching, prognostic factors.

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Hematopoietic stem cell transplantation (HSCT) from an HLA-matched family donor is the treatment of choice for children and young adults with severe aplastic anemia (SAA).[1,2] However, this approach is limited by the availability of HLA-matched donors. Immunosuppressive therapy with a combination of antithymocyte globulin and cyclosporine A is the alternative treatment for patients without a suitable related donor.[3-5] HSCT from a phenotypically HLA-matched unrelated donor is indicated as salvage therapy for patients who fail to respond to one or more courses of immunosuppressive therapy and for patients who experience relapse of disease. The initial results of marrow transplantation from unrelated donors have been far less encouraging than those with grafts from HLA-matched siblings because of a high incidence of graft failure and severe graft-versus-host disease (GVHD). Survival rates from the early series, including a large retrospective analysis by the IBMTR,[6] ranged from 20% to 54%.[7,8] Recently, data on outcomes after unrelated transplantation for SAA were updated by the CIBMTR for the period 1988-1998.[9] Probabilities of overall survival at 5 years were 39% and 36% for 181 and 51 patients receiving transplants from matched or mismatched unrelated donors, respectively. The degree of matching was based on low-resolution typing of HLA-A, -B and –DR. Given these high mortality rates, the decision to transplant SAA refractory patients with a graft from an unrelated donor is often weighed against repeating immunosuppressive therapy or providing supportive care. An optimum conditioning regimen, GVHD prophylaxis, and better donor selection are considered to be essential for improving outcomes for patients who undergo HSCT from an unrelated donor. Two recent studies sponsored by the National Marrow Donor Program (NMDP)[10] and the Japanese Marrow Donor Program (JMDP),[11] while not specifically evaluating the improvement of survival over time, reported encouraging survival rates. The former study[10] tested de-escalating doses of total body irradiation (TBI), from 6 Gy to 2 Gy, in combination with cyclophosphamide and antithymocyte globulin within the conditioning regimen. With a median follow-up of 7 years, survival rates of up to 61% for fully HLA-matched patients, and of 40% for others, were reported. The optimum TBI dose was 1x2 Gy. The Japanese study[11] reported a detailed analysis of the outcome of 154 patients who underwent HSCT from an unrelated donor, attempting to clarify the effect of HLA mismatching as determined by high-resolution DNA typing. The probability of overall survival at 5 years was 56% and risk factor analysis suggested that higher survival rates were attained when HLA-A or -B matched donors were used, while DRB1 matching had no impact. Here we report the outcome of 89 SAA patients who underwent HSCT from an unrelated donor and were consecutively reported to a French Registry (SFGM-TC). Our aim was to determine whether the outcome of such transplantations improved from 1989 to 2004 and, if so, to determine whether the improvement resulted from changes in selection of patients, changes in transplantation technique, or both. For the recent period, we also evaluated the impact of HLA matching at the allelic level regarding ten loci (HLA-A, -B, -C, -DRB1 and -DQB1) analyzed by high-resolution DNA-based methods.

Design and Methods

Patients

We retrospectively analyzed the outcome of 89 patients (median age 17 years, range 0-52) who received HSCT from a voluntary unrelated donor from January 1989 to June 2004 for idiopathic (n=73), post-hepatic (n=4), or paroxysmal nocturnal hemoglobinuria (PNH) clone-associated SAA (n=12). Congenital forms of aplastic anemia were not considered. The search for an unrelated donor was initiated at the discretion of the referring physicians. Cases were consecutively reported to a French Registry (SFGM-TC) by 25 centers. Updating of follow-up and missing data were requested from the centers in December 2005. The analysis was performed in March 2006 when the median follow-up of survivors was 37 months (range 4-191). Patient-, disease-, and transplant-related characteristics of the 89 patients studied are shown in Table 1. Patients were divided into two cohorts based on year of transplantation, using the median date of HSCT within the whole population (September 1999) as the cut-off. We thus compared patients transplanted within two time-periods: 1989-1998 and 1999-2004. These two time periods were also those that best modeled changes in post-transplantation survival over time. The majority of patients (73 out of 81 evaluable patients) received immunosuppressive therapy before HSCT. The median interval between diagnosis and HSCT was 13 months (range 2-145 months). The conditioning regimen included irradiation in 39 patients (TBI: n=36, total lymphoid irradiation: n=3), serotherapy in 69 (antithymocyte globulin n=68, anti-CD3 monoclonal antibody n=1) and fludarabine in 15. Marrow (n=87) or peripheral blood stem cell (n=2) grafts were not T-cell-depleted and the median cell-dose infused was 2.6 (range 0.3-102) $x10^8$ nucleated cells/kg. GVHD prophylaxis consisted of the association of cyclosporine A/methotrexate in 71% of patients. Primary or secondary graft failure occurred in five and seven patients, respectively, and five patients received a second transplant from the same donor after graft failure. Other patients engrafted with neutrophil (absolute neutophil count >500/μL) and platelet (platelet count >50000/μL) recovery occurring, respectively at a median of 21 days (range 10-99) and 34 days (range 14-400) after transplant.

Recipient-donor HLA matching

The HLA matching between recipient and donor was based on HLA search determinants according to the standard techniques: serology and DNA typing. As shown in

Table 1. Patient-, disease-, and transplant-related characteristics of patients undergoing HSCT from unrelated donors for SAA between 1989 and 2004, by period of transplantation.

Variables	*N. evaluable*	*Year of Transplant* 1989-1998 n=37	1999-2004 n=52	*p value*
Age of patients	89			
≤ 17 years	48	21 (57%)	27 (52%)	NS
> 17 years	41	16 (43%)	25 (48%)	
SAA subtype	89			
Post-hepatic	4	2 (5%)	2 (4%)	NS
Idiopathic	73	31(84%)	42 (81%)	
PNH	12	4 (11%)	8 (15%)	
Treatment of SAA before HSCT (ATG and/or steroids and/or CsA)	81			
Yes	73	31 (91%)	42 (87%)	NS
No	8	2 (9%)	6 (13%)	
Mean interval diagnosis-HSCT	89	27 months	25 months	NS
Interval diagnosis-HSCT	89			
≤ 1year	40	16 (43%)	24 (46%)	NS
> 1year	49	21 (57%)	28 (54%)	
Median age of donors	89	36 years	37 years	NS
Sex mismatch	89			
Yes	41	18 (49%)	23 (44%)	NS
No	48	19 (51%)	29 (56%)	
Donor CMV serology	88			
Positive	38	18 (50%)	20 (38%)	NS
Negative	50	18 (50%)	32 (62%)	
Cell dose ($x10^8$ nucleated cells/kg)	81			
≤ 2.6	41	17 (52%)	24 (50%)	NS
> 2.6	40	16 (48%)	24 (50%)	
GVHD prophylaxis	89			
CsA	14	7 (19%)	7 (13%)	NS
CsA + MTX ± steroids	69	26 (70%)	43 (83%)	
MTX ± steroids	4	2 (5%)	2 (4%)	
Other	2	2 (5%)	0 (0%)	
Irradiation-based conditioning (TBI and/or TLI)	87			
Yes	39	17 (49%)	22 (42%)	NS
No	48	18 (51%)	30 (58%)	
Serotherapy in conditioning (ATG and/or monoclonal antibody)	88			
Yes	69	23 (64%)	46 (88%)	0.006
No	19	13 (36%)	6 (12%)	
Fludarabine in conditioning	89			
Yes	15	0 (0%)	15 (29%)	0.0003
No	74	37 (100%)	37 (71%)	
HLA generic matching /6 loci HLA-A, -B, -DR	89			
No mismatch	81	31 (84%)	50 (96%)	0.05
≥ 1 mismatch	8	6 (16%)	2 (4%)	
HLA allelic matching /10 loci HLA-A, -B, -C, -DRB1, -DQB1	89			
No mismatch	31	5 (14%)	26 (50%)	0.0004
1 mismatch (n=13), or allelic typing not available for 10 antigens (n=45)	58	32 (86%)	26 (50%)	

PNH: paroxysmal nocturnal hemoglobinuria; ATG: antithymocyte globulin; CsA: cyclosporine A; CMV: cytomegalovirus; GVHD: graft versus host disease; MTX: methotrexate, TBI: total body irradiation; TLI: total lymphoid irradiation.

Table 1, most donors (91%) were matched at the generic level (or *antigen-level*) for HLA-A, B, and DR. Based on this low-resolution typing, only eight patients received transplants from donors determined to be mismatched for one (n=7, three patients at the HLA-A and four at the HLA-B locus) or two (n=1, at the HLA-B and -DR loci) of these six antigens. At this same generic level, 56 out of 89 patient/donor pairs were typed for the ten HLA-A, -B, -C, -DR and –DQ loci, 12 of them being mismatched for at least one locus.

In 44 (49%) of the 89 recipient-donor pairs, molecular analyses of HLA-A, -B, -C, -DRB1 and -DQB1 loci were performed by high-resolution DNA-based methods prior to transplantation. Most of these recipient-donor pairs (36 out of 44) were studied in the more recent time period (1999-2004). Of those, 31 (70%) were matched for the ten antigens at the allelic level while 13 were not matched for at least one antigen. Eleven patients had one mismatch (at the HLA-A, -B, -C, -DRB1 and -DQB1 locus in one, one, six, two and one patients, respectively) and the other two had two (HLA-A and –B) and four (the two HLA-B and –C loci) mismatches. When allelic HLA typing was incomplete for at least one of the ten alleles for donor and/or recipient, the patient/donor pair was considered as HLA non-identical in the analysis. Thus, for risk factor analyses, we considered the HLA matching at the allelic 10/10 level as a binary variable, i.e. *10/10 matched pairs* (n=31) versus *10/10 mismatched or not typed at the allelic level* (n=58). In addition, confirmatory risk factor analyses only including patients fully typed at the allelic level were performed and are mentioned in the results.

Statistical methods

The primary study end-point was survival. Patient-, disease-, and treatment-related variables in the two time periods are compared in Table 1. In addition to the date of transplantation, several other continuous variables were considered as binary variables with a cut-off value fixed at the median value within the patient population (17 years for recipient age, 1 year for the interval between diagnosis and HSCT, and 2.6×10^8/kg nucleated cells for the cell dose infused). Differences in categorical variables were evaluated by χ^2 analysis. The Fisher's exact test was also used to confirm the results of χ^2 testing, when at least one factor modality was related to fewer than five individuals. Comparisons of continuous variables medians and means were performed using the Mann-Whitney's and Student's test, respectively.

In evaluation of engraftment, patients who died before day +21 without engraftment were not considered evaluable. Patients who failed to engraft were excluded from analyses of acute and chronic GVHD, which were defined using published criteria.[12,13] Probabilities of overall survival were estimated from the time of first transplantation according to the Kaplan-Meier product-limit method and compared using log rank statistics. Univariate analysis, using the competing risk method described by Fine and

Table 2. Outcome (probability ± 95% confidence interval) of HSCT from unrelated donors for SAA between 1989 and 2004, by period of transplantation.

Outcome (Competing risks except for survival)	*Year of transplant*		
	1989-1998	*1999-2004*	*p*
Graft failure (2 years)	20±7%	10±4%	NS
Grade II-IV acute GVHD (100 days)	60±9%	44±7%	NS
Grade III-IV acute GVHD (100 days)	27±8%	22±5%	NS
Chronic GVHD (2 years, limited + extensive)	32±9%	26±6%	NS
Survival (5 years)	29±7%	50±7%	0.008

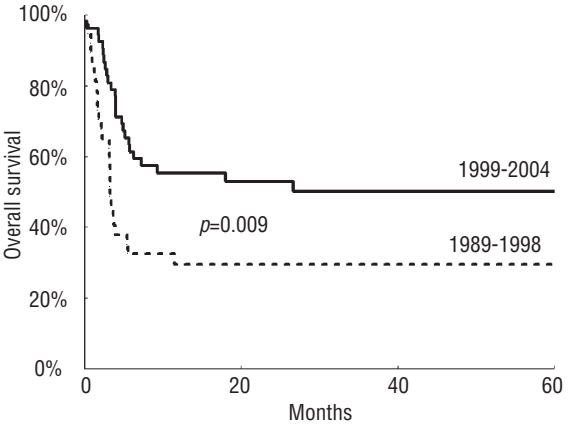

Figure 1. Probability of survival after HSCT from unrelated donors for SAA according to the period of transplantation: 1989-1998 (n=37) or 1999-2004 (n=52).

Gray,[14] was employed to assess prognostic factors for acute and chronic GVHD, with death and secondary graft failure as competing events. Only death was treated as a competing risk for graft failure in the univariate study. Cumulative incidences were compared at 100 days for acute GVHD, and at 2 years for chronic GVHD and graft failure. In multivariate regression analysis of risk factors for 5-year survival, the Cox proportional hazards model was used.

All tests were two sided and the type 1 error rate was fixed at 0.05. Statistica 7 software was used for data management and analysis by the Kaplan-Meier and Cox methods. The R Package *cmprsk*, developed by Gray, was used for competing risk analysis.

Results

Survival after unrelated HSCT has improved over time

The two time periods that best modeled changes in post-transplantation survival were 1989-1998 (n=37) and 1999-2004 (n=52). Five-year probabilities (± 95% confidence interval) of survival for the two cohorts were 29±7% and 50±7%, respectively ($p<0.01$, Figure 1). The overall 5-year probability of survival of the entire cohort was 42±5%.

Table 1 shows patient-, disease-, and transplant-related variables, including HLA matching, for the groups of patients transplanted during the two successive periods. No statistically significant differences between the two cohorts were noted for patient and donor age, etiology of acquired aplastic anemia, and mean time between diagnosis and transplantation. Transplant characteristics that differs significantly between the two periods were HLA matching and pre-transplantation conditioning, the use of serotherapy (antithymocyte globulin in all but one patient who received an anti-CD3 monoclonal antibody) and fludarabine being more frequent in the recent 1999-2004 period. Generic HLA matching by serology or low-resolution DNA typing for HLA-A, -B and –DR tended to be more frequent in the recent period ($p=0.05$), while this difference became highly significant when HLA matching was considered at the allelic high-resolution level for the ten HLA-A, -B, -C, -DRB1 and -DQB1 loci ($p=0.0004$). No differences were noted concerning the use of irradiation within the conditioning regimen, the cell dose injected, or the immunosuppressive regimen used for GVHD prophylaxis.

Engraftment and GVHD

In a competing risk analysis of the entire cohort (n=89), the cumulative incidences of graft failure, grade II-IV or grade III-IV acute GVHD, and chronic GVHD were estimated at 14±4% at 2 years, 50±5% and 24±4% at 100 days, and 28±5% at 2 years after HSCT (limited: 17±4%, extensive: 11±3%), respectively (Figure 2). Comparisons of probabilities of graft failure (primary, secondary, or both), grade II-IV or grade III-IV acute GVHD, and chronic GVHD, between the two time-periods are shown in Table 2. In contrast to overall survival, there was no significant change in the risk of these complications over time.

Risk factor analysis for survival

A univariate Cox proportional hazards regression model showed significant differences in survival by period of transplantation with a higher risk of death for the cohort transplanted in 1989-1998 (relative risk=RR [95% confidence interval], 2.12 [1.22 to 3.68]) compared with that of patients transplanted more recently (Table 3). A second multivariate model adjusting for patient-, disease-, and transplant-related variables showed this same association between the earlier time-period of transplantation and lower survival, with a higher statistical significance ($p<0.0001$, Table 3). The only other two factors found to be significantly deleterious for survival were age over 17 years ($p<0.01$) and the absence of serotherapy within the conditioning regimen ($p=0.04$). In a third model, adjustment for the same variables but also for the HLA matching at the allelic level for ten antigens reduced the impact of the time-period under the level of significance ($p=0.08$) in favor of the HLA matching ($p<0.01$). In this last model, the negative impact of older age (over 17 years) was exemplified, remaining the only other prognostic factor together with

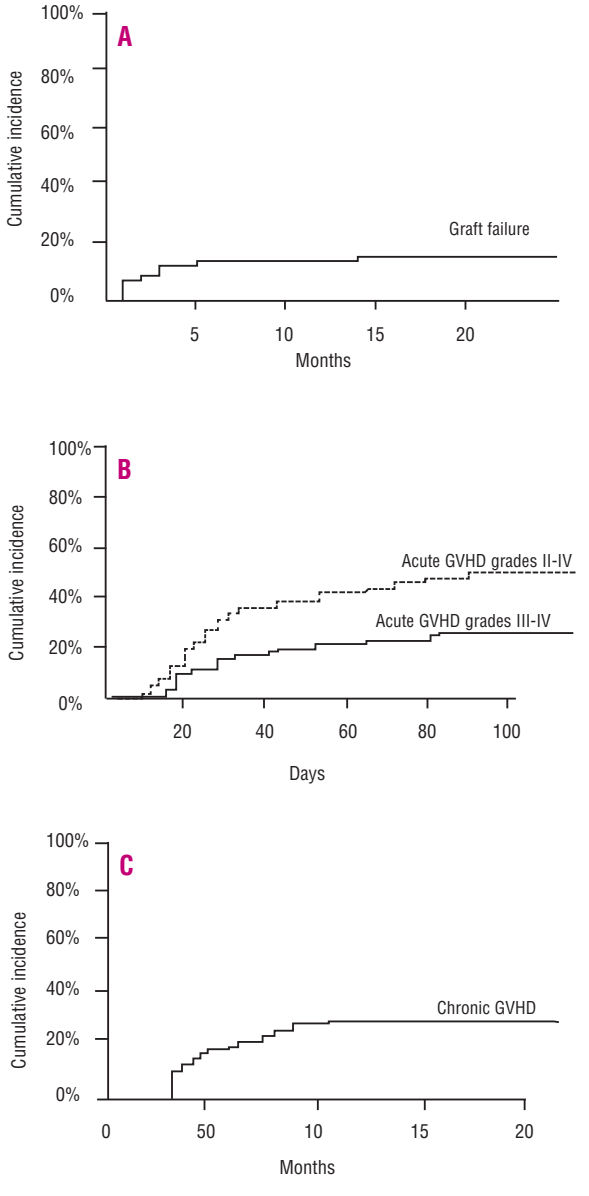

Figure 2. Cumulative incidences of (A) graft failure, (B) acute GVHD, and (C) chronic GVHD after HSCT from unrelated donors for SAA between 1989 and 2004.

Table 3. Relative risk of death after HSCT from an unrelated donor for SAA between 1989 and 2004.

Risk factors	*No adjustment*		*Adjusted for related variables*		*Adjusted for related variables including HLA matching*	
	Relative Risk	*95% CI*	*Relative Risk*	*95% CI*	*Relative Risk*	*95% CI*
Year of transplant before or in 1998	2.12[b]	1.22-3.68	3.95[c]	2.06-7.58	1.80	0.91-3.53
Patient age >17 years			2.92[b]	1.53-5.57	5.14[c]	2.53-10.41
Sex mismatch			1.17	0.67-2.06	1.48	0.82-2.65
Interval diagnosis-HSCT < 1 year			1.13	0.62-2.06	2.16[a]	1.11-4.18
Cell dose ≤2.6×10^8 nucleated cells/kg			1.00	0.99-1.01	1.00	0.99-1.01
Irradiation-based conditioning			1.00	0.98-1.03	1.01	0.99-1.03
No serotherapy in conditioning			2.02[a]	1.07-3.82	1.23	0.63-2.43
No fludarabine in conditioning			1.17	0.49-2.81	1.55	0.64-3.75
HLA allelic matching /10 loci					2.91[b]	1.37-6.20

[a]$p<0.05$; [b]$p<0.01$; [c]$p<0.0001$.

HLA matching. Overall, the survival rate reached 55±9% for patients fully HLA-matched with their donor at the allelic level for 10/10 antigens, and even 78±11% for those of them younger than 17 years (n=14). Of note, all the risk factors for survival identified in multivariate analyses were also found to be significant in univariate analyses (*data not shown*). Furthermore, the impact of HLA matching was also found to be significant if patients not fully typed for the ten antigens at the allelic level were excluded from analysis, thus performed on 44 donor/recipient pairs (p=0.01). Of note, when only eight antigens were considered for the definition of HLA matching, the allelic matching for HLA-A, -B, -C and -DRB1 alleles was associated with significantly improved survival (p=0.05 when including patient/donor pairs with incomplete allelic typing for at least one of the eight alleles, then considered as HLA-non-identical, and p=0.03 when considering only fully typed patients/donors, n=45). In contrast, when HLA-A, -B, -DRB1 and -DQB1, but not HLA-C, were considered for the definition of HLA allelic matching, no impact on overall survival was found.

Causes of death

GVHD, fungal infections, interstitial pneumonia and secondary malignancies - mostly Epstein-Barr virus (EBV)-related lymphoproliferative disorders (six out of eight cases) - were the primary causes of death. However, as shown in Table 4, causes of deaths were not significantly different between the two time-periods, even though microbiologically documented fatal infections - especially of fungal origin - were less frequent in the recent period.

Risk factor analysis for engraftment and GVHD

We further aimed to explain in what manner better HLA matching was associated with improved survival over time. We thus conducted a univariate competing risk analysis for graft failure, acute grade III-IV GVHD and chronic GVHD (limited or extensive). The impact of HLA allelic matching for ten loci was found to be a protective factor against graft failure (p=0.05), but not against acute or chronic GVHD. When considering only eight alleles for the risk of graft failure, the same discordance as for survival was observed with the combination of HLA-A, -B, -C and -DRB1 loci (p=0.03), in comparison with the other combination of HLA-A, -B, -DRB1 and -DQB1 (p=NS). At the allelic level, HLA matching for ten loci had no impact on

Table 4. Causes of death after HSCT from unrelated donors for SAA between 1989 and 2004, by period of transplantation.

	Total	Year of transplant 1989-1998 n=37	Year of transplant 1999-2004 n=52	p value
Documented infection	12	8 (22%)	4 (8%)	NS
Fungal	9	6 (16%)	3 (6%)	NS
Bacterial	1	1 (3%)	0 (0%)	NS
Viral	2	1 (3%)	1 (2%)	NS
GVHD	11	5 (14%)	6 (12%)	
Acute grade III-IV	8	4 (11%)	4 (8%)	NS
Chronic	3	1 (3%)	2 (4%)	NS
Interstitial pneumonia	8	3 (8%)	5 (10%)	NS
Secondary malignancy	8	4 (11%)	4 (8%)	NS
Rejection/poor graft function	2	1 (3%)	1 (2%)	NS
Multi-organ failure	5	1 (3%)	4 (8%)	NS
Other	5	4 (11%)	1 (2%)	NS

the occurrence of acute or chronic GVHD. However, considering a larger number of patient/donor pairs typed at the generic low-resolution level for the ten loci (n=56 versus 49 at the allelic level), the risk of acute grade III-IV GVHD was significantly higher when at least one mismatch existed (p=0.03). Here again, this impact was also found regarding the eight HLA-A, -B, -C and -DR loci (p=0.02), but not the HLA-A, -B, -DR and -DQ combination (p=NS). In addition to HLA matching, other factors found to affect the risk of graft failure were older age of the recipient (>17 years at transplantation, p=0.03) and a low cell-dose received (< 2.6×10^8/kg nucleated cells, p=0.005). For acute grade III-IV GVHD, the other risk factor identified was the absence of serotherapy within the conditioning regimen (p=0.02), while older age of the recipient (p=0.02) and the absence of fludarabine within the conditioning regimen (p=0.02) were associated with the development of chronic GVHD. Of note, the interval between diagnosis and HSCT did not have a significant impact on graft failure, acute or chronic GVHD, or survival.

Discussion

There are two major conclusions of this study. First, the survival rate after transplantation from an unrelated donor improved from 29% in 1989-1998 to 50% in 1999-2004. This is consistent with previous results from different collaborative studies which reported low overall long term survival of 36% to 39% in the periods 1988-1995[15] and 1988-1998[9], and better results with up to 56% and 55% long-term survival rates in the more recent 1993-2000[11] and 1994-2004[10] periods, respectively. In this latter study,[10] a 7-year survival rate of 61% was reported for donor/patient pairs fully HLA-matched at the allelic level for 10/10 loci. For these fully matched patients, we observed a similar survival rate of 55% and even of 78% among recipients younger than 17 years. These results can be compared with survival rates described after transplants from HLA-identical sibling donors in young patients who received a course of immunosuppressive therapy before HSCT,[16] as was the case in almost all of the patients in the present series. Second, only transplant-related, but not patient- and disease-related variables changed over time. Among those, HLA matching at the allelic level was the only one found to affect survival in multivariate analyses, which makes it mostly responsible for the improvement in survival observed over time. Indeed, in risk factor analyses for survival, the addition of HLA matching in the multivariate model lowered the prognostic value of the transplantation period and became the only variable influencing outcome significantly, together with recipient age. Together with the study of Deeg *et al*.,[10] our study is the first to incorporate HLA typing at the allelic level for the ten class I and class II loci in the analysis of outcome. Of note, while we found that HLA matching at the allelic level had an important prognostic value for survival, it had no impact in multivariate analysis when considered at the generic level for the six HLA-A, -B and –DR antigens. Although these data should be considered with caution given the small number of patient/donor pairs not fully matched according to these criteria (8 out of 89), they highlight the importance of considering HLA matching at the allelic rather than the generic level in risk factor analyses. In the study by Deeg *et al*.,[10] as in ours, 10/10 allelic HLA matching and young recipient age (cut-off value of 20 years in the study by Deeg *et al*. versus 17 years in ours) were identified as the two major factors associated with better survival, in a very similar number of patients (87 in the study by Deeg *et al*. versus 89 in ours). With regard to these two major prognostic factors identified in multivariate analyses, one may conclude that a certain degree of mismatching could be tolerated in children, but not in adults. In that sense, we further compared survival of adults (>17 years, n=24) vs. children (<17 years, n=34) in the group of patients who were not 10/10 matched or not typed at the allelic level (n=58) and still observed a significant advantage for children (p=0.003, *data not shown*). As a practical consequence for decision making, this would reinforce the fact that a certain degree of mismatching could be tolerated in younger patients with severe disease. Kojima *et al*. also showed that allelic mismatching of HLA-A and –B antigens, but not of HLA-DRB1, was the most crucial risk factor for survival of patients with SAA who received transplants from unrelated donors. We extended these results from the six HLA-A, -B and –DRB1 alleles to the 10/10 level. Even though not conducting a *locus per locus* prognostic analysis, we observed that matching for the HLA-A, -B, -C and -DRB1 loci had a significant prognostic value for graft failure, acute GVHD and survival while this was not the case when considering the HLA-A, -B, -DRB1 and -DQB1 combination of

eight alleles. This might suggest that matching of HLA-C could be of particular importance and underlines the fact that matching of HLA-C and -DQB1 should be incorporated into algorithms for unrelated donor selection,[17] notably for SAA patients.[18]

Although we did not observe significant changes in the incidence of graft failure and GVHD between the two time-periods, the better HLA matching was found to be a protective factor against these transplant-related complications. However, other factors such as the low cell-dose received, because of the risk of graft failure, and the absence of serotherapy within the conditioning regimen, because of the risk of acute GVHD, were identified, as in previous reports.[19,20] Even in the recent years, the problem of acute and chronic GVHD persists since this remains a frequent complication and one of the main causes of death. Of note, the use of fludarabine within the conditioning regimen was one of the only three variables found to be significantly different between the two time periods, together with the use of antithymocyte globulin and HLA matching. Even though not affecting survival, the use of fludarabine was associated with a lower incidence of chronic GVHD. This might reinforce the exploration of fludarabine-based conditioning regimens for alternative donor transplants in acquired SAA, which provided an encouraging 2-year survival rate of 73% in a recent series of the EBMT.[21] Theoretically, the use of fludarabine might constitute an alternative to TBI to overcome graft rejection, as recently suggested in the unrelated donor setting,[21] and even for patients receiving HLA-identical sibling HSCT who are heavily transfused and allo-immunized.[22] We found no effect of the use or not of irradiation within the conditioning regimen on any outcome endpoint in this study. However, in view of the good results recently reported by the NMDP,[10] the use of low-dose TBI is probably of interest in the unrelated donor setting while, in the setting of HLA-identical related donors, several studies indicate that irradiation-based conditioning regimens should be avoided.[16,23] Altogether, these findings emphasize the need for further studies specifically evaluating the role of conditioning regimens in the setting of transplants from unrelated donors. Other factors not taken into account in that study may have contributed to the improved outcomes. Possibilities include better transfusion support both pre-transplantation and post-transplantation, the systematic use of leukocyte-depleted blood products[24] and other measures such as cotrimoxazole prophylaxis and intravenous immune globulins, which were strongly associated with improved survival in previous reports.[25] As recently reported for leukemia patients,[26] the use of new antibacterial and antifungal drugs may have played a role since we observed that microbiologically documented fatal infections, especially of fungal origin, were less frequent in the recent 1999-2004 period, although not statistically significantly so. The six patients who died of EBV-related lymphoproliferative disorders, who all received antithymocyte globulin, confirm the significant risk of this complication when using antithymocyte globulin in the setting of unrelated HSCT. Serial monitoring of EBV-DNA levels and early use of rituximab[27] may reduce this incidence in the near future.

In contrast to earlier reports,[28,29] the interval between diagnosis and HSCT had no significant impact on transplant outcome. This result should certainly be considered with caution given the numbers of patients, but was also observed in other recent studies,[9,10] and may be related to earlier referral of patients for transplantation. It is also consistent with the recommendation of performing transplantation from unrelated donors only after failure of a first course of immunosuppressive therapy rather than upfront at the time of diagnosis, in keeping with current guidelines.[30]

In summary, from the French experience, survival after unrelated transplantation for SAA has improved over the past 15 years. The data we reviewed suggest that HLA matching at the allelic level was an important factor in this progress. Results for young patients who are fully HLA-matched at the allelic level with their donor are comparable to those observed after related HSCT. Given these results, transplantation from an unrelated donor should be recommended as a salvage therapy for children and young adults who do not respond to immunosuppressive therapy. In the older population, even though results seem to have improved over time, the outcome after unrelated HSCT for SAA remains quite poor. This underscores that this approach should be further compared with alternative modalities of HSCT, or non-transplant strategies such as supportive care or repetition of immunosuppressive treatments.

Authors' Contributions

SM co-designed the study, enrolled patients, analyzed results, and wrote the manuscript; MLB-A collected and verified HLA data and the degree of matching, and performed the statistical analysis; ZC collected clinical data from the SFGM-TC registry; JMB enrolled patients, contributed to data registration and quality control; CG enrolled patients, contributed to data registration and quality control; KY enrolled patients, contributed to data registration and quality control; PB enrolled patients, contributed to data registration and quality control; AM-C enrolled patients, contributed to data registration and quality control; NMiL enrolled patients, contributed to data registration and quality control; JK enrolled patients, contributed to data registration and quality control; NMaiL enrolled patients, contributed to data registration and quality control; GS co-designed the study, enrolled patients, analyzed results and provided comments on the manuscript.

Conflict of Interest

The authors reported no potential conflicts of interest.

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