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Reducing transplant-related mortality after allogeneic hematopoietic stem cell transplantation

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A B S T R A C T

Background and Objectives. Transplant-related mortality (TRM) following allogeneic hematopoietic stem cell transplantation (HSCT) has been reported to be related to disease stage, duratiion of disease and type of donor. Furthermore, the outcome of transplants performed in the 1990s appears to be better than that of transplants done in the previous decade. The aims of this study were to determine whether these relationships still hold and whether the outcome of transplants is continuing to improve.

Design and Methods. We analyzed 1180 consecutive patients with leukemia (n=979) or other hematologic malignancies (n=201) undergoing HSCT in 4 time periods: before 1990, 1991-1995, 1996-2000, and 2001-2002. Changes during these eras include increasing patient age, more unrelated transplants, more patients with advanced disease, different graft-versus-host disease (GvHD) prophylaxis, and different management of infections.

Results. The actuarial 2-year transplant-related mortality (TRM) differed significantly between the transplant eras ($p<0.001$) with a significant interaction with disease phase ($p=0.018$). In patients in first remission (n=585) TRM was 34%, 25%, 21% and 6% in the four transplant eras. The reduction in TRM was less evident in patients in second remission (n=284) (37%, 35%, 30%, 25%) and absent in relapsed patients (n=311) (TRM=45%, 41%, 29%, 51%). This is a consequence of reductions in GvHD, infections and multiorgan failure among patients in remission but not among those who relapse. The actuarial 2-year survival has improved significantly in patients in first remission (54%, 66%, 72%, 78%) but not in those in second remission (38%, 46%, 52%,45%), or relapsed patients (31%, 25%, 36%, 21%).

Interpretation and Conclusions. In conclusion, TRM has been significantly reduced in first remission patients, suggesting an allograft should be considered in this phase, when appropriate, without delay. There has been no improvement in survival for patients beyond first remission, due to persisting high risk of infections and organ toxicity, a possible consequence of prolonged pre-transplant chemotherapy and neutropenia.

Key words: transplant related mortality, allogeneic HSCT.

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Transplant-related mortality (TRM) is a major concern for patients who are eligible for an allogeneic hematopoietic stem cell transplant (HSCT), and for hematologists who refer patients to a transplant center. Potentially fatal complications include graft-versus-host disease (GvHD) in both its acute and chronic form, infections, and organ failure, such as veno occlusive disease and interstitial pneumonia: all of these are inter-connected and it is often difficult to identify primary causes of death.

A typical example is a patient with acute GvHD, who is treated with steroids, develops a cytomegalovirus (CMV) infection, and then interstitial pneumonia: although many investigators would question the primary cause of death, they would still agree that the patient died of a transplant-related complication. When a fatal complication occurs in the absence of the underlying disease it is referred to as TRM. The rate of TRM has been reported to be higher in patients with advanced disease, in those with disease of long duration, and among patients whose donors were not HLA identical siblings.[1] Age is an additional powerful predictor of transplant mortality:[2,3] if the risk of TRM is 1 in patients less than 20 years old, it is 1.5 in the 20-40-year old group, and 2.0 in the over 40-year old ones.

Thus, there are well established risk factors which can be used to calculate the transplant risk for a given patient.3 The question is: have things changed over the years, and is the predictive effect of these variables always the same? Some studies have shown that transplants in the nineties have a better outcome than those performed in the eighties.[4] It would be valuable to know whether this improvement is ongoing, also in the most recent years.

Table 1. Clinical data of 1180 patients according to the transplant era.

	<=1990	*1991-95*	*1996-2000*	*2001-02*	*P*
Number	373	277	367	163	
Alternative donors (%)	3.5	15	41	50	<0.0001
Median donor age (years)	27	35	39	40	<0.0001
Median recipient age (years)	27	35	38	39	<0.0001
Patients over 50 years (%)	0	4	11	21	<0.0001
Median Interval					
Diagnosis- HSCT (days)	336	306	481	426	<0.0001
Diagnosis: leukemia (%)	94	83	77	71	<0.0001
First complete remission (%)	53	56	46	38	<0.000
Conditioning regimen (%)					
CY-TBI	92	64	53	51	
BU-CY	8	24	3	4	
THIO-CY	0	12	40	37	
Other	0	0	4	8	<0.0001
Intensity of the conditioning					
Reduced intensity (%)	0	1	15	30	0.0001
GvHD prophylaxis (%)					
MTX	7	0	0	0	
CyA	62	17	0	0	
CyA+MTX	15	63	98	98	
In vivo TCD	1	1	1	27	<0.0001
Ex vivo TCD	15	19	1	2**	
Stem cell source: BM(%)	99	100	84	85	<0.0001
Median stem cell dose					
BM ($\times 10^8$/kg)	2.3	4.6	4.4	4.1	<0.000
PB ($\times 10^8$/kg)	–	13.2	11.2	7.3	<0.000

*CY: cyclophosphamide; TBI: total body irradiation; BU: busulfan; THIO: thiotepa; MTX: methotrexate; CyA: Cyclosporin; TCD: T cell depletion; BM: bone marrow; PB: peripheral blood. ** the numbers don't add up because some patients had more than one GvHD prophylaxis procedure.*

To answer this question question we studied 1180 patients with hematologic malignancies, who underwent an allogeneic HSCT in our Unit at San Martino Hospital in Genova between 1976 and 2002. We looked at known prognostic variables such as age, phase of the disease and donor type, but we also looked at changes in transplant protocols and TRM over 4 defined time periods. The last patient entered in the analysis was grafted in December 2002, so this allows for a minimum follow-up of 1 year for surviving patients.

Design and Methods

Patients

The patients' characteristics are presented in Table 1, divided according to period in which the patients were transplanted. These were consecutive allogeneic transplants performed between 1976 and 2002, for patients with hematologic malignancies. The diagnoses were acute leukemia (AL) (n=549), chronic myeloid leukemia (CML) (n=430), myelodysplasia (MDS) (n=90), lymphoproliferative disorders (LPD) (n=70) and other (n=41). There were 585 patients in first remission/chronic phase (1st CR), 284 in second remission (including accelerated phase CML) and 311 patients with more advanced disease (17 had leukemia in 3rd CR, 27 had blast crisis of CML and the remaining had evidence of active disease at the time of transplant).

Conditioning regimens

The major conditioning regimens are also outlined in Table 1. Patients received either cyclophosphamide 60 mg/kg/day×2 and total body irradiation (10-12 Gy in fractionated doses),[5] or cyclophosphamide combined with busulfan or thiotepa, as described elsewhere.[6,7]

Stem cell source and harvesting

Bone marrow (BM) was the source of stem cells for the majority of patients (90%); peripheral blood (PB) stem cells were given to the remaining 10% of patients. BM was harvested under general anesthesia from theposterior iliac crests. Following a randomized

study which showed that small volume aspirations yielded significantly greater numbers of cells and colony-forming units (CFU),[8] we have been more aware of harvest procedures and we have, in fact, doubled the BM cell dose from 2.2-2.4×10⁸/kg in the 1980s to 4.6×10⁸/kg in the 1990s (Table 1). The number of PB stem cells infused from harvests obtained by priming with granulocyte colony-stimulating factor (G-CSF) remained stable in the early and late 1990s (13.2 and 11.2×10⁸/kg, respectively).

GvHD prophylaxis

Five patients received syngeneic grafts and were given no GVHD prophylaxis. Methotrexate alone was given to 31 patients, cyclosporine A alone was given to 281, a combination of methotrexate and cyclosporine A to 754 and *ex vivo* T-cell depletion was used for 109. Table 1 outlines the proportions of patients receiving these regimens in the different transplant periods. *Ex vivo* T-cell depletion was performed with Campath 1G (kindly provided by H Waldman and J Hale, Cambridge, UK). A number of patients receiving unrelated or family mismatched allografts received rabbit antithymocyte globulin (Thymoglobuline, Sangstat, Lyon, France) in the conditioning regimen, at doses ranging from 7.5 to 15 mg/kg as described elsewhere.

CMV monitoring and pre-emptive therapy

Patients were monitored for CMV antigenemia as of 1/9/1991 (UPN 590). Patients received foscarnet or ganciclovir as a single agent until patient UPN 730 (19/6/1993). Following the study in which we showed increased mortality among patients with high numbers of CMVAg-positive cells, as of UPN 731 (24/6/93) patients with 1-4 CMVAg-positive cells have been treated with single agent therapy (foscarnet or ganciclovir) and patients with more than 4 CMVAg-positive cells have been treated with a combination of foscarnet + ganciclovir.[10]

Intravenous immunoglobulin

Patients were given high dose intravenous immunoglobulin G (IgG). In different time periods we compared 400 mg/kg/week of non-specific IgG with 200 mg/kg/week of CMV-specific IgG.[11] A second trial was run in HLA identical siblings, comparing 400 vs 100 mg non-specific IgG (*unpublished data*). A third study was run in alternative donor transplants with 400 mg/kg/week of non-specific IgG vs 200 mg/kg/week of IgG enriched with anti-endotoxin IgM (*unpublished data*). None of these studies showed significant differences in the two treatment arms. We are currently running a study of 100 mg/kg/week IgG vs no IgG.

Gut decontamination

Gut decontamination was achieved with oral neomycin and colimycin in the 1980s and with quinolones in the 1990s. We are currently using ciprofloxacin orally unless the patient becomes febrile.

At that point the patient is treated with intravenous antibiotics (an aminoglycoside and a cephalosporin would be first choice) and vancomycin would be added after 3 days if the patient does not become afebrile.

Antifungal prophylaxis-therapy

Oral nystatin and then mepartricin (an absorbable polyene) were used until the advent of fluconazole, which is now standard fungal prophylaxis until day +75. Secondary prophylaxis with amphotericin or voriconazole was given if the patient came to tran plantation with a known history of *Aspergillus* infection.

Statistical analysis

The patients' data were collected prospectively and updated at each outpatient attendance. The data were analyzed with the SPSS 12 package. All the comparisons between transplant periods were carried out using the χ^2 test for categorical variables and the non-parametric Mann-Whitney test for continuous variables.

The end-point for survival analyses were death without relapse (transplant related mortality, TRM), xdeath due to relapse (relapse-related death, RRD) or death due to any cause. The survival curves for TRM and RRD were estimated using the cumulative incidence accounting for the fact that these endpoints are competing causes of death. Univariate and multivariate survival analyses were carried out using the Cox proportional hazard model; the interaction analysis was carried out including all the variables found to be significant in the multivariate analysis.

Results

Differences in the 4 transplant periods

All the variables studied were significantly different between the 4 transplant periods (Table 1). Thus, over the period studied, there were changes in the type of disease treated, an increasing proportion of patients with advanced disease, increases in the ages of both patients and donor, increasing use of alternative donors, decreasing use of TBI, different GVHD prophylaxis, decreasing use of T-cell depletion and increasing doses of BM cells (Table 1). As to the *diagnoses,* there was a significant increase in the percentage of patients with diagnoses other than leukemia (lymphoma, myeloma, myelodysplasia) from 6% to 29%

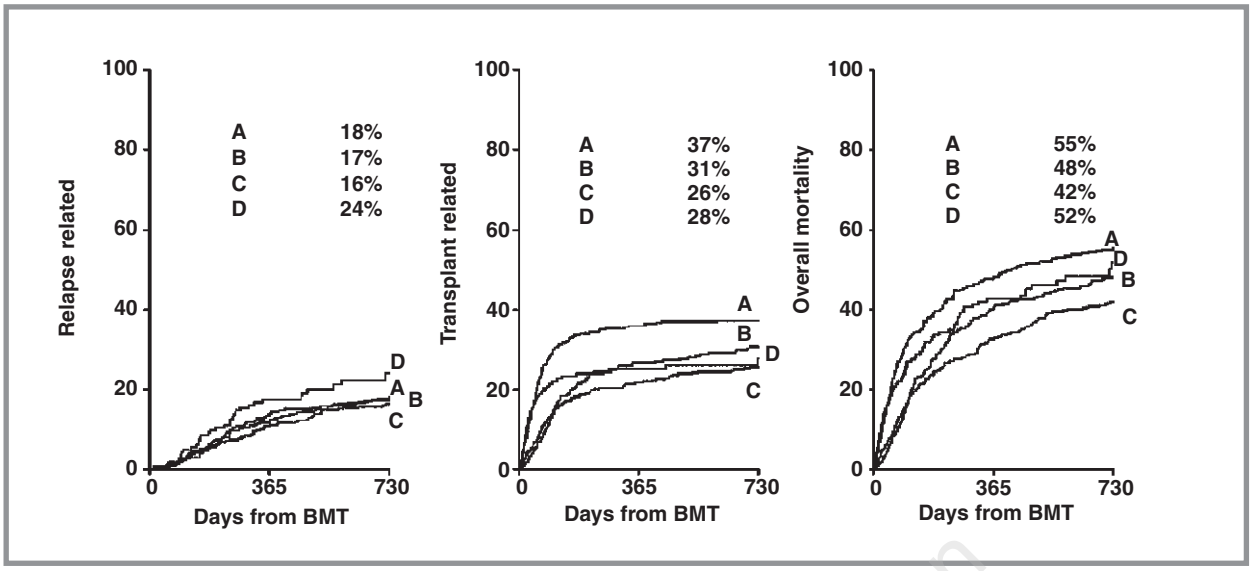

Figure 1. Overall cumulative incidence of relapse-related death (left) , transplant related-mortality (center) and overall mortality (right) in the four transplant periods : A: up to 1990, B: 1991-95; C: 1996-2000; D: 2001-02.

(p<0.0001). The age of recipients increased in each transplant period with a net increment of 12 years between the first and the last transplant period (p<0.0001). The upper age limit in the 4 periods was 50, 55, 66 and 65 years. Similarly the proportion of patients over 50 years in the 4 periods was 0%, 4, 11%, 21% (p<0.0001). As to the donor type, the use of alternative donor grafts increased across the 4 periods (3.5%, 15%, 41%, 50%), due to an increase of one antigen mismatched family donors (4% to 15%) and unrelated donors (1% to 36%). The *conditioning regimen* changed, with a decrease in the use of TBI. *Ex vivo T-cell depletion* was used in approximately 20% of the patients in the 1980s and early 1990s. In our Unit we have been using *in vivo T-cell depletion*, namely rabbit ATG, for alternative donor transplants: ATG was given to 5% of patients receiving a transplant from an alternative donor in the period 1991-95, to 71% in the period 1996-2000, and to 100% in the period 2001-2002. As to post-transplant GVHD prophylaxis, most patients currently receive the standard cyclosporine and methotrexate regimen (Table 1).

Transplant-related mortality, relapse-related death and overall mortality

Figure 1 illustrates the cumulative incidence of TRM, RRD and overall mortality at 2 years for all patients, divided according to transplant period. TRM has decreased in the most recent years, whereas RRD is at best unchanged. The overall mortality has decreased.

Univariate and multivariate analyses on TRM

The results of univariate and multivariate Cox analyses on TRM are listed in Table 2. In univariate analysis the significant predictors of TRM were transplant period, donor age, recipient age, cell dose, the interval between diagnosis and transplant, disease phase, donor type, diagnosis. In multivariate analysis significant predictors remained transplant period, recipient age, disease phase, cell dose and donor type.

Interaction analysis

All the variables with a significant impact on TRM (that is, donor and recipient age, disease phase, donor type and cell dose) were also analyzed for interactions with transplant periods. The only variable with a significant interaction with the transplant period was the disease phase (p for interaction=0.018), indicating that the change of TRM across transplant periods was different between disease phases. Therefore the cumulative incidence of TRM was constructed for patients allografted in first CR, second CR and beyond second CR (Figure 2): this shows a significant reduction of TRM in first CR patients, but not in those in second CR or relapsed patients.

Univariate and multivariate analyses on RRD

The results of univariate and multivariate COX analyses on TRM are presented in Table 3. In univariate analysis significant predictors of TRM were transplant period and disease phase. In multivariate analysis significant predictors were disease phase and stem cell source. There was no interaction with transplant period.

Causes of death

We then looked at causes of death. Leukemia has remained stable as a cause of death between 16% and 23% of patients (Table 4). Deaths related to acute GVHD

Table 2. Univariate and multivariate Cox analysis for transplant-related mortality.

	Univariate HR (95% Conf Int)	*p*	*Multivariate HR (95% Conf Int)*	*p*
Transplant period				
Up to 1990	1		1	
1990-1995	0.76 (0.59-0.98)	0.001	0.56 (0.41-0.77)	<0.001
1995-2000	0.61 (0.47-0.79)		0.30 (0.21-0.43)	
2001-2002	0.72 (0.51-1.01)		0.32 (0.20-0.51)	
Donor sex				0.46
Male	1	0.29	1	
Female	0.90 (0.74-1.10)		1.08 (0.88-1.33)	
Donor age	1.012 (1.004-1.019)	0.002	1.010 (1.000-1.020)	0.05
Recipient sex				
Male	1	0.08	1	0.27
Female	0.83 (0.68-1.02)		0.89 (0.71-1.09)	
Recipient age	1.008 (1.000-1.017)	0.05	1.02 (1.01-1.04)	<0.001
Cell dose	0.90 (0.87-0.95)	<0.001	0.92 (0.87-0.98)	0.01
Interval diagnosis-transplant (years)	1.04 (1.00-1.08)	0.98 0.05	(0.93-1.03)	0.45
Disease phase				
1st remission	1		1	
2nd remission	1.27 (0.98-1.63)	<0.001	1.16 (0.88-1.54)	0.006
beyond 2nd remission	2.01 (1.60-2.52)		1.60 (1.20-2.12)	
Donor				
HLA id.sibling	1	<0.001	1	<0.001
Alternative	1.91 (1.55-2.36)		3.17 (2.34-4.30)	
Source				
BM	1	0.28	1	0.52
PB	1.24 (0.85-1.80)		1.21 (0.69-2.12)	
Diagnosis				
Leukemia	1	0.009	1	0.08
Other	1.39 (1.09-1.78)		1.34 (0.97-1.85)	

HR: hazard ratio. For all variables the reference condition, assigned a risk of 1, is in bold.

have dropped from 20% to 5% , whereas those related to chronic GVHD have remained stable between 2% and 4%. The rate of fatal interstitial pneumonia peaked in the 1991-95 period at 12%, and is currently down at 1%. The incidence of post-transplant infections has actually tended to increase over the 4 periods. Second tumors were the cause of death in 9 patients in transplanted before 1990, and in 1 patient transplanted in each of the other periods. Figure 3 illustrates the 4 major causes of transplant-related deaths (acute GvHD, Infections, interstitial pneumonia and multiorgan failure in patients stratified according to phase of disease at transplant. In patients transplanted in first CR there has been substantial reductions in GVHD, interstitial pneumonia and multiorgan failure, but not in infections. In patients beyond first CR there has been a reduction in interstitial pneumonia as a cause of death, but not in GvHD, infections and multiorgan failure. Currently a patient not in first CR has a 30% risk of dying of infections or multiorgan failure compared with the 8% risk in a patient in first CR, irrespective of donor type.

Graft versus host disease

Protocols for GVHD prophylaxis have changed significantly over the years: Cyclosporine A was used mainly as a single drug in the first 2 transplant periods but is now used together with methotrexate (Table 1). Despite the decreased use of *ex vivo* T-cell-depletion, the risk of developing grade III-IV GVHD decreased in the 4 transplant periods as follows: 17%, 8%, 7%, 2% in recipients of HLA identical sibling grafts and 44%, 25%, 19%, 7% in those grafted from an alternative donor (Figure 4). The figures for chronic GVHD are 27%, 36%, 38%, 28% in recipients of

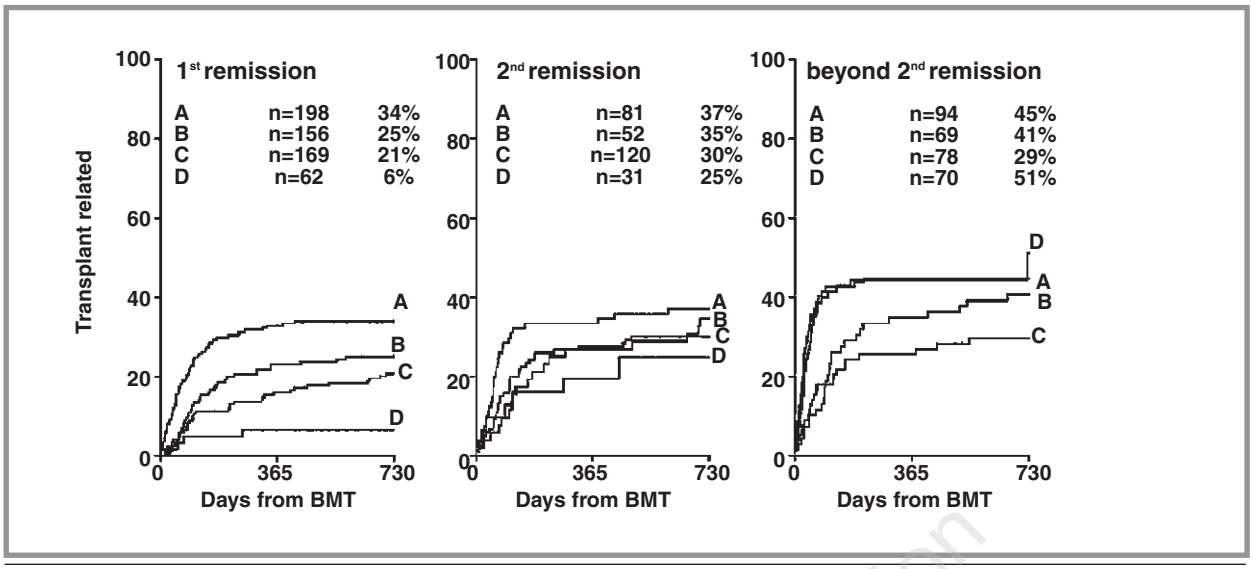

Figure 2. Cumulative incidence of transplant-related mortality in patients allografted in first complete remission/chronic phase (left), in second remission (middle) and with more advanced disease (> 2nd CR) (right). The four curves A, B, C, D represent cumulative incidence of TRM at 2 years in the four transplant periods. There is a significant reduction of TRM in first CR but not in patients transplanted with more advanced disease.

Table 3. Univariate and multivariate Cox analysis for relapse-related mortality.

	Univariate *HR (95% Conf Int)*	*Multivariate* *p*	*HR (95%Conf Int)*	*p*
Transplant period				
Up to 1990	1		1	
1990-1995	0.77 (0.56-1.08)	0.03	0.68 (0.46-1.01)	0.053
1996-2000	0.70 (0.51-0.95)		0.64 (0.41-0.99)	
2001-2002	1.15 (0.77-1.72)		1.09 (0.61-1.95)	
Donor sex				
Male	1	0.19	1	0.22
Female	1.17 (0.92-1.50)		1.18 (0.91-1.52)	
Donor age	0.99 (0.98-1.01)	0.68	1.005 (0.991-1.020)	0.48
Recipient sex				
Male	1	0.08	1	0.59
Female	0.83 (0.68-1.02)		1.07(0.83-1.40)	
Recipient age	0.97 (0.76-1.24)	0.80	1.001 (0.983-1.018)	0.94
Cell dose	1.026 (0.99-1.06)	0.13	1.096 (1.036-1.160)	0.001
Interval diagnosis-transplant (years)	0.99 (0.93-1.05)	0.75	0.91 (0.85-0.97)	0.008
Disease phase				
1st remission	1		1	
2nd remission	2.52 (1.85-3.45)	<0.001	3.29 (2.32-4.67)	<0.001
beyond 2nd remission	4.32 (3.22-5.81)		6.19 (4.37-8.78)	
Donor				
HLA id.sibling	1	0.14	1	0.10
Alternative	0.77 (0.55-1.09)		0.68 (0.44-1.07)	
Source				
BM	1(ref)	0.20	1	0.02
PB	1.29 (0.87-1.90)		0.46 (0.24-0.88)	
Diagnosis				
Leukemia	1	0.10	1	0.07
Other	1.31 (0.95-1.80)		0.68 (0.45-1.03)	

Abbreviations and variables as in Table 2.

Table 4. Proportion of patients alive and causes of death in different transplant eras.

	Year of transplant				
Cause	*<1990*	*1991-95*	*1996-00*	*2001-02*	*Total*
alive (n)	126	120	193	86	525
(%)	33.8	43.3	52.6	52.8	44.5
leukemia (n)	89	50	60	30	229
(%)	23.9	18.1	16.3	18.4	19.4
acute GvHD (n)	73	13	30	9	125
(%)	19.6	4.7	8.2	5.5	10.6
infections (n)	23	33	41	21	118
(%)	6.2	11.9	11.2	12.9	10.0
sec.tumors (n)	9	1	1	1	12
(%)	2.4	0.4	0.3	0.6	1.0
chronic GvHD (n)	12	9	15	3	39
(%)	3.2	3.2	4.1	1.8	3.3
Interstitial pneumonia (n)	17	33	12	1	63
(%)	4.6	11.9	3.3	0.6	5.3
MOF (n)	24	18	15	12	69
(%)	6.4	6.5	4.1	7.4	5.8
Total	373	277	367	163	1180

GvHD: graft vs host disease; Int.Pneumonia: interstitial pneumonia; sec.tumors: secondary tumors; MOF: multiple organ failure.

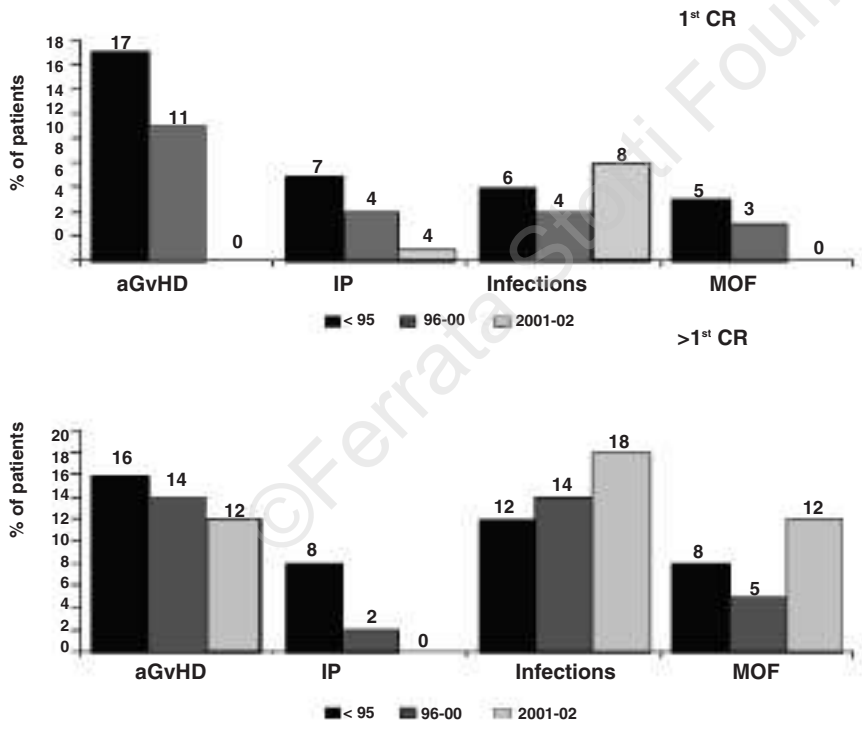

Figure 3. Four major causes of transplant related death in patients allografted from alternative donors: graft vs host disease (GVHD), interstitial pneumonia (IP), infections and multi organ failure (MOF). In patients allografted in 1st CR (upper part of the graph) deaths from acute GVHD, IP and MOF have almost been eliminated while 8% of patients still die of infections. However in patients allografted in more advanced disease (>1st CR) this reduction is not as evident.

HLA identical sibling grafts and 20%, 68%, 40%, 21% in those grafted from alternative donors.

Survival

Survival has improved overall in the 4 periods: for patients in first CR the actuarial 2-year survival is currently 81% and 71% for patients grafted from an identical sibling or an alternative donor, respectively. The corresponding TRM is 3% and 15%. Finally we looked at differences in actuarial survival for patients grafted in 1st CR or more advanced phase in the four transplant periods (Figure 5): the difference increases over time, because patients in first CR are doing progressively better, while the survival of patients with more advanced disease has remained low.

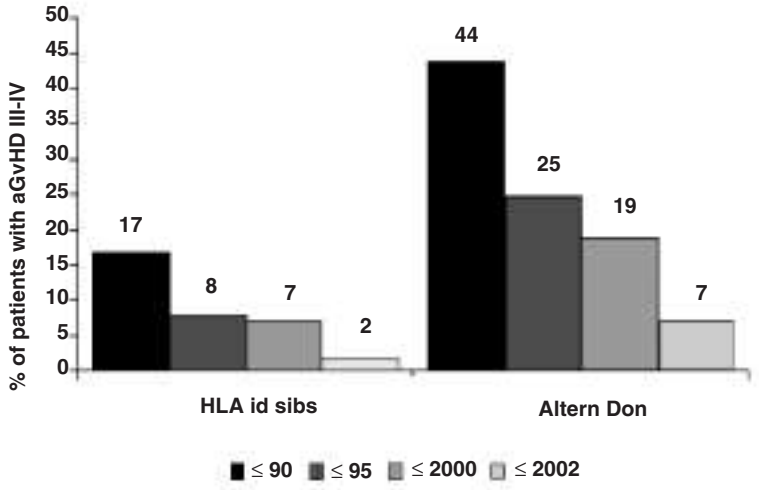

Figure 4. Proportion of patients with acute graft vs host disease (GVHD) grade III-IV in the four transplant periods, divided according to donor type: HLA identical siblings (on the left) or alternative donors (on the right). Severe GVHD has decreased significantly in both transplant settings.

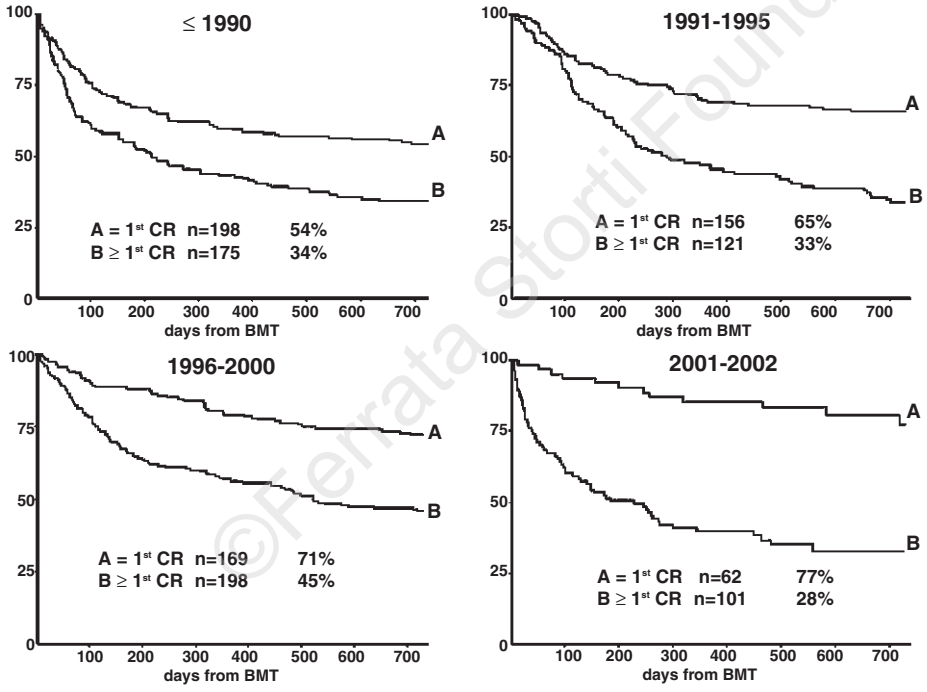

Figure 5. Actuarial survival in the four transplant periods. The differences in actuarial 2-year survival are significant in all four periods: however before 1990 the difference was 20% whereas in 2001-2002 the difference had increased up to 49%.

Discussion

In this study we have shown that: (i) TRM following an allogeneic HSCT has progressively decreased in the past two decades resulting in improved survival and (ii) this effect is seen predominantly in patients transplanted in first remission, and not in patients with advanced disease. As a consequence the advantage of being allografted in first remission rather than in relapse has increased significantly over time. This improving outcome of patients undergoing allogeneic HSCT in encouraging. It is important to note that the reduction of TRM is progressive, apparently also in the last years, since other reports had shown improvements when comparing transplants in the 1980s and in the 1990s, but not further.[4] The current rate of TRM for patients in first remission is 6% overall, being 3% in recipients of HLA identical sibling transplants and 15% in those grafted from alternative donors. Considering CML in chronic or

accelerated phase, the current TRM is 0% for sibling transplants and 9% for alternative transplants; in acute leukemias in first CR these figures are 4% and 12%, respectively. This is an important message for physicians caring for patients with hematologic malignancies, and should encourage them to consider an allogeneic HSCT when the patient is in first remission, rather than waiting until the disease has advanced, and sometimes become refractory to chemotherapy or radiotherapy.

Overall, TRM has decreased, despite a very significant increase in the patients' age, and in the proportion of patients with alternative donor transplants and advanced disease. TRM was reduced in every age group, regardless of donor type. Changes in conditioning regimens, such as the reduced intensity conditioning (RIC) regimen in the older age group (above 45 years), may have played a role. We have been using one of these regimens (thiotepa 10 mg/kg + cyclophosphamide 10 mg/kg) since 1995:7 with a median follow up of 6 years, results are similar to those presented in the original publication,suggesting that this procedure can ensure longterm engraftment and survival. The entire field of RIC transplants is expanding greatly, as witnessed by the increased use in Europe and elsewhere.[12] In the present series the increasing age of patients has been accompanied by an increased use of RIC regimens, although also patients undergoing a conventional transplant with cyclophosphamide-TBI are older now (median 36 years) than in the period before 1990 (median 23 years). We also looked at mortality in patients over the age of 40 receiving cyclophosphamide-TBI: this declined from 60% (before 1990) to 18% (1991-1995 and 1996-2000) and then to 3% (2001-2002). This is not to say that we advocate using cyclophosphamide-TBI for all patients above the age of 40, also because the upper age limit of these patients is between 48 and 50; however we can say that mortality has been reduced also in adults above 40 undergoing a conventional transplant. Together with RIC regimens, it may be appropriate to explore new ways of delivering conventional doses of chemo-radiotherapy: two examples come from targeted busulfan levels in patients receiving myeo-ablative doses of busulfan, apparently with low toxicity also in elderly patients.[13,14]

The second finding is that improvement is confined, although not exclusively, to patients in first remission/chronic phase, with little change for patients with advanced disease. When looking at the four major causesof transplant-related death (acute GVHD, infections, interstitial pneumonia and multiorgan failure), it is clear that for patients in first remission there has been a significant reduction, in some cases an abrogation, of these complications. In contrast, transplants in patients-beyond first remission continue to fail due to infections and multiorgan failure: for HLA identical sibling transplants the current rate of failure due to infections and multiorgan failure is 20% in patients beyond first remission, as compared to 2% in first remission patients. For grafts from alternative donors these figures are 36% and 10%, respectively. This may be because patients coming to transplant beyond first remission have received many courses of chemo-radiotherapy, and have experienced prolonged periods of neutropenia: as a consequence there is colonization with multiple pathogens, including aspergillus, and there is organ toxicity. It is not unexpected that we would see more deaths due to infections and multiorgan failure in these patients than in those being transplanted in first remission. Because patients with advanced disease also have a greater risk of relapsing, survival is greatly affected by disease phase at the time of transplant, as also shown in multivariate Cox analysis. Currently a patient transplanted in our Unit in first remission/chronic phase has a 2-year probability of survival of 77%, independently of his age and donor type, whereas if the patient is in relapse the probability of being alive at 2 years is 23%. This difference has, of course, always existed, but it has become greater with time: indeed, the survival advantage for patients transplanted in first CR compared to patients with more advanced disease was 20% before 1990 and is currently almost 50%. In addition, there is also a trend for a reduced risk of relapse among patients in first remission, and a significantly improved survival in patients who have relapsed after transplant: in other words, 3-year survival following post-transplant relapse before 1990 was significantly poorer (38%) than the current 3-year survival of the same type of patients (56%). This is possibly due to earlier diagnosis, use of donor lymphocyte infusions and second transplants. This is not the case for patients beyond first remission. These results suggest very strongly that patients should be allografted in remission, early in the course of their disease, and this is justified by the current, low TRM and low risk of relapse. However we also need new strategies for patients with advanced disease: ideally conditioning regimens should be non-toxic and have greater anti-tumor effect: radiolabeled antibodies may be one way to deliver an extra dose of radiation in patients with advanced leukemia,[15,16] and the use of anti-CD20 monoclonal antibody, whether radiolabeled or not, may be helpful in lymphoma patients.[17] The recent demonstration of a potent anti-leukemia effect of natural killer cells (NK)18 suggests the potential role of expanding specific NK subpopulations to eradicate leukemia. The final comments must be dedicated to changing transplant protocols or, if you wish, to the question: why has transplant-related mortality decreased? Of course we do not have a simple answer, but rather a list of changes which have occurred over the past 2 decades. GVHD prophylaxis has changed with the advent of combined cyclosporine and methotrexate (1990), the bone marrow cell dose has increased

as a result of standardized harvest procedures (1990), CMV is diagnosed early and treated pre-emptively (1992), we have designed single or combined pre-emptive therapy for different levels of CMV antigenemia (1995), we have been using RIC protocols for patients over 45 years old (1995), we have reduced the use of intravenous high dose IgG (1990-2000), and use antithymocyte globulin for alternative donor grafts (1996), we are using peripheral blood grafts only in selected groups of patients (1996), EBV is diagnosed early and treated pre-emptively with anti-CD20 antibodies (2000). There have also been significant changes in the use of antibiotics and antifungal agents, though mortality due to infection has remained stable at 6% in HLA identical sibling transplants and at 15-19% in transplants from alternative donors. The greatest reduction has been seen in deaths due to acute GvHD and interstitial pneumonia: these 2 complications were the cause of failure in over 20% of all patients before 1990 and currently only in 5%. Therefore one would conclude that appropriate GVHD prophylaxis and early diagnosis and treatment of CMV infections have had the greatest impact on TRM though increasing bone marrow cell dose, tailored conditioning regimens and early treatment of EBV infections may also have played important roles.

In conclusion, for patients who come to transplant in first remission, TRM has been significantly reduced and is now relatively low, with a low risk of relapse, resulting in improved survival. For patients who come to transplant in second remission or in relapse, a minority become long-term survivors, which is of course amazing for patients with advanced stage diseases: however, the lack of major improvement over two decades calls for different transplant approaches, such as optimization of chemo/radiotherapy, different strategies to control infections and post-transplant cell therapy based on minimal residual disease. We hope we will witness improved control of advanced malignancies in the near future, with one or more of these approaches. Until then early transplants should be considered when appropriate, and will yield best results.

AB: design of study and writing the manuscript; MPS: statistical analysis; TL, LG: unrelated transplant data; FG: acute leukemia data; CV, AMR: infectious disease data; DO, SB, CdG, AD, ET, FF, GP, MP: stem cell manipulation; BB, RO: data manager; AL: head nurse; DR: renal complications; GAR, CF: radiology; MTVL: transplant coordinator. The great work of our nursing staff is gratefully acknowledged. The authors reported no potential conflicts of interest.

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