Brief report

Kinetics of minimal residual disease and chimerism in patients with chronic myeloid leukemia after nonmyeloablative conditioning and allogeneic stem cell transplantation

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The kinetics of minimal residual disease (MRD) and chimerism were studied in 15 patients with chronic myeloid leukemia (CML) receiving nonmyeloablative stem cell transplantation (NST) and in 10 patients receiving conventional stem cell transplantation (CST). All NST patients showed T-cell mixed chimerism (MC) while granulocyte and B-cell MC occurred in 80% and 60% of the NST patients, respectively. In CST patients, T-cell MC was detected in 5 patients, of whom 3 were mixed only during the first month. MRD was detected in all NST patients. During the first 3 months the median BCR-ABL/ABL ratio was 0.2% in NST patients compared with 0.01% in CST patients ($P < .01$). However, 12 months after transplantation, the percentage of reverse transcriptase–polymerase chain reaction (RT-PCR)–positive patients was 20% in NST patients and 50% in CST patients. In conclusion, molecular remission can be induced in most patients after NST, albeit with different kinetics from CST. (Blood. 2003;101:469-472)

Introduction

Allogeneic hematopoietic stem cell transplantation (SCT) is the treatment of choice for patients with chronic myeloid leukemia (CML) who have a suitable donor.[1] In older patients and among those with comorbidity, however, there is a high risk of regimen-related toxicity that makes conventional stem cell transplantation (CST) unsuitable for these patients. In recent years, nonmyeloablative stem cell transplantation (NST) has been studied as a safer approach for older patients.[2-4] Such transplantations are more dependent on a graft-versus-leukemia effect, which is known to be powerful in CML patients.[1,5]

Molecular techniques for chimerism and minimal residual disease (MRD) analysis after SCT are routinely used in many laboratories to follow engraftment and predict a threatening relapse of leukemia. In the conventional SCT setting, such analyses have been well evaluated in CML patients.[6-9]

With the introduction of nonmyeloablative transplantations, the significance of chimerism and MRD results to clinical outcome after allogeneic SCT needs to be reevaluated.[10,11]

In the present study, we determined the incidence and kinetics of chimerism and MRD in 15 CML patients receiving NST and compared them with 10 patients receiving CST.

Study design

Patients

Patient characteristics for the 25 CML patients included in this study are shown in Table 1. Fifteen patients received a nonmyeloablative conditioning regimen and allogeneic SCT (NST) between April 1999 and April 2001. Of these 15 patients, 8 were either too old or considered unfit for conventional SCT (CST). Seven patients requested NST after shared decision making. Thirteen patients received CST between January 1999 and March 2001. Three patients were excluded because samples were not available. Of these, 1 patient relapsed 2 years after SCT and 2 patients are alive in clinical remission 18 and 19 months after SCT, respectively. The ethics committee at Karolinska Institute, Huddinge University Hospital, approved this study. Informed consent was provided according to the Declaration of Helsinki.

Conditioning

The NST patients received fludarabine (Flu) 30 mg/m^2/d for 6 days, busulfan (Bu) 4 mg/kg/d for 2 consecutive days, and antithymocyte globulin (ATG) 2 to 10 mg/kg for 4 days.[4]

The CST group was conditioned with cyclophosphamide (Cy) 120 mg/kg with 10 to 12 Gy total body irradiation (TBI) (n = 5) or busulfan 16 mg/kg (n = 5).[12] Details regarding supportive care have been described elsewhere.[13]

Samples

Samples for chimerism and MRD analyses were 5 to 10 mL of peripheral blood.

A median of 8 (range, 3-35) chimerism analyses and 10 (range, 3-30) MRD analyses was performed for each patient.

Lineage-specific chimerism analysis

Polymerase chain reaction (PCR) amplification of variable number of tandem repeats (VNTRs) was used to evaluate various degrees of donor and

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Table 1. Patient characteristics

Patient no.	Age, y	Sex, recipient/donor	CP	HS	Donor	BM/PB	Conditioning	GVHD prophylaxis	Acute GVHD	Chronic GVHD	MRD status, last follow-up (mo)	Follow-up, mo (status)
N1	63	F/F	1	IR	SIB	PB	Flu/Bu/ATG	MMF/CsA/Pr	I	None	− (27)	29 (R)
N2	50	M/M	1	HR	SIB	PB	Flu/Bu/ATG	MTX/CsA	III	None	− (5)	5 (D)
N3	53	F/F	1	NA	SIB	PB	Flu/Bu/ATG	MTX/CsA	I	Lim	− (21)	22
N4	55	F/F	1	IR	MUD	PB	Flu/Bu/ATG	MTX/CsA	None	Lim	− (20)	21
N5	62	F/M	1	IR	SIB	PB	Flu/Bu/ATG	MTX/CsA	None	Lim	− (7)	8.5
N6	56	F/M	1	IR	MUD	BM	Flu/Bu/ATG	MTX/CsA	I	None	+ (7)	8.5
N7	56	F/F	1	IR	SIB	BM	Flu/Bu/ATG	MTX/CsA	None	None	− (6)	6
N8	58	F/F	1	HR	SIB	PB	Flu/Bu/ATG	CsA	II	Lim	+ (29)	31
N9	40	F/M	1	LR	SIB	PB	Flu/Bu/ATG	CsA	II	Ext	− (24)	24
N10	44	M/M	1	LR	SIB	PB	Flu/Bu/ATG	CsA	II	Lim	− (18)	21
N11	50	M/M	1	LR	SIB	PB	Flu/Bu/ATG	MTX/CsA	II	Lim	− (20)	20
N12	49	M/M	1	HR	MUD	BM	Flu/Bu/ATG	MTX/CsA	II	Ext	+ (17)	17 (D)
N13	46	F/M	1	LR	MUD	PB	Flu/Bu/ATG	MTX/CsA	I	None	− (13)	14
N14	36	F/M	1	LR	MUD	PB	Flu/Bu/ATG	MTX/CsA	None	None	− (13)	14
N15	51	M/F	1	HR	SIB	PB	Flu/Bu/ATG	MTX/CsA	II	Lim	+ (8)	8
C1	35	M/M	1	LR	MUD	BM	TBI/Cy/ATG	MTX/CsA	I	Ext	− (28)	34
C2	31	F/F	1	IR	MUD	PB	TBI/Cy/ATG	MTX/CsA	I	None	+ (29)	33
C3	33	F/M	1	IR	MUD	PB	TBI/Cy/ATG	MTX/CsA	III	Ext	− (28)	32
C4	44	M/F	1	HR	SIB	BM	Bu/Cy	MTX/CsA	II	Lim	− (24)	30
C5	53	M/F	1	IR	MUD	BM	fTBI/Cy/ATG	MMF/CsA	I	None	+ (24)	25
C6	49	M/M	1	LR	MUD	PB	Bu/Cy/ATG	MTX/CsA	III	Ext	− (5)	7 (D)
C7	35	F/M	1	LR	MUD	BM	Bu/Cy/ATG	MTX/CsA	II	Lim	− (18)	22
C8	50	M/F	1	LR	SIB	PB	Bu/Cy	MTX/CsA	I	Lim	+ (16)	21
C9	44	F/F	1	IR	MUD	BM	Bu/Cy/ATG	MTX/CsA	I	None	+ (11)	19 (R)
C10	40	M/M	1	IR	MUD	PB	fTBI/Cy/ATG	MTX/CsA	None	None	+ (8)	9 (R)

CP indicates chronic phase; HS, Hasford score at diagnosis; BM, bone marrow; PB, peripheral blood stem cells; GVHD, graft-versus-host disease; MRD, minimal residual disease; N1 to N15, patients with nonmyeloablative conditioning; F, female; IR, intermediate risk; SIB, sibling donor; Flu, fludarabine; Bu, busulfan; ATG, antithymocyte globulin; MMF, mycophenolate mofetil; CsA, cyclosporine A; Pr, prednisolone; R, relapse; M, male; HR, high risk; MTX, methotrexate; D, dead; NA, not available; Lim, limited; MUD, matched unrelated donor; LR, low risk; Ext, extensive; C1 to C10, patients with conventional conditioning; TBI, total body irradiation; Cy, cyclophosphamide, and fTBI, fractionated TBI.

recipient chimerism in $CD3^+$, $CD19^+$, and $CD45^+$ cells as previously described.[14]

Reverse transcriptase (RT)–PCR analysis for BCR-ABL

Quantification was done by competitive PCR using plasmid constructs containing a modified *BCR-ABL* fusion gene.[15] Dr N. C. P. Cross, Hammersmith Hospital, London, kindly provided pNC210/G (p210) and pNC190/G (p190) competitor plasmids.

BCR-ABL and *ABL* transcript numbers were estimated by comparing the competitor and sample band intensity to find the equivalence point. Results were expressed as the ratio between *BCR-ABL* and *ABL* transcript numbers *(BCR-ABL/ABL).*

Results and discussion

Sensitivity of BCR-ABL detection

RNA from K562 cells was serially diluted in RNA from HL-60 cells in a total amount of 20 μg RNA. After cDNA synthesis and 40 cycles of PCR amplification, a sensitivity of 10^{-6} was obtained.

Patients

In the NST group, 13 patients are alive with a median follow-up of 20 months (range, 6-29 months). Two patients died of graft-versus-host disease (GVHD) and progressive disease 5 and 17 months after SCT, respectively (Table 1). One patient (N1) relapsed 7 months after SCT.

In the CST group, 9 patients are alive with a median follow-up of 25 months (range, 9-34 months). One patient died of GVHD 7 months after SCT. Two patients relapsed 9 and 16 months after SCT, respectively.

Chimerism results

NST. T-cell mixed chimerism (MC) was detected in all 15 patients, and all but 2 (N7 and N15) converted to donor chimerism (DC) at the end of this study (Figure 1). The median time for T-cell DC to occur was 87 days (range, 28-145 days) and was significantly delayed (21 days [range, 14-60 days]) compared with the CST group ($P < .01$). Granulocyte and B-cell MC were found in 12 (80%) and 9 (60%) of the patients, respectively. Median times to granulocyte and B-cell DC were 29 days (range, 14-128 days) and 30 days (range, 14-190 days), respectively.

CST. Five patients showed T-cell MC after SCT. Three patients had MC only the first month, whereas 2 patients (C9 and C10) had increasing levels of recipient cells in all cell fractions; both relapsed.

In accordance with other NST studies, we found a high incidence of MC.[10,14,16] The engraftment of T cells lagged behind granulocytes and B cells, which is in agreement with some but not other studies.[10,17]

MRD results

NST. All patients in this group had detectable *BCR-ABL* transcripts after SCT. The *BCR-ABL/ABL* ratio during the first 3 months was, with a median of 0.2%, significantly more compared with 0.01% in CST patients ($P < .01$) (Figure 1). This probably reflects the lower antitumor effect of nonmyeloablative conditioning. Eleven patients became MRD negative within 7 months (median, 3.5 months; range, 1-7 months). Three patients (N6, N12, N15)

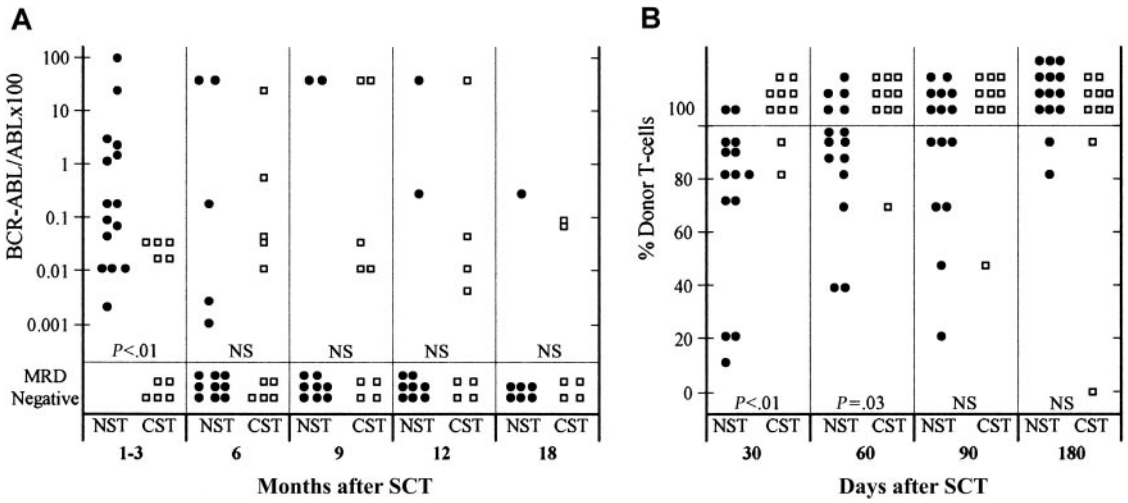

Figure 1. The incidence and level of MRD and T-cell chimerism after SCT. MRD levels (A) and T-cell chimerism engraftment (B) are shown for both patient groups at different time points after SCT. ● represents NST (nonmyeloablative) patients; □, CST (conventional stem cell transplantation patients). Differences in levels between the 2 patient groups are calculated at each time point using the Mann-Whitney *U* test. NS indicates not significant.

were *BCR-ABL* positive during the entire posttransplantation period. However, in 2 patients, the follow-up was less than 9 months.

CST. After SCT, 7 of 10 patients showed at least 1 positive PCR assay for *BCR-ABL*. The percentage of MRD-positive patients was 50% at 1 year (Figure 1). Long persistent expression of *BCR-ABL* in the absence of clinical relapse has been observed in many studies.[6,18-21] For these patients, it is important to monitor the change in transcript levels with quantitative analysis.[15,22]

Chimerism and MRD

NST. A switch from T-cell MC to DC was usually seen before (n = 5) or at the time (n = 4) of MRD negativity. In the former group, the median interval between T-cell DC and MRD negativity was 40 days (range, 24-90 days). One patient (N3) had T-cell MC with no signs of the *BCR-ABL* transcript for 1 month. The short interval between T-cell DC and MRD negativity suggests that complete DC is not necessary for disease response.[11]

Twelve patients with MC in the granulocyte cell population were all PCR positive for *BCR-ABL*. Among those, a *BCR-ABL/ABL* ratio of at least 0.1% was found in 10.

CST. MC beyond 1 month after SCT was seen in only 2 patients (C9 and C10). Both had increasing *BCR-ABL* levels, and MC in B cells and granulocytes appeared only at *BCR-ABL/ABL* ratios of more than 1%. Three patients (C2, C5, and C8) with persistent *BCR-ABL* transcript (ratio less than 0.1%) more than 1 year after SCT had DC in all cell fractions.

GVHD and MRD

The incidence of acute GVHD was 73% and 90% in the NST and CST groups, respectively (Table 1). Interestingly, 4 NST patients without acute GVHD became MRD negative. This suggests that an allogeneic graft-versus-leukemia (GVL) effect may be seen even in the absence of GVHD, which is in accordance with a report in patients with acute leukemia.[23]

In conclusion, despite high *BCR-ABL* levels during the early posttransplantation period and a high incidence of mixed chimerism, nonmyeloablative transplantation for CML patients may induce molecular remission in most patients. Close monitoring of chimerism and MRD is needed to guide the timely introduction of immune-therapeutic interventions.

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