ORIGINAL ARTICLE

BCR-ABL1 transcripts (MR$^{4.5}$) at post-transplant 3 months as an early predictor for long-term outcomes in chronic myeloid leukemia

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Background/Aims: The aim of this study was to identify the role of *BCR-ABL1* transcript level as a predictor for post-transplant relapse and outcome in patients who underwent allogeneic stem cell transplantation (SCT) for chronic phase (CP) chronic myeloid leukemia (CML).

Methods: Of 101 patients receiving allograft in CML CP, 85 had available quantitative reverse transcriptase polymerase chain reaction data at post-transplant 3 months. These patients were divided into two groups according to molecular response (MR$^{4.5}$), defined as a *BCR-ABL1* transcript level ≤ 0.0032% on the international scale, at 3 months based on receiver operating characteristic curve analysis of relapse.

Results: The 4-year overall survival and event-free survival (EFS) were 80.6% and 57.3%, respectively, and the cumulative incidence of relapse at 4 years was 29.6% after a median follow-up of 126.4 months. We performed multivariate analyses including potential variables to evaluate the early predictive role of MR$^{4.5}$ at 3 months and found that MR$^{4.5}$ at 3 months was associated with a higher EFS (p = 0.028) and showed a trend for a lower relapse rate (p = 0.089).

Conclusions: our results imply that frequent molecular monitoring and immune suppressive therapy modulation are required for patients without reduction of *BCR-ABL1* transcripts to this level after SCT.

Keywords: Leukemia, myelogenous, chronic, BCR-ABL positive; Allogeneic stem cell transplantation; Post-transplant relapse

INTRODUCTION

Since imatinib mesylate (IM), the first *BCR-ABL1* tyrosine kinase inhibitor (TKI), became a first-line therapy for chronic phase (CP) chronic myeloid leukemia (CML), more potent second generation (2G) TKIs have been introduced into routine practice. However, allogeneic stem cell transplantation (SCT) remains an important treatment for patients with advanced-phase CML at di-

agnosis, those with a T315I mutation in *BCR-ABL1*, or those who fail to respond durably to TKIs [1]. Nonetheless, relapse after allogeneic SCT is observed in 20% to 40% of patients [2-4]. Previous studies showed the role of TKIs as an option for salvage therapy in patients with post-transplant relapse [5,6] and emphasized the importance of post-transplant monitoring by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).

Several studies have suggested that early detection of *BCR-ABL1* transcripts by qRT-PCR is associated with an increased risk of relapse [7,8]. Asnafi et al. [8] evaluated the predictive role of day 100 *BCR-ABL1* quantification using qRT-PCR, whereas other studies emphasized serial measurement of *BCR-ABL1* transcripts [9,10].

However, the sensitivity of PCR technology has recently increased and more stringent standardization of PCR assays is now available [11]. In an IM discontinuation study of post-transplant relapse that included transplant patients who sustained undetectable molecular residual disease (UMRD) for more than 2 years from IM therapy, we found an association between a previous allograft and sustained molecular response. This implies the importance of immunologic effects, such as donor-derived cytotoxic T-cells, in CML patients receiving allogeneic SCT [12]. Therefore, it is necessary to identify early predictors for post-transplant relapse.

The purpose of this study was to identify a uniform *BCR-ABL1* transcript cutoff on the international scale (IS) that predicts post-transplant relapse and outcomes in patients who undergo allogeneic SCT for CP CML.

METHODS

Patient selection

A total of 110 consecutive patients with CML underwent allogeneic SCT at Seoul St. Mary's Hospital between May 2001 and December 2013. Because our aim was to investigate an early predictor for post-transplant relapse in CP CML, nine patients with advanced disease: six in accelerated phase and three in blast phase (BC) at the time of transplant were excluded and 101 CP patients were finally evaluated. All of the patients received grafts from either a human leukocyte antigen (HLA) identical sibling or an unrelated donor, and HLA matching for unrelated SCT was based on molecular typing for HLA-A, -B, -C, and DRB1.

The transplantation procedures were performed as described below. Thirty-seven patients were given a reduced-intensity conditioning regimen consisting of either fludarabine (150 mg/m²) with intravenous busulfan (6.4 mg/kg) or fludarabine (125 mg/m²) with cyclophosphamide (120 mg/kg). Sixty-four patients were given a standard conditioning regimen consisting of either fraction-

ated total body irradiation (total body irradiation 1,200 cGy) and cyclophosphamide (120 mg/kg) or intravenous busulfan (12.8 mg/kg) and cyclophosphamide (120 mg/kg). Prophylaxis for graft-versus-host disease (GVHD) was administered using a combination of short-course methotrexate with either cyclosporine (for related transplants) or tacrolimus (for unrelated transplants). Granulocyte colony stimulating factor (5 µg/kg) was subcutaneously administered daily to all patients from day +7 after transplantation until the absolute neutrophil count (ANC) was > 3.0 × 10⁹/L. This study was approved by the Institutional Review Board of Seoul St. Mary's Hospital.

Molecular monitoring

After allogeneic SCT, molecular testing by qRT-PCR was performed at 1, 3, 6, 9, and 12 months and subsequently at 6-month intervals if the patient did not experience relapse. Results were expressed as the ratio of *BCR-ABL1* copy number to *ABL1* copy number on the IS. For patients who lacked available records for *BCR-ABL1* transcript at 1 and 3 months post-transplant, cryopreserved samples (cells or mRNA) were used for qRT-PCR testing. The quality of RNA was assessed using Experion automated electrophoresis (Applied Bio-Rad, Hercules, CA, USA), and only qRT-PCR results with more than 50,000 *ABL1* transcripts were analyzed. Major molecular response was defined as a *BCR-ABL1* transcript level of ≤ 0.1% on the IS. Molecular response (MR$^{4.5}$) was defined as a reduction in the *BCR-ABL1* transcript level to ≤ 0.0032%.

Definitions

Disease phase and response were defined according to recent criteria [1]. To examine post-transplantation outcomes, neutrophil engraftment was defined as the first of 3 consecutive days with an ANC > 0.5 × 10⁹/L, and platelet engraftment was defined as the first of 5 consecutive days with a platelet count > 20 × 10⁹/L without transfusion support. Failure to engraft by day 28 was considered a primary engraftment failure, and secondary engraftment failure was defined as initial engraftment with documented donor-derived hematopoiesis followed by the loss of graft function without disease recurrence. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were graded according to clinical consensus criteria [13,14]. Overall survival (OS) was calculated from

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the day of transplantation, with patients alive at the time of last follow-up being administratively censored, and event-free survival (EFS) was counted from day 0 to any type of relapse or death while in remission. Relapse was defined by ratio of *BCR-ABL1* to *ABL1* > 0.1% on the IS for two consecutive analyses and therapeutic intervention was made. Transplant-related mortality (TRM) was defined as death due to any cause other than relapse.

Statistical analysis

The aim of this study was to identify a predictive marker for post-transplant relapse and outcomes based on *BCR-ABL1* transcript levels on the IS at one early time point in patients that underwent allogeneic SCT for CP CML. Probabilities of OS and EFS were calculated by the Kaplan-Meier method and compared by the log-rank statistic, whereas those for TRM and relapse were plotted according to cumulative incidence estimates and compared with the Gray test. The prognostic significance of presenting and transplantation covariates with respect to OS and EFS were determined using the Cox proportional hazard regression model, including variables with a $p \leq 0.1$ from univariate analyses. Factors were considered to be statistically significant if they had an associated $p < 0.05$ as determined by the likelihood ratio test. The prognostic significance of covariates affecting the competing events of TRM and relapse were determined using the proportional hazard model for the sub-distribution of competing risks. The incidence of aGVHD and cGVHD was calculated using cumulative incidence estimates. Statistical studies were performed with the SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) and the cumulative incidence analyses were carried out with R (freely distributed on the web, http://cran.r-project.org/).
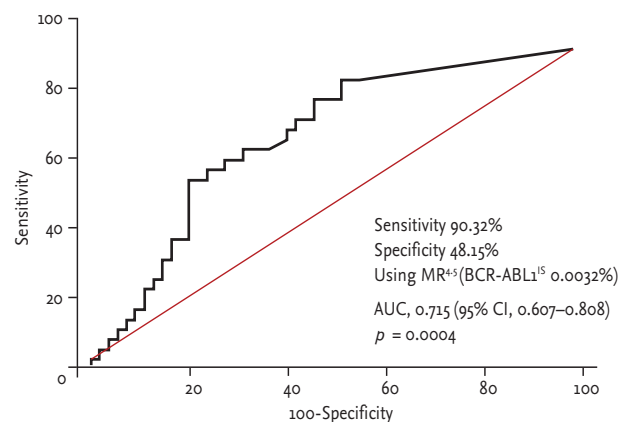
RESULTS

Patient demographics and characteristics

The study included a total of 101 patients (60 men and 41 women) with a median age of 32 years (range, 13 to 54). Of the 101 patients, 47 were TKI-naïve at the time of transplantation, in most case during the period before the national health insurance program covered IM, and 51 received IM as their frontline therapy. The remaining three received frontline treatment with 2G-TKIs: one

with dasatinib (DAS), one with nilotinib, and one with bosutinib. Upon the failure of front-line TKI therapy, 17 patients were given other TKIs as second-line therapy, of whom eight were administered a third-line TKI. All patients received grafts from either matched siblings (n = 58) or unrelated donors (n = 43) (Table 1).

Prediction of relapse using *BCR-ABL1* transcript levels at 3 months after SCT

To evaluate the predictive role of the *BCR-ABL1* transcript levels at 3 months after SCT, 85 patients who had available qRT-PCR data at 3 months after SCT were analyzed. We performed a receiver operating characteristic (ROC) curve analysis based on results of the association between *BCR-ABL1* transcript levels at 3 months and relapse (Fig. 1). With consideration of the predictive marker as a test for post-transplant relapse in higher risk patients, the MR4.5 cutoff was chosen in favor of sensitivity over specificity. At 3 months after SCT, 32 patients achieved MR4.5 (MR4.5 group) and 53 patients did not achieve MR4.5 (no MR4.5 group). In comparison of clinical characteristics, the two groups were comparable for sex distribution, age, disease phase at diagnosis and transplant, and Sokal score, but in the MR4.5 group, prior TKI therapy was more frequent and the median interval from diagnosis to transplant was longer. In terms of transplant characteristics, there were no differences in conditioning intensity, donor type, and GVHD prophylaxis between the two groups. However, patients in the

Sensitivity 90.32%
Specificity 48.15%
Using MR4.5 (BCR-ABL1^IS 0.0032%)
AUC, 0.715 (95% CI, 0.607–0.808)
$p = 0.0004$

Figure 1. Receiver operating characteristic curves of *BCR-ABL1* transcript level on the international scale (IS) at post-transplant 3 months. MR, molecular response; AUC, area under the receiver operating characteristic curve; CI, confidence interval.

Table 1. Clinical characteristics of patients and transplants

| Parameter | Total (n = 101) | BCR-ABL1 transcript at post-transplant 3 months[a] | | |
		MR$^{4.5}$ (n = 32)	No MR$^{4.5}$ (n = 53)	p value
Age, yr	32 (13–54)	33.5 (20–51)	32 (14–54)	0.350
Sex, male/female	60 (59)/41 (41)	15 (47)/17 (53)	34 (64)/19 (36)	0.118
Disease phase at diagnosis, CP/AP/BP	79 (78)/17 (17)/5 (5)	22 (69)/8 (25)/2 (6)	45 (85)/6 (11)/2 (4)	0.203
Disease phase at transplant, CP	101 (100)	32 (100)	53 (100)	-
Disease status at transplant				
No MCyR/MCyR	63 (62)/38 (38)	17 (53)/15 (47)	39 (74)/14 (26)	0.054
> 10% MR/≤ 10% MR	63 (62)/38 (38)	17 (53)/15 (47)	39 (74)/14 (26)	0.054
No MMR/MMR	90 (89)/11 (11)	26 (81)/6 (19)	49 (93)/4 (7)	0.167
Previous therapy before SCT				0.003
No prior TKI	47 (47)	10 (31)	34 (64)	
Prior TKI(s)	54 (54)[b]	22 (69)	19 (36)	
Interval from diagnosis to transplant, mon	11.6 (4.0–132.2)	13.2 (4.0–58.7)	10.4 (4.8–132.2)	0.023
Sokal score, low/intermediate/high/NA	31 (31)/ 22 (22)/ 30 (30)/ 18 (18)	8 (25)/ 4 (12)/ 13 (41)/ 7 (22)	19 (36)/ 16 (30)/ 11 (21)/ 7 (13)	0.069
EBMT score				0.043
0 and 1	23 (23)	4 (13)	17 (32)	
2	38 (38)	11 (34)	22 (42)	
3 and 4	38 (38)	16 (50)	14 (26)	
5	2 (2)	1 (3)	0	
Donor type				0.407
Related	58 (57)	58 (57)	58 (57)	
Unrelated	43 (43)	43 (43)	43 (43)	
Donor/recipient sex match				
F to M/others[c]	18 (18)/83 (82)	6 (19)/26 (81)	10 (19)/43 (81)	0.407
Source of graft				
BM/PBSC-based[d]	72 (71)/29 (29)	18 (56)/14 (44)	43 (81)/10 (19)	0.014
CD34$^+$ cells (× 10^6/kg)	4.1 (1.2–20.0)	4.7 (1.3–20.0)	3.7 (1.2–17.9)	0.285
CD3$^+$ cells (× 10^7/kg)	5.35 (0.2–109.7)	8.59 (0.3–57.3)	5.49 (1.7–109.7)	0.549
Conditioning intensity, standard/RIC	64 (63)/37 (37)	22 (69)/10 (31)	32 (60)/21 (40)	0.437
GVHD prophylaxis, CsA + MTX/ FK506 + MTX	52 (52)/49 (49)	14 (44)/18 (56)	28 (53)/25 (47)	0.417

Values are presented as median (range) or number (%).

MR, molecular response; CP, chronic phase; AP, accelerated phase; BP, blast phase; MCyR, major cytogenetic response; MMR, major molecular response; SCT, stem cell transplantation; TKI, tyrosine kinase inhibitor; NA, not available; EBMT, European Group for Blood and Marrow Transplantation; F, female; M, male; BM, bone marrow; PBSC, peripheral blood stem cell; RIC, reduced-intensity conditioning; GVHD, graft-versus-host disease; CsA, cyclosporine; MTX, methotrexate.

[a]85 patients had available quantitative reverse transcriptase polymerase chain reaction records at 3 months after SCT.

[b]36 patients were treated with imatinib (IM) prior to SCT and 15 patients were treated with IM and second generation (2G)-TKI(s). Another 3 patients received 2G-TKI as initial therapy.

[c]Others included male-to-male (n = 42), female-to-female (n = 12), and male-to-female (n = 29).

[d]PBSC-based graft sources included PBSC (n = 23) and BM plus PBSC (n = 6).

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Table 2. Transplant outcomes

Parameter	Total (n = 101)	BCR-ABL1 transcript at post-transplant 3 months[a]		
		MR$^{4.5}$ (n = 32)	No MR$^{4.5}$ (n = 53)	p value
Hematologic reconstitution and engraftment				
Primary graft failure	1	-	-	-
Secondary graft failure	0	-	-	-
Engraftment kinetics[b]				
Days to ANC > 0.5 × 10^9/L	13 (0–38)	12 (7–25)	14 (0–25)	0.164
Days to PLT > 20 × 10^9/L	19 (0–53)	15 (0–28)	22 (0–53)	0.165
Acute GVHD				0.616
Grade I	7 (7)	1 (3)	5 (9)	
Grade II	17 (17)	7 (22)	7 (13)	
Grade III	4 (4)	1 (3)	2 (4)	
Grade IV	2 (2)	0	1 (2)	
CI (≥ grade II) at 100 days	22.8 ± 4.2	25.3 ± 7.9	18.9 ± 5.4	0.475
Chronic GVHD, E/N				0.385
Limited	19/93 (20)	6/31 (19)	13/53 (25)	
Extensive	35/93 (38)	14/31 (45)	16/53 (30)	
CI (extensive) at 4 years	35.0 ± 7.8	45.3 ± 9.3	30.2 ± 6.4	0.199
Cumulative incidence of relapse				
3 mon	2.0 ± 1.4	0	0	-
1 yr	23.4 ± 4.3	10.0 ± 5.6	30.2 ± 6.4	0.034
4 yr	29.6 ± 4.6	13.3 ± 6.3	37.9 ± 6.8	0.018
Cumulative incidence of TRM				
3 mon	2.0 ± 1.4	0	0	-
1 yr	8.1 ± 2.7	0	7.5 ± 3.7	0.121
4 yr	12.2 ± 3.3	6.7 ± 4.6	9.5 ± 4.1	0.628
Event-free survival				
3 mon	95.0 ± 2.2	100	100	-
1 yr	68.6 ± 4.7	90.0 ± 5.5	62.3 ± 6.7	0.007
4 yr	57.3 ± 5.0	80.0 ± 7.3	50.9 ± 6.9	0.008
Overall survival				
3 mon	97.0 ± 1.7	100	100	-
1 yr	86.8 ± 3.4	96.7 ± 3.3	86.8 ± 4.7	0.145
4 yr	80.6 ± 4.0	90.0 ± 5.5	81.1 ± 5.4	0.274

Values are presented as median (range), number (%), or mean ± SE.

MR, molecular response; ANC, absolute neutrophil count; PLT, platelet; GVHD, graft-versus-host disease; E/N, number of events/number of evaluable patients; CI, cumulative incidence; TRM, transplant-related mortality.

[a]85 patients had available quantitative reverse transcriptase polymerase chain reaction records at 3 months after stem cell transplantation.

[b]Engraftment kinetics were evaluated in all patients, except for one patient who had primary graft failure.

MR$^{4.5}$ group showed a higher European Group for Blood and Marrow Transplantation (EBMT) score and more frequent use of peripheral blood stem cells (PBSCs) as a source (Table 1).

Engraftment and GVHD

Transplant outcomes are shown in Table 2. All patients except for one achieved primary engraftment. The median times to neutrophil and platelet engraftment were

Table 3. Univariate analyses of potential variables affecting transplantation outcomes

Variable	OS at 4 years		EFS at 4 years		Relapse at 4 years		TRM at 4 years	
	RR (95% CI)	p value	RR (95% CI)	p value	RR (95% CI)	p value	RR (95% CI)	p value
Increasing age, yr	1.03 (0.98–1.08)	0.210	1.00 (0.96–1.03)	0.777	0.96 (0.92–1.00)	0.041	1.07 (1.02–1.12)	0.006
Age of patient, yr								
≤ 35	1		1		1		1	
> 35	2.01 (0.81–4.94)	0.130	0.78 (0.41–1.48)	0.446	0.22 (0.08–0.64)	0.005	5.51 (1.51–20.10)	0.010
Patient sex								
Male	1		1		1		1	
Female	0.66 (0.25–1.74)	0.400	0.96 (0.52–1.78)	0.902	0.99 (0.45–2.05)	0.980	1.04 (0.33–3.24)	0.950
Disease phase at diagnosis								
Chronic phase	1		1		1		1	
Accelerated phase	1.45 (0.47–4.44)	0.518	0.84 (0.35–1.99)	0.685	0.80 (0.27–2.35)	0.680	1.02 (0.22–4.66)	0.980
Blast phase	3.85 (0.87–17.12)	0.077	3.26 (0.99–10.70)	0.052	2.19 (0.46–10.39)	0.320	2.48 (0.27–22.73)	0.420
Disease status at transplant								
MCyR								
No	1		1		1		1	
Yes	1.33 (0.50–3.49)	0.566	0.86 (0.47–1.60)	0.637	0.57 (0.28–1.16)	0.120	3.14 (0.71–13.90)	0.130
MR, %								
> 10	1		1		1		1	
≤ 10	1.37 (0.52–3.61)	0.520	1.00 (0.54–1.87)	0.993	0.68 (0.33–1.41)	0.300	3.14 (0.71–13.90)	0.130
MMR								
No	1		1		1		1	
Yes	1.15 (0.27–4.97)	0.854	1.43 (0.51–4.01)	0.495	1.24 (0.42–3.66)	0.690	1.44 (0.196–10.50)	0.720
Treatment prior to SCT								
No TKI	1		1		1		1	
IM	1.17 (0.45–3.04)	0.744	0.98 (0.50–1.92)	0.958	0.93 (0..43–2.05)	0.860	1.12 (0.35–3.59)	0.850
IM and 2G-TKI(s)[a]	0.67 (0.15–3.11)	0.609	1.10 (0.46–2.59)	0.835	1.14 (0.40–3.31)	0.800	0.47 (0.06–4.00)	0.490
Interval from diagnosis to transplant, yr	1.00 (0.97–1.03)	0.810	1.01 (1.00–1.03)	0.198	1.01 (0.98–1.03)	0.700	1.00 (0.97–1.03)	0.860
EBMT score								
0–2	1		1		1		1	
≥ 3	2.01 (0.82–4.95)	0.130	1.36 (0.74–2.51)	0.321	0.72 (0.33–1.57)	0.400	3.56 (1.09–11.60)	0.035
Sokal score								
Low	1		1		1		1	
Intermediate	1.03 (0.33–3.26)	0.956	0.94 (0.42–2.10)	0.884	1.02 (0.42–2.47)	0.970	0.71 (0.14–3.72)	0.680
High	0.49 (0.13–1.91)	0.307	0.74 (0.33–1.64)	0.452	0.69 (0.26–1.80)	0.440	0.57 (0.10–3.06)	0.510
NA	1.12 (0.33–3.84)	0.854	0.81 (0.33–1.98)	0.638	0.42 (0.12–1.39)	0.150	1.97 (0.51–7.60)	0.320
Year of transplant								
2001–2004	1		1		1		1	
2005–2008	0.61 (0.08–4.58)	0.628	1.20 (0.42–3.40)	0.732	1.39 (0.49–3.93)	0.530	1.02 (0.15–6.99)	0.990
2009–2012	0.59 (0.14–2.58)	0.485	0.91 (0.38–2.17)	0.828	0.92 (0.29–2.87)	0.880	0.44 (0.06–3.51)	0.440
Donor type								
Related	1		1		1		1	
Unrelated	1.59 (0.64–3.90)	0.316	1.51 (0.83–2.77)	0.180	1.12 (0.55–2.31)	0.750	2.04 (0.66–6.33)	0.220

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Table 3. Continued

Variable	OS at 4 years		EFS at 4 years		Relapse at 4 years		TRM at 4 years	
	RR (95% CI)	*p* value	RR (95% CI)	*p* value	RR (95% CI)	*p* value	RR (95% CI)	*p* value
Donor/recipient sex match								
F to M	1		1		1		1	
Others	0.82 (0.27–2.47)	0.721	1.11 (0.49–2.50)	0.805	1.42 (0.48–4.17)	0.520	0.65 (0.18–2.36)	0.510
Source of graft								
BM	1		1		1		1	
PBSC-based	0.65 (0.22–1.95)	0.441	0.34 (0.14–0.81)	0.014	0.08 (0.01–0.55)	0.011	1.29 (0.40–4.21)	0.670
Conditioning intensity								
Standard	1		1		1		1	
RIC	0.69 (0.26–1.82)	0.455	0.69 (0.36–1.32)	0.262	0.93 (0.45–1.91)	0.830	0.52 (0.15–1.85)	0.310
GVHD prophylaxis								
CsA-based	1		1		1		1	
FK506-based	1.15 (0.47–2.84)	0.785	1.52 (0.83–2.80)	0.179	1.39 (0.67–2.87)	0.380	1.51 (0.48–4.74)	0.480

OS, overall survival; EFS, event-free survival; TRM, transplant-related mortality; RR, risk ratio; CI, confidence interval; MCyR, major cytogenetic response; MR, molecular response; MMR, major molecular response; SCT, stem cell transplantation; TKI, tyrosine kinase inhibitor; IM, imatinib mesylate; 2G-TKI, second generation tyrosine kinase inhibitor; EBMT, European Group for Blood and Marrow Transplantation; NA, not available; BM, bone marrow; PBSC, peripheral blood stem cell; RIC, reduced-intensity conditioning; GVHD, graft-versus-host disease; CsA, cyclosporine.

[a]This group included two patients who received 2G-TKI as initial therapy.
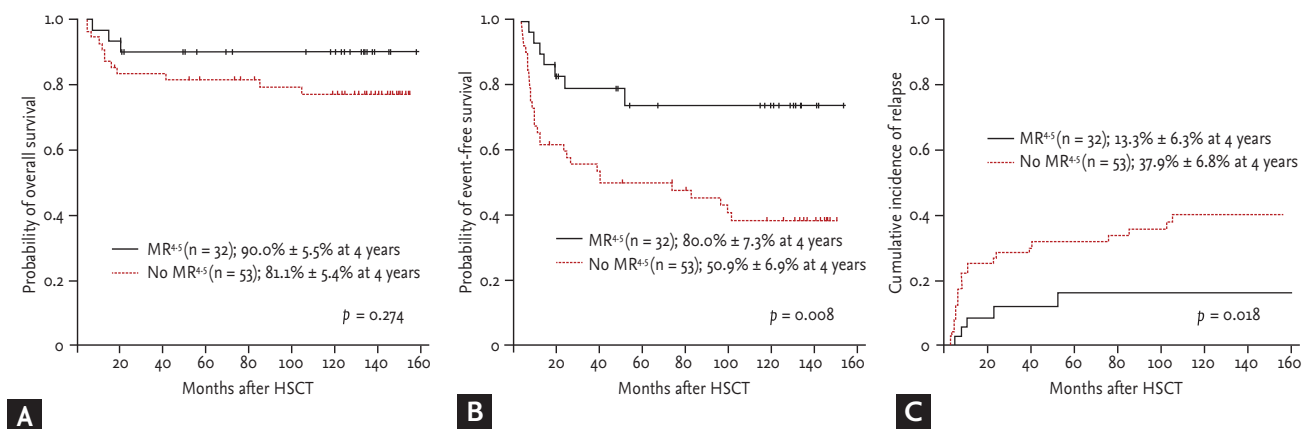
Figure 2. Transplantation outcomes according to *BCR-ABL1* transcript levels at 3 months after allogeneic stem cell transplantation (SCT). Probabilities of (A) overall survival and (B) event-free survival, and (C) cumulative incidence of relapse according to MR[4.5] at 3 months after SCT. HSCT, hematopoietic stem cell transplantation; MR, molecular response.

13 and 19 days after SCT, respectively. The cumulative incidence of clinically significant aGVHD (≥ grade II) at 100 days was 22.8%, and of the 93 patients who were evaluated, chronic extensive GVHD developed in 35 patients (38.0%). The 4-year cumulative incidence of chronic extensive GVHD was 35.0%. There were no differences in the occurrence of aGVHD and cGVHD between the two groups (MR[4.5] group vs. no MR[4.5]); cumulative inci-

dence of aGVHD (≥ grade II) at 100 days was 25.3% and 18.9%, respectively (*p* = 0.475) and cumulative incidence of cGVHD at 4 years was 45.3% and 30.2% (*p* = 0.199).

Prediction of outcomes by MR[4.5] at post-transplant 3 months

With a median follow-up of 126.4 months (range, 3.1 to 154.8) for survivors, the 4-year OS and EFS was 80.6 %

Table 4. Multivariate analyses of independent variables affecting transplantation outcome

Variable[a]	Patients with post-SCT qRT-PCR at 3 months (n = 85)		
	No.	RR (95% CI)	p value
Relapse			
Age, yr			
≤ 35	54	1	
> 35	31	0.26 (0.08–0.86)	0.028
Source of graft			
PBSC-based	24	1	
BM	61	7.04 (0.94–52.44)	0.057
MR$^{4.5}$ at 3 months			
Yes	32	1	
No	53	2.46 (0.87–6.95)	0.089
Transplant-related mortality			
Age, yr			
≤ 35	54	1	
> 35	31	8.05 (0.66–97.80)	0.100
EBMT score			
0–2	54	1	
> 3	31	3.34 (0.47–23.60)	0.230
MR$^{4.5}$ at 3 months			
Yes	32	1	
No	53	2.46 (0.46–13.30)	0.290
Event-free survival			
Source of graft			
PBSC based	24	1	
BM	61	2.71 (0.94–7.79)	0.064
MR$^{4.5}$ at 3 months			
Yes	32	1	
No	53	2.73 (1.12–6.68)	0.028
Overall survival			
Disease phase at diagnosis			
Chronic phase	67	1	
Accelerated phase	14	1.04 (0.22–4.86)	0.961
Blast phase	4	2.52 (0.32–19.79)	0.379
MR$^{4.5}$ at 3 months			
Yes	32	1	
No	53	2.04 (0.55–7.58)	0.285

SCT, stem cell transplantation; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; RR, risk ratio; CI, confidence interval; PBSC, peripheral blood stem cell; BM, bone marrow; EBMT, European Group for Blood and Marrow Transplantation; MR, molecular response.

[a]Only factors identified as significant in univariate analysis were analyzed in this multivariate analysis.

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and 57.3%, respectively. Of the 101 patients, 12 died of transplant-related toxicities after transplantation, including aGVHD (n = 3), cGVHD (n = 6), infections (n = 2), and veno-occlusive disease (n = 1), and nine patients died after post-SCT relapse. Of the 101 patients, 36 relapsed after SCT and for post-relapse therapy, received TKI therapy (n = 28), donor lymphocyte infusion (DLI, n = 1), immunosuppressant withdrawal (n = 5), follow-up loss (n = 1), and conservative care (n = 1). The cumulative incidence of relapse at 4 years was 29.6%. In analysis evaluating the predictive role of *BCR-ABL1* transcript levels after SCT, $MR^{4.5}$ at 3 months was associated with a lower relapse rate (13.3% ± 6.3% vs. 37.9% ± 6.8% at 4 years, p = 0.018) and higher EFS (80.0% ± 7.3% vs. 50.9% ± 6.9% at 4 years, p = 0.008), but did not influence OS (90.0% ± 5.5% vs. 81.1% ± 5.4%, p = 0.274) (Fig. 2). With additional information, among the 16 CP patients who had no available qRT-PCR data at 3 months after SCT, five patients relapsed at a median of 2.8 months (range, 1.3 to 24.7) after SCT and one patient died.

Multivariate analyses including potential clinical parameters

Potential predictive factors affecting relapse, EFS, and OS were assessed, including age, sex, Sokal risk, disease status at diagnosis and transplant, prior therapy before SCT, time from diagnosis to transplant, EBMT score, year of transplant, donor type, graft source, conditioning regimen, and GVHD prophylaxis (Table 3). In univariate analyses, younger age and BM graft source were potential risk factors for relapse. The BC at the time of diagnosis was a potential factor affecting OS, and the graft source was associated with EFS. In multivariate analyses including the potential variables affecting relapse and EFS respectively, $MR^{4.5}$ at 3 months remained a predictive factor for higher EFS (risk ratio [RR], 2.73; 95% confidence interval [CI], 1.12 to 6.68; p = 0.028) and showed a trend for a lower relapse rate (RR, 2.46; 95% CI, 0.87 to 6.95; p = 0.089). However, $MR^{4.5}$ at 3 months had no influence on OS (RR, 2.04; 95% CI, 0.55 to 7.58; p = 0.285) (Table 4).

DISCUSSION

In this study, 64% (23 of 36) of post-transplant relapse,

defined as the ratio of *BCR-ABL1* to *ABL1* > 0.1% on the IS for two consecutive tests, occurred in the first 1 years of SCT. Therefore, we evaluated the predictive role of the *BCR-ABL1* transcript levels at 1, 3, 6, and 9 months after SCT by ROC analysis (Supplementary Fig. 1), showing that 3-month data was used with maximized sensitivity in the prediction of patients at risk of relapse. Finally, we observed that $MR^{4.5}$ at post-transplant 3 months was an early predictive factor for post-transplant relapse, and EFS in patients who underwent allogeneic SCT for CP CML. Several previous studies suggested that detection of *BCR-ABL1* transcripts by qRT-PCR is associated with an increased risk of relapse [7,8]. Considering the limitation of differences in *BCR-ABL1* kinetics in individual patients after allogeneic SCT, Asnafi et al. [8] evaluated the predictive role of BCR-ABL quantification at day 100 using qRT-PCR in 38 patients with > 1 year follow-up after conventional non-T-cell depleted SCT. They found that 14 patients with a high BCR-ABL/ABL ratio ($\geq 10^{-4}$) had a higher relapse rate than 24 patients with a negative/low ratio ($< 10^{-4}$).

In contrast, several studies suggested that an occasional positive test for *BCR-ABL1* transcripts that was derived only from proliferating leukemia cells should not be interpreted as clinical relapse. Kaeda et al. [9] observed that *BCR-ABL1* transcripts were detected at low levels in some patients for long periods after SCT without obvious progression. In their study, patients with a transcript level > 0.02% on three consecutive tests or > 0.05% on two consecutive tests were classified as having relapsed and were candidates for DLI. The other patients were classified into three categories: persistently negative for *BCR-ABL1* transcripts, intermittently positive at a low level, and persistently positive at a low level. Only a minority of patients with fluctuating or persistently low levels of *BCR-ABL1* transcripts satisfied their definitions of molecular relapse, whereas a majority of patients who satisfied their criteria for molecular relapse were likely to progress further [9]. Arpinati et al. [10] also reported results of 63 patients who underwent allogeneic SCT and had data from at least three qRT-PCR tests with a median follow-up of > 10 years. Eleven of the 63 evaluable patients never had *BCR-ABL1* detectable transcripts, and none of these relapsed. Six of the 52 patients who had *BCR-ABL1* transcripts detected at least once experienced post-transplant relapse. In their study,

pre-emptive treatment was applied upon achieving transcripts levels in excess of 0.1% that were confirmed by the finding of Philadelphia chromosome-positive metaphases in the bone marrow [10]. The results of the above studies are consistent in that patients with persistent absence of detectable transcripts never relapsed and some patients who intermittently had low levels of transcripts inevitably relapsed. However, because the dose and duration of immune suppressive therapy (IST) can tailored in patients who are at high risk for post-transplant relapse, it is important to evaluate early predictors for post-transplant relapse. In this study, a total of 71 patients had paired qRT-PCR data of 3 and 6 months. Of the patients who did not achieve $MR^{4.5}$ at 3 months, 57% of patients showed *BCR-ABL1* transcript > 0.1% at 6 months and compared with 7% in $MR^{4.5}$ group at 3 months, suggesting that a relatively fair number of these patients did eventually relapse. Moreover, the sensitivity of PCR technology has recently increased and the measurement of molecular responses has become standardized. Therefore, an early uniform *BCR-ABL1* transcript cutoff on the IS for post-transplant relapse may provide additional information to guide clinical decisions on IST modulation.

The notion that the graft-versus-leukemia effect can play a role in maintaining remission was supported by the beneficial effects of DLI [15] and chronic GVHD [16]. The duration and withdrawal of immunosuppressive treatment are known to be important factors influencing the risk of relapse [17]. DLI has proved effective for the treatment of patients who relapse after allogeneic SCT, with stable responses of 60% to 70% in CP recurrence [18-21]. After the introduction of TKIs, the combination of IM with DLI became an option for achieving remission in patients with relapse [22] and many experiences of IM treatment for CML recurrence after SCT have been reported [5,6,23-25]. Wright et al. [6] showed the feasibility of TKIs including IM and/or DAS, with 64% of patients achieving molecular responses. Although frontline 2G-TKI therapy demonstrated faster and deeper responses than IM, the role of 2G-TKIs for management of post-transplant relapse is still limited by the lack of studies with a large series of patients. In our study, $MR^{4.5}$ at 3 months was associated with a lower relapse rate and higher EFS, whereas there was no difference in OS. This might be because the $MR^{4.5}$ cut-off at 3 months

was determined in favor of sensitivity and not specificity, or because of the beneficial effect of TKI therapy for post-transplant relapse; 28 of the 36 relapsed patients received TKI therapy for post-transplant relapse and of them, 21 patients are alive in molecular remission, including UMRD in 16 patients, and seven patients died. Therefore, although the results of our study did not confirm that failure to achieve $MR^{4.5}$ at 3 months indicates pre-emptive treatment with IM and 2G-TKIs, we can suggest that frequent molecular monitoring and IST modulation are required for patients with no reduction in *BCR-ABL1* transcripts to these levels after SCT.

In our study, univariate analyses showed that use of PBSCs as a graft source was associated with a lower relapse rate and higher EFS, and that younger age was a potential factor for higher relapse. Considering that a PBSC source was used more frequently in the $MR^{4.5}$ group, we performed multivariate analyses including potential variables affecting relapse and EFS, and found that $MR^{4.5}$ at 3 months remained associated with higher EFS ($p = 0.028$) and showed a trend for a lower relapse rate ($p = 0.089$). In addition, the incidence of relapse was observed relatively lower in patients with cGVHD, compared with those of the patients without cGVHD ($p < 0.001$), supported by well-known graft versus leukemia effect associated with cGVHD [26,27]. In this study, the incidence of cGVHD was observed relatively lower in younger patients, which may be related with a higher relapse rate in younger age group. The significance of $MR^{4.5}$ at 3 months held within a multivariate analysis' model including cGVHD. Moreover, we observed no differences in aGVHD and cGVHD between the $MR^{4.5}$ group and no $MR^{4.5}$ group, suggesting that $MR^{4.5}$ at 3 months can be used as an independent predictive value.

In conclusion, our data showed that $MR^{4.5}$ at 3 months was associated with a lower relapse rate and higher EFS in patients who underwent allogeneic SCT for CP CML. This suggests that frequent molecular monitoring and intervention are required for patients who do not show a reduction in *BCR-ABL1* transcripts to these levels after SCT. Additionally, the type of graft source was associated with relapse and EFS, and younger age was associated with relapse. However, future studies are needed to evaluate the use of TKIs in patients with a higher risk for relapse after SCT.

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KEY MESSAGE

1. *BCR-ABL1* transcripts (MR$^{4.5}$) at post-transplant 3 months is predictive for relapse in allografted patients with chronic myeloid leukemia.
2. Immune suppressive therapy modulation based on the molecular monitoring is warranted.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

REFERENCES

1. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood 2013;122:872-884.

2. Lee SE, Choi SY, Kim SH, et al. Prognostic factors for outcomes of allogeneic stem cell transplantation in chronic phase chronic myeloid leukemia in the era of tyrosine kinase inhibitors. Hematology 2014;19:63-72.

3. Oyekunle A, Zander AR, Binder M, et al. Outcome of allogeneic SCT in patients with chronic myeloid leukemia in the era of tyrosine kinase inhibitor therapy. Ann Hematol 2013;92:487-496.

4. Khoury HJ, Kukreja M, Goldman JM, et al. Prognostic factors for outcomes in allogeneic transplantation for CML in the imatinib era: a CIBMTR analysis. Bone Marrow Transplant 2012;47:810-816.

5. Kantarjian HM, O'Brien S, Cortes JE, et al. Imatinib mesylate therapy for relapse after allogeneic stem cell transplantation for chronic myelogenous leukemia. Blood 2002;100:1590-1595.

6. Wright MP, Shepherd JD, Barnett MJ, et al. Response to tyrosine kinase inhibitor therapy in patients with chronic myelogenous leukemia relapsing in chronic and advanced phase following allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2010;16:639-646.

7. Olavarria E, Kanfer E, Szydlo R, et al. Early detection of BCR-ABL transcripts by quantitative reverse transcriptase-polymerase chain reaction predicts outcome after allogeneic stem cell transplantation for chronic myeloid leukemia. Blood 2001;97:1560-1565.

8. Asnafi V, Rubio MT, Delabesse E, et al. Prediction of relapse by day 100 BCR-ABL quantification after allogeneic stem cell transplantation for chronic myeloid leukemia. Leukemia 2006;20:793-799.

9. Kaeda J, O'Shea D, Szydlo RM, et al. Serial measurement of BCR-ABL transcripts in the peripheral blood after allogeneic stem cell transplantation for chronic myeloid leukemia: an attempt to define patients who may not require further therapy. Blood 2006;107:4171-4176.

10. Arpinati M, Tolomelli G, Bochicchio MT, et al. Molecular monitoring of BCR-ABL transcripts after allogeneic stem cell transplantation for chronic myeloid leukemia. Biol Blood Marrow Transplant 2013;19:735-740.

11. Cross NC, White HE, Muller MC, Saglio G, Hochhaus A. Standardized definitions of molecular response in chronic myeloid leukemia. Leukemia 2012;26:2172-2175.

12. Lee SE, Choi SY, Bang JH, et al. Predictive factors for successful imatinib cessation in chronic myeloid leukemia patients treated with imatinib. Am J Hematol 2013;88:449-454.

13. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus conference on acute GVHD grading. Bone Marrow Transplant 1995;15:825-828.

14. Lee SJ, Vogelsang G, Flowers ME. Chronic graft-versus-host disease. Biol Blood Marrow Transplant 2003;9:215-233.

15. Cullis JO, Jiang YZ, Schwarer AP, Hughes TP, Barrett AJ, Goldman JM. Donor leukocyte infusions for chronic myeloid leukemia in relapse after allogeneic bone marrow transplantation. Blood 1992;79:1379-1381.

16. van Rhee F, Lin F, Cross NC, et al. Detection of residual leukaemia more than 10 years after allogeneic bone marrow transplantation for chronic myelogenous leukaemia. Bone Marrow Transplant 1994;14:609-612.

17. Inamoto Y, Flowers ME, Lee SJ, et al. Influence of immunosuppressive treatment on risk of recurrent malignancy after allogeneic hematopoietic cell transplantation. Blood 2011;118:456-463.

18. Mackinnon S, Papadopoulos EB, Carabasi MH, et al. Adoptive immunotherapy evaluating escalating doses

of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: separation of graft-versus-leukemia responses from graft-versus-host disease. Blood 1995;86:1261-1268.

19. Dazzi F, Szydlo RM, Craddock C, et al. Comparison of single-dose and escalating-dose regimens of donor lymphocyte infusion for relapse after allografting for chronic myeloid leukemia. Blood 2000;95:67-71.

20. Kolb HJ, Schattenberg A, Goldman JM, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood 1995;86:2041-2050.

21. Collins RH Jr, Shpilberg O, Drobyski WR, et al. Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. J Clin Oncol 1997;15:433-444.

22. Savani BN, Montero A, Kurlander R, Childs R, Hensel N, Barrett AJ. Imatinib synergizes with donor lymphocyte infusions to achieve rapid molecular remission of CML relapsing after allogeneic stem cell transplantation. Bone Marrow Transplant 2005;36:1009-1015.

23. Olavarria E, Craddock C, Dazzi F, et al. Imatinib mesylate (STI571) in the treatment of relapse of chronic myeloid leukemia after allogeneic stem cell transplantation. Blood 2002;99:3861-3862.

24. Wassmann B, Klein SA, Scheuring U, et al. Hematologic and cytogenetic remission by STI571 (Glivec) in a patient relapsing with accelerated phase CML after second allogeneic stem cell transplantation. Bone Marrow Transplant 2001;28:721-724.

25. Kim YJ, Kim DW, Lee S, et al. Early prediction of molecular remission by monitoring BCR-ABL transcript levels in patients achieving a complete cytogenetic response after imatinib therapy for posttransplantation chronic myelogenous leukemia relapse. Biol Blood Marrow Transplant 2004;10:718-725.

26. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED. Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. N Engl J Med 1981;304:1529-1533.

27. Baron F, Maris MB, Sandmaier BM, et al. Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. J Clin Oncol 2005;23:1993-2003.

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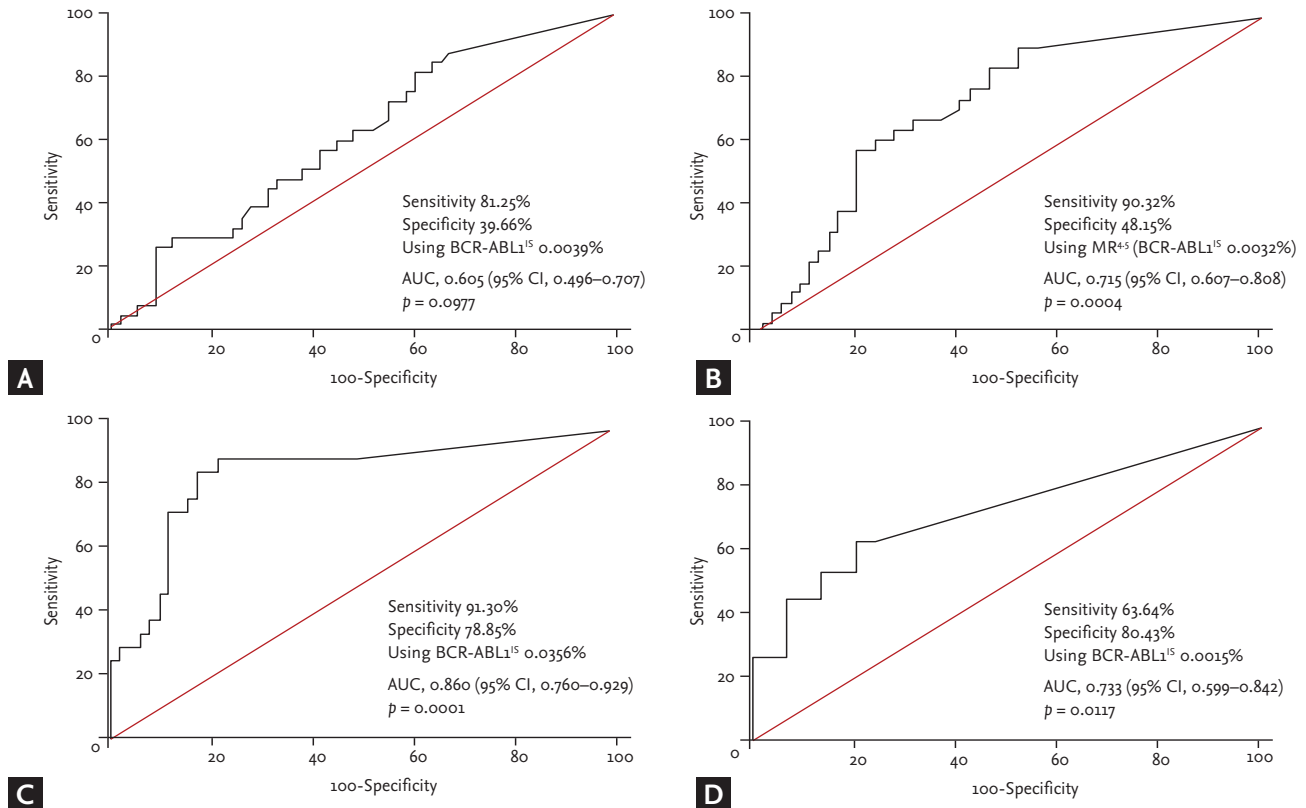
Supplementary Figure 1. Receiver operating characteristic (ROC) curves of *BCR-ABL1* transcript level on the international scale (IS) at post-transplant (A) 1, (B) 3, (C) 6, and (D) 9 months. At post-transplant 1, 3, 6, and 9 months, 90, 85, 75, and 57 patients had available quantitative reverse transcriptase polymerase chain reaction data. AUC, area under the ROC curve; CI, confidence interval; MR, molecular response.