Clinical impact of pre-transplant use of multiple tyrosine kinase inhibitors on the outcome of allo-HSCT for CML

Running Title: Use of multiple TKIs before allo-HSCT for CML

Takeshi Kondo[1], Tokiko Nagamura-Inoue[2], Arinobu Tojo[3], Fumitaka Nagamura[4], Naoyuki Uchida[5], Hirohisa Nakamae[6], Takahiro Fukuda[7], Takehiko Mori[8], Shingo Yano[9], Mineo Kurokawa[10], Hironori Ueno[11], Heiwa Kanamori[12], Hisako Hashimoto[13], Makoto Onizuka[14], Minoko Takanashi[15], Tatsuo Ichinohe[16], Yoshiko Atsuta[17, 18], and Kazuteru Ohashi[19]; on behalf of JSHCT adult CML/MPN Working Group.

1: Department of Hematology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

2: Department of Cell Processing/Transfusion, The Institute of Medical Science, Tokyo University, Tokyo, Japan

3: Department of Hematology and Oncology, The Institute of Medical Science, Tokyo University, Tokyo, Japan

4: Center for Translational Research, The Institute of Medical Science, Tokyo University, Tokyo, Japan

5: Department of Hematology, Federation of National Public Service Personnel Mutual Aid Associations Toranomon Hospital, Tokyo, Japan

6: Department of Hematology, Osaka City University Hospital, Osaka, Japan

7: Department of Hematopoietic Stem Cell Transplantation, National Cancer Center

Hospital, Tokyo, Japan

8: Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo, Japan

9: Hematopoietic Cell Therapy Center, Jikei University School of Medicine, Tokyo, Japan

10: Department of Cell Therapy and Transplantation Medicine, The University of Tokyo Hospital, Tokyo, Japan

11: Department of Hematology, National Hospital Organization Tokyo Medical Center, Tokyo, Japan

12: Department of Hematology, Kanagawa Cancer Center, Yokohama, Japan

13: Department of Hematology/Division of Stem Cell Transplantation, Kobe General Hospital/Institute of Biomedical Research and Innovation, Kobe, Japan

14: Department of Hematology/Oncology, Tokai University School of Medicine, Isehara, Japan

15: Blood Service Headquarters, Japanese Red Cross Society, Tokyo, Japan

16: Department of Hematology and Oncology, Research Institute for Radiation Biology and Medicine (RIRBM), Hiroshima University, Hiroshima, Japan

17: Japanese Data Center for Hematopoietic Cell Transplantation, Nagoya, Japan

18: Department of Healthcare Administration, Nagoya University Graduate School of Medicine, Nagoya, Japan

19: Hematology Division, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan

Correspondence: Takeshi Kondo, Department of Hematology, Graduate School of Medicine, Hokkaido University, Kita 15 Nishi 7, Kita-ku, Sapporo 060-8638, Japan; e-mail: t-kondoh@med.hokudai.ac.jp

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Abstract

Tyrosine kinase inhibitors (TKIs) are widely used to treat patients with chronic myelogenous leukemia in the chronic phase (CML-CP), and outcomes of TKI treatment for patients with CML-CP have been excellent. Since multiple TKIs are currently available, second-line or third-line TKI therapy is considered for patients who are intolerant of or resistant to the previous TKI treatment. Therefore, allogeneic hematopoietic stem cell transplantation (allo-HSCT) is considered only for patients with disease progression or for patients after treatment failure with multiple TKIs. To reflect the current clinical situation of patients with CML-CP, we tried to clarify whether prior TKI treatment affects the outcome of allo-HSCT. Data from 237 patients for whom the number of pre-transplant TKIs varied from one to three were used for analysis. Before allo-HSCT, 153 patients were treated with one TKI, 49 patients were treated with two TKIs and 35 patients were treated with three TKIs. In addition to conventional risk factors, i.e., disease status at transplantation and patient's age, the use of three TKIs before transplantation was identified as a significant adverse factor for prognosis. Non-relapse mortality rate was higher in patients treated with three TKIs than in patients treated with one or two TKIs. Our results suggest that allo-HSCT could be considered for young patients with CML-CP who manifest resistance to second-line TKI therapy and who have an appropriate donor.

Introduction

Chronic myelogenous leukemia (CML) is a myeloproliferative disorder characterized by the presence of the Philadelphia chromosome (Ph), which results from a reciprocal translocation between chromosomes 9 and 22. As a result of formation of t(9;22)(q34;q11), a novel fusion gene, *Bcr-abl*, is formed, and the subsequent chimeric tyrosine kinase, BCR-ABL, is constitutively active and plays a critical role in the pathogenesis of CML. Until 2001, allogeneic hematopoietic stem cell transplantation (allo-HSCT) was the only curable treatment for CML and was thus proactively considered for transplant-eligible patients [1,2]. However, after the introduction of the tyrosine kinase inhibitor (TKI) imatinib, which blocks the ATP-binding site of BCR-ABL, allo-HSCT has lost its position as the primary treatment option for patients with newly diagnosed CML in the chronic phase (CP). The International Randomized Study of Interferon versus STI571 (IRIS) for patients with newly diagnosed CML-CP showed that overall survival (OS) of patients treated with imatinib was 88% at 6 years [3]. In addition, the use of second-generation TKIs, dasatinib, nilotinib and bosutinib, has shown excellent results in patients with imatinib resistance or imatinib intolerance [4-8]. Furthermore, dasatinib and nilotinib showed higher efficacy than that of imatinib in treatment of patients with newly diagnosed CML-CP [9,10]. Therefore, allo-HSCT is now considered for patients with disease progression or for patients after treatment failure with multiple TKIs [11].

Since there has been an interest in the possible impact of prior TKI treatment

on the outcome of allo-HSCT, several retrospective studies on this issue were carried out. However, the early studies were based on imatinib use before transplantation, and the numbers of patients in recent studies, including cases treated with second-generation TKIs, were small [12-18]. Many patients who undergo allo-HSCT have received second-line or third-line TKI therapy. Therefore, it should be determined whether prior TKI treatment affects the outcome of allo-HSCT. To clarify this issue, we analyzed observational data provided by the Japan Society for Hematopoietic Cell Transplantation (JSHCT).

Methods

Patients

Data for patients of at least 20 years of age who were diagnosed as having CML-CP between 2001 and 2012 and underwent allo-HSCT were collected through the Transplant Registry Unified Management Program (TRUMP) [19-21]. Initially, data for 378 patients were collected. The criteria for inclusion of patients were 1) initially diagnosed as having CML-CP, 2) treated with at least one TKI, 3) available data for disease status at transplantation, 4) receiving first allo-HSCT and 5) available data for survival status. Data for 141 patients were excluded from the analysis since those patients did not fulfill the criteria for analysis (Supplementary Figure 1). The data for the excluded patients are shown in Supplementary Information. Other missing data were dealt with as missing data. Data for 237 patients were used for analysis. The

study was approved by the data management committees of the JSHCT as well as by the Ethical Committee of Hokkaido University Hospital.

Pre-transplant treatments and disease status at transplantation

All patients were diagnosed as having CML-CP and were primarily treated with a tyrosine kinase inhibitor. After the second-generation TKIs dasatinib and nilotinib became available, treatment for some patients was changed to second-line or third-line TKI treatment because of intolerance or treatment failure. Therefore, the number of TKIs used before transplantation varied from one to three. Disease status at transplantation was determined according to the WHO classification 2008 and categorized as chronic phase 1 (CP1), chronic phase 2 (CP2), chronic phase 3 or later (CP3-), accelerated phase (AP) and blastic crisis (BC) [22]. Data for cytogenetic and/or molecular response at transplantation were also collected if available.

Conditioning regimens, donor source and post-transplant monitoring

The intensity of the conditioning regimen, myeloablative or reduced intensity, was defined as previously described [23]. Briefly, a myeloablative conditioning regimen includes one of the following treatments: total body irradiation $\geq$ 5 Gy in a single dose or $\geq$ 8 Gy fractionated or busulfan > 8 mg/kg orally or intravenous equivalent dose. Donor sources were related bone marrow (rBM), unrelated BM (uBM), related peripheral blood (rPB) and unrelated cord blood (CB). HLA matching was performed

on the basis of low-resolution antigens of HLA-A and HLA-B and high-resolution molecular typing of HLA-DRB1 as previously described [24]. HLA antigen disparities were categorized as either GVH or HVG direction. Relapse was defined by the reappearance of not only blasts or the Philadelphia chromosome but also *BCR-ABL* fusion transcripts. Non-relapse mortality (NRM) was defined as death during continuous remission.

Statistical analysis

Group characteristics associated with patient-related variables were compared with the chi-squared test for categorical variables and analysis of variance (ANOVA) for continuous variables. OS was defined as the time between transplantation and death due to any cause. Leukemia-free survival (LFS) was defined as the time interval from transplantation to the first event, either relapse or death, in patients who achieved complete remission. The probabilities of OS and LFS were calculated using the Kaplan-Meier method and were statistically analyzed by the log-rank test. Cumulative incidences of relapse and NRM were calculated by Gray's test. To examine the factors influencing OS ad LFS, the following variables were initially analyzed in univariate analysis: pre-transplantation TKI use, graft source, donor-recipient gender combination, HLA disparities, disease status at transplantation, interval between diagnosis and transplantation, patient's age at transplantation, conditioning regimen and cytogenetic/molecular status at transplantation. Subsequently, a stepwise multivariate

analysis was performed using the Cox regression model to identify potential prognostic factors for OS and LFS and by the Fine-Gray proportional hazard regression model for relapse incidence (RI) and NRM. $P<0.05$ was used as a significance level.

Results

Patient characteristics

The demographics of patients are shown in Table 1. A total of 237 patients (155 men, 82 women; median age, 42 years; range, 20–67 years) were initially diagnosed as having CML-CP and eventually underwent allogeneic HSCT. The male predominance in the incidence of CML in Japan was consistent with a previous study [25]. All of the patients had a history of pre-transplant TKI use. Before transplantation, 153 patients were treated with a single TKI (TKI=1), 49 patients were treated with second-line TKI (TKI=2) and 35 patients were treated with third-line TKI (TKI=3). The TKIs used before transplantation were imatinib alone in 151 patients, dasatinib alone in two patients, imatinib and dasatinib in 44 patients, imatinib and nilotinib in five patients, and three TKIs in 35 patients. As for disease status at transplantation, 97 patients remained in CP1, 50 patients were in CP2, seven patients were in CP3-, 32 patients were in AP, and 51 patients were in BC. Therefore, 140 patients underwent allo-HSCT at an advanced disease status. The other variables, including graft source, donor-recipient gender combination (male recipient/female donor v.s. others), patient's age, GVHD prophylaxis, duration from diagnosis to transplantation, conditioning

intensity, HLA disparities, ABO mismatch and cytogenetic/molecular status at transplantation, are shown in Table 1. Either the chi-squared test or ANOVA showed that duration from diagnosis to transplantation, conditioning regimen, disease status at transplantation and molecular response at transplantation were significantly different depending on the number of pre-transplant TKIs used.

Overall survival and leukemia-free survival

The median follow-up period after transplantation was 727 days (range, 13-4191 days). Two-year OS was 63.7% (95% CI, 57.0%-69.7%) and LFS was 57.0% (95% CI, 50.2%-63.1%) in all of the patients (Supplementary Figure 2). According to the number of pre-transplant TKIs used, two-year OS and LFS were 64.3% and 57.9% in patients with TKI=1, 69.2% and 62.2% in patients with TKI=2 and 53.9% and 46.1% in patients with TKI=3, respectively (Figure 1). OS and LFS in patients with TKI=3 were significantly worse than those in the other groups (TKI=1/2). Two-year OS and LFS were 66.7% and 60.7% in patients with TKI=1/2 and 53.9% and 46.1% in patients with TKI=3, respectively (p=0.020 in OS and p=0.027 in LFS, Supplementary Table 1). We also analyzed the prognostic impact of donor source, donor-recipient gender combination, HLA disparities, disease status at transplantation, duration from diagnosis to transplantation, patient's age at transplantation, intensity of the conditioning regimen, cytogenetic status of the Philadelphia chromosome at transplantation, and molecular status of *bcr-abl* transcript at transplantation. In addition to TKI=3, donor source (cord

blood), HLA disparities (both GVH and HVG directions), advanced disease stage at transplantation and patient's age (≥50 y.o.) had negative impacts on both OS and LFS, and no major cytogenetic response (no MCyR) had a negative impact on LFS (Supplementary Table 1 and Supplementary Figure 3). These factors were subjected to a stepwise multivariate analysis using the Cox regression model. As shown in Table 2, TKI=3 remained an adverse risk factor for both OS and LFS. HLA disparities (≥2 loci for GVH direction), advanced age (≥50 y.o.) at transplantation and advanced disease stage were also adverse risk factors for both OS and LFS, while CB as a graft source and no MCyR were not adverse risk factors after multivariate analysis. This is possibly because CB as a graft source and no MCyR were significantly associated with adverse risk factors identified by multivariate analysis (Supplementary Information).

Relapse incidence and NRM

Since TKI=3 was identified as an adverse risk factor for both OS and LFS, we tried to determine the reason for patients with TKI=3 having a lower survival rate. We analyzed the cumulative relapse incidence (RI) and NRM by Gray's test. Two-year RI and NRM were 24.0% and 17.0% in patients with TKI=1/2 and 20.1% and 33.8% in patients with TKI=3, respectively (p=0.840 in RI, p=0.005 in NRM, Supplementary Table 2 and Supplementary Figure 4a). In addition to the number of pre-transplant TKIs used, we also analyzed the clinical impacts of other factors that were used in the analysis of OS and LFS. As a result, CB as a graft source, HLA disparities for GVH

direction (≥2 loci), disease status at transplantation (advanced stage), patient's age at transplantation (≥50 y.o.) and cytogenetic status at transplantation (no MCyR) were significant adverse factors for RI. In addition to TKI=3, male recipient/female donor combination, pre-transplant duration (longer than 3 years), patient's age at transplantation (≥50 y.o.) and intensity of the conditioning regimen (reduced intensity conditioning) were identified as significant factors for NRM (Supplementary Table 2 and Supplementary Figure 4). These factors were subjected to a stepwise multivariate analysis using the Fine-Gray proportional hazard regression model. As shown in Table 3, advanced disease stage at transplantation was significantly associated with higher RI, and TKI=3 remained a significant adverse factor associated with higher NRM rate. Moreover, male recipient/female donor combination and patient's age at transplantation (≥50 y.o.) were identified as risk factors for NRM. On the other hand, CB as a graft source, HLA disparities for GVH direction, pre-transplant duration (≥3 years), intensity of conditioning regimen and cytogenetic status at transplantation were not significant factors after multivariate analysis, since these factors were tightly associated with significant adverse risk factors identified by multivariate analysis (Supplementary Information).

Discussion

After TKIs became available for treatment of CML, the effect of pre-transplant TKI use on the outcome of allo-HSCT was analyzed. Previous studies with a large number of

patients showed that pre-transplant imatinib use has no negative effect on the outcome of all-HSCT for CML [12-15]. There have also been a few studies on second-generation TKI use before allo-HSCT. Although the results of those studies suggested that second-generation TKI use before allo-HSCT did not increase transplant-related toxicity, the number of patients was small and cases diagnosed not only as chronic phase but also as accelerated phase or blastic crisis were included in those studies [16-18].

Since treatment of CML-CP with a second-generation TKI has shown good therapeutic responses in imatinib-intolerant, imatinib-resistant and newly diagnosed patients, allo-HSCT is now considered only for patients for whom treatment with at least 2 TKIs failed and for patients who progressed to an advanced phase. To reflect the current clinical situation of patients with CML-CP, our study was based on patients who were primarily treated with a TKI and underwent allo-HSCT. Thus, compared with previous studies, one prominent feature of our analysis is the inclusion of a substantial number of patients treated with two or three TKIs before transplantation (49 patients with TKI=2 and 35 patients with TKI=3). Therefore, we tried to clarify the clinical impact of the use of multiple TKIs before allo-HSCT.

From our analysis, TKI=3 was identified as an adverse risk factor for both OS and LFS, and the reason for patients with TKI=3 having a low survival rate was a higher rate of NRM. Moreover, HLA disparities for GVH direction, advanced age and advanced disease status at transplantation were associated with low rate of both OS and

LFS. As expected, lower survival rates were ascribed to increased NRM rate in patients of advanced age (≥50 y.o.) and higher RI in patients with advanced disease status at transplantation. HLA disparity for GVH direction was a significant factor for both OS and LFS after multivariate analysis; however, it was significantly associated with RI only in univariate analysis. On the other hand, male recipient/female donor combination was a significant risk factor for NRM by multivariate analysis, though it was not a significant adverse factor for OS or LFS. Therefore, HLA disparities for GVH direction and male recipient/female donor combination are potential clinical factors that affect patient prognosis.

Intriguingly, the results of our study showed that patients who received three TKIs (imatinib, nilotinib and dasatinib) before allo-HSCT had a lower survival rate than that of patients treated with one or two TKIs. The reason for patients with TKI=3 having inferior survival was increased NRM. On the other hand, OS and LFS were similar in patients with TKI=1 and patients with TKI=2. It is difficult to determine the mechanism underlying the increased NRM in patients with TKI=3. Each TKI has multiple off-targets, which may have some effects on the immune system or vascular endothelial cells [26-28]. A possible explanation is that prolonged and multiple use of TKIs, i.e., TKI=3, deteriorates the condition of patients via such off-target effects. Another possible explanation is that patients with TKI=3 might received chemotherapy in addition to TKIs because of disease progression. In fact, the proportions of patients with advanced phase at transplantation was higher in patients with TKI=3 than in

patients with TKI=1. Although the proportions of advanced phase in patients with TKI=2 was similar to that in patients with TKI=3, the reason of high NRM rate in patients with TKI=3 may be caused by other treatment than TKI use.

Although treatment with three TKIs before allo-HSCT was shown to be a poor prognostic factor in our analysis, it should be pointed that most of the patients, 235 out of 237 patients, were primarily treated with imatinib. Because most of the analyzed patients were diagnosed before 2007, only available TKI was imatinib at the time of diagnosis. In the current situation in CML treatment, a second-generation TKI is frequently chosen for both first-line and second-line treatment. Therefore, analysis for patients who are treated with TKIs other than imatinib and undergo all-HSCT is still required.

In addition to TKI=3, HLA disparities for GVH direction, advanced disease stage at transplantation and advanced age were identified as adverse risk factors for both OS and LFS in our analysis (Table 3). Classically, the EBMT scoring system was proposed to predict a patient's risk according to transplant-related parameters including donor type (HLA-identical sibling vs. others), disease stage at transplantation (CP1 vs. AP vs. others), age at transplantation (<20 y.o. vs. 20 to 40 y.o. vs. ≥40 y.o.), interval from diagnosis to transplantation (<1 yr vs. ≥1 yr) and donor/patient sex-compatibility (male recipient/female donor vs. others) [29]. From our analysis, age at transplantation and disease stage at transplantation were strong factors influencing both OS and LFS, and donor/patient sex-compatibility was associated with NRM. However,

different from the EBMT score, age of 50 years at transplantation clearly stratified patients in association with outcome (Table 3, Supplementary Figure 1i). Regarding donor source, there was no difference in either OS or LFS between rBM and uBM (Supplementary Figure 1a). Recent progress in the management of transplantation-related toxicity may change the current risk factors of allo-HSCT for CML.

To determine the indication and timing of allo-HSCT for patients with CML, we need to evaluate the risks and benefits of the treatment. After treatment failure with imatinib, a second-generation TKI is a choice of therapy. However, based on previous reports, long-term survival of patients who received second-line and third-line TKI treatment is not so good. It was reported that two-year OS and PFS rates in imatinib-resistant/intolerant patients were 91% and 80% with second-line dasatinib treatment, 87% and 64% with second-line nilotinib treatment and 92% and 79% with second-line bosutinib treatment, respectively [8,30,31]. Moreover, previous studies suggested that disease progression frequently occurs during third-line TKI treatment [32,33].

The long-term outcomes of patients with newly diagnosed CML, who were eligible for allo-HSCT and were randomized by availability of a matched family donor between allo-HSCT and best available drug treatment, have recently been reported. In that study, patients with an available related donor and low adapted EBMT score had significantly higher survival probability than did patients who received treated drug

treatment [34]. Our results indicated that TKI=3 is a significant factor for survival after allo-HSCT besides disease progression and patient's age. Allo-HSCT could be considered for young patients with CML showing resistance to second-line TKI therapy who did not have disease progression and who have an appropriate donor.

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Authorship

Contribution: T.K. designed the study, reviewed and analyzed data, and wrote the paper, T.N-I., A.T., and K.O. interpreted data and revised the manuscript, F.N. supervised statistical analysis, and N.U., H.N., T.F., T.M., S.Y., M.K., H.U., H.K., H.H., M.O., M.T., T.I., and Y.A. contributed to the data collection and provided critique to the manuscript. All authors read and approved the final manuscript.

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Figure Legends

Figure 1. Kaplan-Meier estimates of OS and LFS after allo-HSCT according to number of pre-transplant TKIs used. 2-year OS and 2-year LFS were 64.3% and 57.9% in patients with TKI=1, 69.2% and 62.2% in patients with TKI=2, and 53.9% and 46.1% in patients with TKI=3, respectively.

Figure 1.

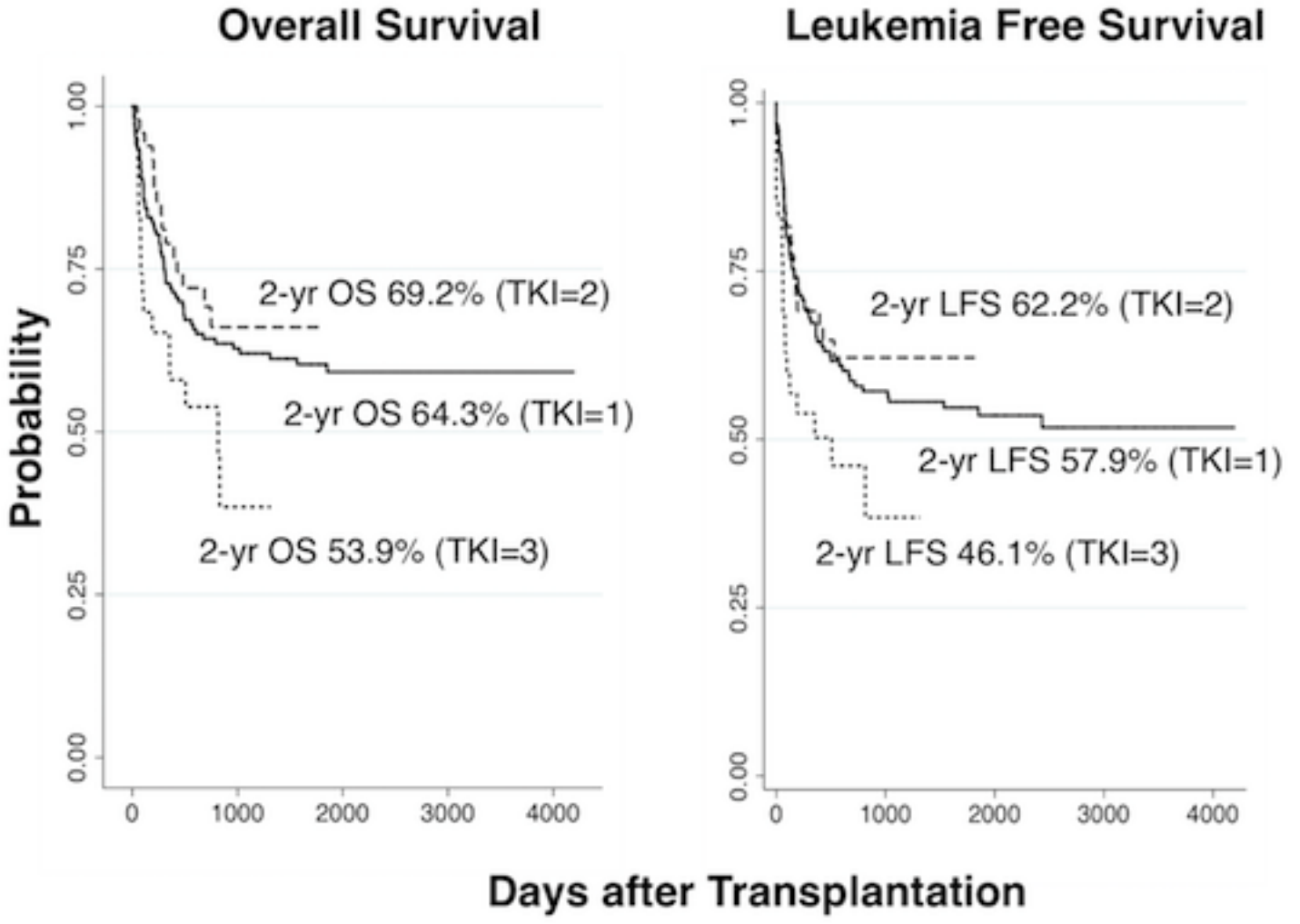
Overall Survival
Leukemia Free Survival
Probability
1.00
0.75
0.50
0.25
0.00
2-yr OS 69.2% (TKI=2)
2-yr OS 64.3% (TKI=1)
2-yr OS 53.9% (TKI=3)
2-yr LFS 62.2% (TKI=2)
2-yr LFS 57.9% (TKI=1)
2-yr LFS 46.1% (TKI=3)
0
1000
2000
3000
4000
Days after Transplantation

Table 1. Clinical Characteristics of Patients

Number of TKI use	Total	TKI=1	TKI=2	TKI=3	missing data	χ2-test, p	ANOVA, p
Patient number	**237**	**153**	**49**	**35**	**0**		
Gender (F/M)	82/155	59/94	13/36	10/25	0	0.219	
Graft source (rBMT/rPBSCT/uBMT/CBT)	**43/49/87/58**	**34/33/54/32**	**4/9/18/18**	**5/7/15/8**	**0**	**0.182**	
CBT (yes/no)	58/179	32/121	18/31	8/27	0	0.079	
Male recipient, female donor/Other combinations	**48/176**	**31/118**	**11/33**	**6/25**	**13**	**0.800**	
Age at SCT, median y.o. (range)	42 (20-67)	42 (20-67)	42 (21-66)	44 (23-66)	0		0.239
Age (20-49 / 50-)	**170/67**	**109/44**	**39/10**	**22/13**	**0**	**0.238**	
GVHD prophylaxix (CyA-based/FK506-based/others)	123/112/2	88/63/2	21/28/0	14/21/0	0	0.127	
Duration from diagnosis to SCT, median days (range)	**631 (95-4166)**	**559 (95-3664)**	**637 (131-4166)**	**1105 (267-4110)**	**0**		**<0.001**
Duration from diagnosis to SCT (1 year≥ / >1 year)	62/175	50/103	11/38	1/34	0	0.001	
Duration from diagnosis to SCT (2 year≥ / >2 year)	**134/103**	**96/57**	**29/20**	**9/26**	**0**	**<0.001**	
Duration from diagnosis to SCT (3 year≥ / >3 year)	174/63	124/29	34/15	16/19	0	<0.001	
Conditioning (MAST/RIST)	**194/43**	**121/32**	**46/3**	**27/8**	**0**	**0.048**	
HLA disparities (gvh direction 0-1/≥2)	188/49	123/30	36/13	29/6	0	0.497	
HLA disparities (hvg direction 0-1/≥2)	**195/42**	**128/25**	**38/11**	**29/6**	**0**	**0.619**	
ABO mismatch (match/mismatch)	109/127	72/80	19/30	18/17	1	0.460	
CML status at SCT (CP1/CP2/CP3-/AP/BC)	**97/50/7/32/51**	**74/32/2/18/27**	**12/12/2/7/16**	**11/6/3/7/8**	**0**	**0.027**	
Cytogenetic Response at SCT (CCyR/PCyR/Others)	73/43/109	45/31/68	19/5/23	9/7/18	12	0.435	
Molecular Response at SCT (MMR/Others)	**28/161**	**9/106**	**11/33**	**8/22**	**48**	**0.003**	

Table 2. Multivariate analysis of Variables affecting OS and LFS

Outcome		OS			LFS		
Factors		HR	95% CI	p-value	HR	95% CI	p-value
Pre-transplant TKI number	TKI=1/2	1			1		
	TKI=3	2.011	1.161 - 3.483	0.013	1.708	1.020 - 2.860	0.042
HLA disparities (GVH direction)	0-1	1					
	≥ 2	1.817	1.130 - 2.923	0.014	1.626	1.027 - 2.572	0.038
Age at SCT	50 yo＞	1			1		
	≥ 50 yo	2.805	1.834 - 4.292	<0.001	2.731	1.827 - 4.083	<0.001
CML status at SCT	CP1	1			1		
	CP2	1.851	1.036 - 3.311	0.038	1.611	0.937 - 2.769	0.084
	CP3-	2.197	0.794 - 6.078	0.130	1.671	0.613 - 4.553	0.316
	AP	1.532	0.765 - 3.067	0.228	1.729	0.914 - 3.270	0.092
	BC	2.461	1.421 - 4.261	0.001	2.353	1.416 - 3.911	0.001

Table 3. Multivariate Analysis of Variables affecting RI and NRM

Outcome	Factors		HR	95% CI	p-value
RI	Disease status at SCT	CP1	1		
		CP2	2.163	0.858 – 5.455	0.100
		CP3-	1.110	0.158 – 7.784	0.920
		AP	2.378	0.902 – 6.269	0.080
		BC	5.299	2.385 – 11.770	<0.001
NRM	Pre-transplant TKI Number	TKI=1/2	1		
		TKI=3	2.554	1.185 – 5.504	0.017
	Male recipient/Female donor	no	1		
		yes	2.107	1.022 – 4.346	0.044
	Age at SCT	-49	1		
		50-	3.016	1.520 – 5.984	0.002