Prognostic Values of D816V *KIT* Mutation and Peri-transplant *CBFB-MYH11* MRD Monitoring on Acute Myeloid Leukemia with *CBFB-MYH11*

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Abstract

Given the controversies in the prognostic value of *KIT* mutations and optimal thresholds and time points of MRD monitoring for AML with *CBFB-MYH11*, we retrospectively evaluated 88 patients who underwent allogeneic hematopoietic stem cell transplantation (Allo-HSCT, n=60) or autologous HSCT (Auto-HSCT, n=28). The D816V *KIT* mutation was significantly associated with post-transplant relapse, contrasting with other types of mutations in *KIT*. Pre- and post-transplant (3 months after transplant) *CBFB-MYH11* MRD assessments were useful in predicting post-transplant relapse and poor survival. The optimal threshold was determined as a 2 log reduction at both time points. In multivariate analysis, the D816V *KIT* mutation and *CBFB-MYH11* MRD assessments were independently associated with post-transplant relapse and survival. Stratification by D816V *KIT* and pre-transplant *CBFB-MYH11* MRD status further distinguished the risk of relapse and survival. In conclusion, this study demonstrated the differentiated prognostic value of the D816V *KIT* mutation in AML with *CBFB-MYH11* and clarified optimal time points and thresholds for *CBFB-MYH11* MRD monitoring in the setting of HSCT.

Background

Acute myeloid leukemia (AML) with *CBFB-MYH11* is a heterogeneous disease entailing different prognoses. Various prognostic factors including *KIT* mutations and measurable residual disease (MRD) status have been proposed. Many types of *KIT* mutations have been identified but their prognostic value remains controversial. Furthermore, *CBFB-MYH11* quantification by real-time quantitative polymerase chain reaction (RT-qPCR) is known useful as an MRD tool for predicting relapse, but there is no consensus regarding sample sources, thresholds, and time points for MRD assessment with *CBFB-MYH11*.

Objectives

We have evaluated the prognostic value of different types of KIT mutations and *CBFB-MYH11* quantification in AML patients with *CBFB-MYH11* who underwent Allo-HSCT or Auto-HSCT. Furthermore, we determined optimal thresholds and time points of CBFB-MYH11 quantification during the peri-transplant period.

Material & Methods

We retrospectively evaluated 96 consecutive AML patients with *CBFB-MYH11* in remission who underwent HSCT at the Catholic Hematology Hospital between 2009 and 2019. 88 patients were finally included in this study. All patients were treated with intensive induction and one or two cycles of consolidation chemotherapy. Prognostic impacts of the KIT mutation types and MRD assessment on overall survival (OS), disease-free survival (DFS) and the cumulative incidence of relapse (CIR) were evaluated. We tested different thresholds for levels of *CBFB-MYH11* or log reduction compared to levels at diagnosis. Receiver operating characteristic (ROC) curve analysis was used to determinate optimal time points for MRD assessment with *CBFB-MYH11*.

Results

Table 1. Patient-, disease-, and transplant-related characteristics according to *KIT* mutations

Variables	Overall (n=88)	KIT unmutated (n=58)	KIT mutated (n=30)	p
Age at transplantation, years, n (%)				
Median (range)	45 (18-69)	44 (18-69)	45 (20-65)	0.930
Sex, n (%)				1.000
Male	53 (60)	35 (60)	18 (60)	
Female	35 (40)	23 (40)	12 (40)	
AML type, n (%)				0.603
De novo	84 (96)	56 (97)	28 (93)	
Therapy-related	4 (4)	2 (3)	2 (7)	
WBC count per liter at diagnosis, n (%)				
Median (range)	37.51 (0.26-869.7)	39.8 (0.26-274.7)	24.43 (3.14-869.7)	0.755
Additional cytogenetic abnormalities, n (%)				
Trisomy 22	17 (19)	10 (17)	7 (23)	0.493-
Trisomy 8	8 (9)	6 (10)	2 (7)	0.711
Loss of sex chromosome	1 (1)	1 (2)	0	1.000
Del(7q)	6 (7)	4 (7)	2 (7)	1.000
Complex karyotype	7 (8)	5 (9)	2 (7)	1.000
KIT mutations n (%)				
Exon 17-D816V	15 (18)	0	15 (50)	-
Exon 17-D816Y	10 (12)	0	10 (33)	-
Exon 17-D816H	3 (4)	0	3 (10)	-
Exon 17-N822K	2 (2)	0	2 (7)	-
Exon 8	7 (8)	0	7 (23)	-
CBFB-MYH11 transcripts at pre-HSCT				
Median copies (range)*	497.5 (0-96000.0)	469.0 (0-96000.0)	756.5 (0-28300.0)	0.763
Persistent transcripts	76 (86)	47 (81)	29 (97)	0.052
Log reduction at pre-HSCT				
≥ 2 log reduction	77 (88)	52 (90)	25 (83)	0.395
Persistent transcripts / no transcript	65 (84) / 12 (16)	41 (79) / 11 (21)	24 (96) / 1 (4)	
≥ 3 log reduction	38 (49)	26 (50)	12 (48)	
< 2 log reduction	11 (12)	6 (10)	5 (17)	
FLT3 mutations, n (%)				
FLT3-ITD	0	0	0	-
FLT3-TKD	9 (10)	8 (14)	1 (3)	0.146
Missing data	5 (6)	5 (9)	0	-
Disease status at HSCT, n (%)				0.090
CR1	81 (92)	51 (88)	30 (100)	
CR2	7 (8)	7 (12)	0 (0)	
Donor type, n (%)				0.327
Matched sibling	28 (32)	18 (31)	10 (33)	
Matched unrelated	22 (25)	12 (21)	10 (33)	
Haploidentical	10 (11)	6 (10)	4 (13)	
Autologous	28 (32)	22 (38)	6 (20)	
Stem cell source, n (%)				0.180
Peripheral blood	63 (72)	38 (66)	25 (83)	
Bone marrow	13 (15)	10 (17)	3 (10)	
Peripheral blood and bone marrow	12 (14)	10 (17)	2 (7)	
Conditioning intensity, n (%)				0.901
Myeloablative	52 (59)	34 (59)	18 (60)	
Reduced-intensity	36 (41)	24 (41)	12 (40)	
CD34⁺ cells x 10^6/kg in graft				
Median (range)	3.86 (0.37-16.99)	3.78 (0.37-13.50)	4.35 (1.67-16.99)	0.092

Abbreviations: AML, acute myeloid leukemia; CR1, first complete remission; CR2, second complete remission; HSCT, hematopoietic stem cell transplantation; n, number; WBC, white blood cells

Figure 1. Survival outcomes according to *KIT* mutations (A) or D816V *KIT* mutations (B)

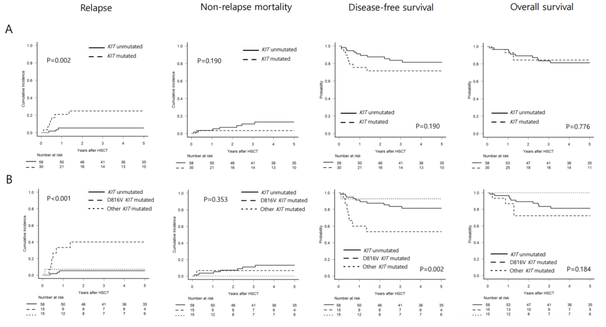
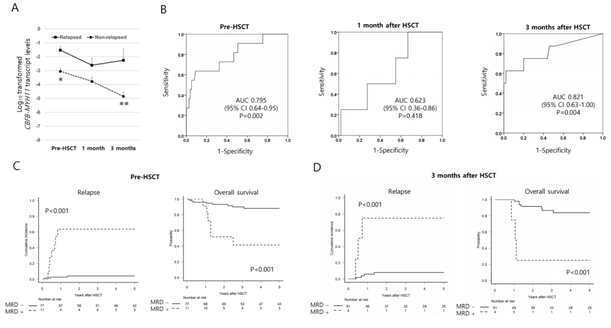


Figure 2. Optimal time points and thresholds for *CBFB-MYH11* MRD monitoring



(A) Changes of *CBFB-MYH11* levels according to the occurrence of relapse (numbers of relapsed patients vs. non-relapsed patients; pre-HSCT, 11 vs. 71; 1 month, 4 vs. 51; 3 months, 8 vs. 50) during the peri-transplant period. Results are expressed as mean ± SEM. * $p < 0.05$, ** $p < 0.01$. AUC indicates area under the curve.

(B) Receiver operating characteristic (ROC) curve analysis of *CBFB-MYH11* levels at each time point. *CBFB-MYH11* levels were normalized to the number of *ABL1* transcripts and expressed as copy numbers per 10^5 copies of *ABL1*.

(C) Cumulative incidence of relapse and overall survival according to MRD positivity defined by 2 log reduction in *CBFB-MYH11* levels at pre-HSCT (C) and 3 months after HSCT (D).

Table 2. Multivariate analyses to evaluate the prognostic independency of D816V *KIT* mutation and *CBFB-MYH1* MRD status at each time point for predicting post-transplant relapse and disease-free survival

Variables	Pre-HSCT N	Pre-HSCT HR (95% CI)	Pre-HSCT P value	3 months after HSCT N	3 months after HSCT HR (95% CI)	3 months after HSCT P value
Cumulative incidence of relapse						
CBFB-MYH1 levels						
≥ 2 log reduction	77	1		51	1	
< 2 log reduction	11	19.3 (4.52-82.34)	<0.001	4	22.15 (5.26-93.32)	<0.001
D816V KIT mutation						
Unmutated	73	1		45	1	
Mutated	15	11.6 (2.65-50.89)	0.001	10	8.21 (1.96-34.35)	0.004
Disease-free survival						
CBFB-MYH1 levels						
≥ 2 log reduction	77	1		51	1	
< 2 log reduction	11	5.16 (1.97-13.49)	<0.001	4	9.83 (2.49-38.76)	0.001
D816V KIT mutation						
Unmutated	73	1		45	1	
Mutated	15	4.16 (1.64-10.55)	0.003	10	3.29 (0.95-11.39)	0.061

Discussion

We showed that the D816V *KIT* mutation, a subtype of exon 17 mutations, had differentiated prognostic impact on post-transplant relapse and survival in a unique cohort of transplanted patients in AML with *CBFB-MYH11*. On the other hands, Other types of mutations in exons 17 (D816H, D816Y, or N822K) and 8 showed no difference in survival outcomes with *KIT* unmutated patients. The conflicting values of *KIT* mutations in previous reports in AML with *RUNX1-RUNX1T1* or *CBFB-MYH11* may be caused by a lack of information about each site of mutation in the *KIT* gene.

Furthermore, our data, including the information of each type of *KIT* mutation, confirmed the feasibility of peri-transplant *CBFB-MYH11* MRD monitoring to identify patients with a high risk of relapse but with different optimal time points and cut-off compared to those of previous studies; optimal time points were pre-HSCT or 3 months after HSCT, and optimal cutoffs were 2 log reduction of BM *CBFB-MYH11* level at both pre-HSCT and 3 months after HSCT.

In addition, our data showed that the D816V *KIT* mutation held the prognostic impact irrespective of pretransplant *CBFB-MYH11* MRD status defined by 2 log reduction and multivariate analysis supports its independent prognostic power.

Conclusion

In conclusion, this study demonstrated the differentiated prognostic impact of the D816V *KIT* mutation among various *KIT* mutations in AML with *CBFB-MYH11* and clarified optimal time points and thresholds for *CBFB-MYH11* MRD monitoring in the setting of HSCT. Both the D816V *KIT* mutation and peri-transplant MRD monitoring with *CBFB-MYH11* were independently associated with post-transplant relapse and survival, providing a platform for decision-making or risk-adapted therapeutic approaches.