European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 08, Issue 02, 2021

BCR-ABL type of mutation and hematology response to imatinib mesylate in chronic phase CML patient: a retrospective study in a tertiary referral hospital in Indonesia

Mohamad Arif[1], Mardiah Suci Hardianti[2*], Johan Kurnianda[2], Ibnu Purwanto[2], Kartika Widayati Taroeno-Hariadi[2], Susanna Hilda Hutajulu[2]

[1]Departement of Internal Medicine, Faculty of Medicine,Universitas Islam Sultan Agung, Semarang, Indonesia

[2]Division of Hematology and Medical Oncology, Department of Internal Medicine, Faculty of Medicine Public Healthand Nursing, Universitas Gadjah Mada - Dr. Sardjito Hospital, Yogyakarta, Indonesia

Abstract

Background: The BCR-ABL mutation types were reported influencing the treatment of Chronic Myelogenous Leukemia (CML) due to different responses to the use of tyrosine kinase inhibitor. However, the clinical score system, that is, EUTOS score is also considered as one essential factor in determining the treatment outcome. This study aims at observing the hematology response among chronic phase CML patients atSardjito Hospital Yogyakarta, Indonesia based on BCR-ABLmutation types, namely b3a2 and non-b3a2, with evaluation of EUTOS scores.

Methods: A retrospective cohort of 72 subjects from January 2013 to November 2018 was analyzed according to mutation types and EUTOS scores to observe the hematological response within 6 months after initiation of Imatinib therapy.

Results: Forty-eight subjects (66.7%) had b3a2 mutations, while others had non-b3a2 mutations. The median of spleen size was significantly higher in non-b3a2 mutations ($p = 0.024$). Only 49 subjects (68%) achieved the complete hematological response (CHR) within 6 months. The non-b3a2 mutation subjects were likely to have worse clinical and laboratory profiles than the b3a2 mutation subjects did ($p > 0.05$). The b3a2 mutation subjects tended toachieve earlier CHR than non-b3a2 mutation subjects (HR 1.031; $p = 0.919$). The latter subjects with high EUTOS scores had longer time to achieve the CHR ($p = 0.781$).

Conclusion: Cases with non-b3a2 mutations were presented with worse clinical and laboratory profiles than those with b3a2 mutations. In addition, the non-b3a2 mutations with higher EUTOS scores tended to have longer time to achieve the CHR.

Keywords: Chronic Myelogenous Leukemia, imatinib, hematological response, BCR-ABL b3a2; EUTOS score.

A. Introduction

CML is one hematological malignancy type remarked by the clonal expansion of hematopoietic cells resulted from the reciprocal translocation between parts of chromosome 9 and 22 [1]. This translocation produces a new hybrid gene called the *BCR-ABL* gene which encodes the formation of oncoproteins circulating in the cytoplasm and has a strong tyrosine kinase activity. These oncoproteins will continuously activate the replication signal of hematopoietic cells as the CML's pathophysiological hallmark [2]. The breakpoints in the BCR gene, specifically in chromosome 22 mostly occur at between the exon e12 (b2) and exon e13 (b3) or between the exon e13 (b3) and exon e14 (b4). The differences in these breakpoints will produce different chimeric transcripts [3].

Patients with transcriptions of mutations b2a2 express higher activity of tyrosine kinase through the higher CrKL pathway compared to the type of mutation b3a2[4,5]. This higher activity in b2a2 mutation would result in less suppression of tyrosine kinase inhibitors. On the other hand, the less active tyrosine kinase of b3a2 mutation was observed to be more responsive to treatment with tyrosine kinase inhibitors (TKI) compared with b2a2 mutation[4–8].

The goal of CML treatment is to achieve complete remission, i.e. hematological remission (CHR), cytogenetic remission (CCR), and molecular remission (CMR)[9]. Evaluation of cytogenetic responses and molecular responses cannot yet be done routinely in most Indonesian health services; meanwhile, the hematological response is still routinely done in Indonesia. This study aims to determine the time to achieve complete hematological response (CHR) in chronic CML patients who received Imatinib mesylate based on the type of mutation of the BCR-ABL gene (b3a2 and non-b3a2) in a tertiary referral hospital in Yogyakarta Indonesia.

B. Materials and Methods

Patient Selection

This study was a retrospective cohort study to observe the time to achieve CHR in chronic phase CML patients receiving Imatinib therapy from January 2013 to December 2018. This was conducted at Dr Sardjito Hospital, a tertiary hospital in Yogyakarta, Indonesia. We included all patients diagnosed with chronic phase CML, aged > 18 years, with available data on BCR-ABL type of mutation, received regular imatinib therapy, and had periodic evaluation of hematological responses during the first 6 months. Patients were excluded in the presence of other co-morbid diseases that may change blood profile and imitated the symptoms of CML (stage III-V kidney disorders, chronic heart failure, diabetes mellitus, liver cirrhosis, COPD). Patients with CML patients but also with chronic infection/ severe acute infection for first 6 months of observation (tuberculosis, HIV, viral hepatitis, pneumonia), evidenced with imatinib toxicity (hematological and non-hematological), or allergic to imatinib were also excluded. All data were obtained from the medical records.

Ethical consideration was approved by the Medical and Health Research Ethics Committee of Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada/ Dr Sardjito Hospital, Yogyakarta (reference number: KE/FK/1326/EC/2018).

Statistical analysis

The main outcome of this study is survival data (event rate) which was analyzed by Kaplan-Meier method. Hazard ratio (HR) of BCR-ABL b3a2 mutations compared with BCR-ABL non-b3a2 mutations were analyzed based on proportional hazard assumptions. Sub-analysis of hematological responses to prognostic scores was performed by using EUTOS scores that composed of spleen size and basophils count which finally categorized patients into high or low risk group. The analysis of this study used SPSS for Windows version 22 software. The normality test was assessed using Kolmogorov-Smirnov for any numerical data, with the normally distributed data would be analyzed with independent t-test, and otherwise, Mann-Whitney test would be performed. Statistical significance was determined if p value <0.05.

C. Results

From the 83 subjects recruited, 11 subjects were excluded, i.e 4 subjects had other co-morbid diseases (1 subject to stage 3 kidney failure, 1 subject to COPD, 1 subject to chronic heart failure, 1 subject to liver cirrhosis), 7 subjects experienced severe chronic infection/acute infection (3 TBC subjects, 1 hepatitis B subject, 3 pneumonia subjects). A total of 72 subjects were finally included. Forty-eightsubjects were with b3a2 type of mutation and 24 subjects were with non b3a2.

Table 1 described the clinical and laboratory profiles of the patient which were associated with anemia, leukocytosis, thrombocytosis, with splenomegaly in general with wide range of severity. Mean hemoglobin level was 10.9 g/dl with median of 10.2 g/dl (5-19.99 g/dl). Mean leukocytes count was 160.8 x 10^3 / mm^3 with the median was 65 x 10^3/mm3 (3.7-741 x 10^3 / mm^3). Mean platelet count was 597.8 x 10^3 / mm^3 with median of 500 x 10^3 / mm^3 (59-2204 x 10^3/mm^3). Large proportion of the subjects had an enlarged spleen in their initial presentation (88.9%) with average initial spleen size was 11.7 cm from the bottom of the costal arch.

Based on *BCR-ABL* mutation type, the clinical and laboratory phenotypes of both mutations were presented in Table 2. Median hemoglobin level among subjects with non-b3a2 mutation was lower compared with b3a2 mutation (9.8 g/dl and 10.3 g/dl, respectively; p = 0.423), while non-b3a2 mutation had higher median leukocyte compared with b3a2 mutations (120 x 10^3/mm^3 and 53 x 10^3/mm^3, respectively; p = 0.181). On the contrary, the median platelet count among b3a2 mutation was higher than non-b3a2 mutation (504 x 10^3/mm^3 and 443 x 10^3/mm^3 respectively; p = 0.558). The spleen was palpable in 41 subjects (85%) with b3a2 mutation while more patients in non b3a2 mutation group had palpable spleen with 23 subjects (95.8%) (p=0.185). Subjects with non b3a2 mutations had larger median spleen size (16.0 cm) compared to b3a2 mutation (median 8.0 cm) (p = 0.024) (Table 2). From 72 subjects, only 49 subjects had complete data for the calculation of EUTOS score. Median EUTOS score of b3a2 mutation group was lower compared to the non-b3a2 mutation

(52.2 and 86.3, respectively; p = 0.146) although significance was not reached (Table 2). None of those 72 subjects had the progression to the acceleration phase or blast crisis during the 6 months of observation.

From 49 subjects who achieved a CHR within the first 6 months, cases with b3a2 mutation was fairly prevalent to experience CHR than those with non-b3a2 mutation (68.8% or 33 subjects and 66.7% or 16 subjects, respectively). Among subjects with CHR within the first 6 months, most patients achieved CHR in the first 3 months (85.7% in b3a2 mutation and 87.5% in non-b3a2 mutation), with earlier treatment response occurred more often in the b3a2 mutation group (Figure 1). Figure 2 illustrated Kaplan-Meier curve of the time to achieve CHR based on mutation which resulted similar time between b3a2 mutation and non-b3a2 mutation (mean 3.71 months; p = 0.908) (Table 3). According to the EUTOS score, during the first 6 months of observation, subjects with high EUTOS score (> 87) had longer time to achieve CHR (mean 4 months and median 3 months) compared with low EUTOS score (≤ 87) (mean 3.6 months and median 3 months) although the difference was not statistically significant (p = 0.578) (Table 4).

Subjects with b3a2 mutation hada hazard ratio (HR) of 1.031 to achieve CHR compared to subjects with non-b3a2 mutation (p = 0.919 and 95% CI 0.568-1.874). Figure 3 showed the Kaplan-Meier curves of hematological response based on EUTOS score. Subjects with high EUTOS score (> 87) had HR of 0.831 to achieve CHR compared to patients with low EUTOS score (≤ 87) (p = 0.627 and 95% CI 0.393-1.755). Sub-group analysis of the CHR based on the type of *BCR-ABL* mutation and EUTOS score showed that subjects with non-b3a2 mutation accompanied with a high EUTOS score had the longest time to achieve CHR (mean 4.4 months and median 5 months). However, statistical calculations showed no significant difference with other group (p = 0.781) (Table 5). Figure 4 showed Kaplan-Meier curve of the hematological response for sub-group analysis among patients with high EUTOS scores that resulted in lower time to achieve CHR in subjects with non-b3a2 mutation compared to b3a2, although only few differences achieved.

D. Discussion

In this study, there were more subjects with b3a2 mutation than non-b3a2 mutation, i.e. 66.7% compared to 33.3%. This result is different from Lucas *et al* with 54% of b3a2 and 56% of non-b3a2, Hanfstein *et al* showed 55% of b3a2 and 45% of non b3a2. Our data is comparable with the report by Jain *et al* that had 60% of b3a2 and 40% of non-b3a2 [4,5,10].

The initial clinical presentations of our study population were similar with other reports from different parts in Indonesia i.e. from Jakarta and Bali. Reksodiputro *et al* from Jakarta reported population of CML with the median hemoglobin level of 9.9 g/dl, the median leukocyte count of 73 x 10^3/mm^3, and the median platelet count of 481 x 10^3/mm^3. Putra *et al* from Bali showed that the average of hemoglobin level of 9.68 g/dl, the average leukocyte count of 227.59 x 10^3/mm^3, the average platelet count of 458.32 x 10^3/mm^3, and as many as 96% of subjects had splenomegaly [8,11]. Erdem and Bilen study from Turkey showed lower average spleen size i.e. 5.7 cm below the costal arch [12]. The IRIS study showed median hemoglobin level of 13 g/dl, median leukocyte count of 17.9 x 10^3/mm^3, the

median platelet count of 336 x 10^3/mm^3. As many as 23% of subjects had splenomegaly, and only 6% had a spleen size of > 10 cm [13]. Kantarjian *et al* showed the median hemoglobin level of 12.5 g/dl, median leukocyte count of 15 x 10^3/mm^3, the median platelet count of 303 x 10^3/mm^3 [14]. These comparative data showed that CML patients in our study population had a tendency for being "too late" to come [13,14].

Based on the type of *BCR-ABL* mutation, the basic characteristics showed that subjects with non-b3a2 mutations had a clinical profile that tended to be worse than the b3a2 mutation type, but these results was not statistically significant (p> 0.05). The EUTOS score and spleen size were statistically different between the two groups. Subjects with the non-b3a2 mutation type had a higher EUTOS score (> 87 i.e. high risks) than the b3a2 (p = 0.048). Subjects with the b3a2 mutation type had a larger spleen size than the b3a2 (p = 0.024). The results of this study are similar to report by Balatzenko and Hanfstein that showed subjects with non-b3a2 mutations had a worse blood cell profile compared to the type of b3a2 mutation [10,15].

During 6 months of observation, 58.3% (42 subjects) achieved a CHR in 3 months and 68% (49 subjects) achieved it in 6 months. These results are similar to the report from other parts in Indonesia. Report from Jakarta showed achievement of CHR in 3 months in 52% cases [8], while report from Bali showed achievement of CHR in 3 months onlyin 37.9% and achievement of CHR in 6 months in 51.7% cases[11]. The results from Turkey showed 100% achievement of a CHR in 3 months [12]. A study from India showed 100% also report achievement of a CHR in 3 months with a median time of 21 days [16]. A study by Kantarjian *et al* showed 95% of subjects (430 subjects out of a total of 454 subjects) achieved a CHR in 3 months with a median time of 21 days [14]. The IRIS study showed that 96% of subjects experienced a CHR in 18 months with a median time to reach a CHR was 1 month [13]. The different study design and specific population among these studies might explain the various results.

There was no differencefound in the achievement of a complete hematological response within 6 months between both groups. Inequivalentproportion cases between b3a2 and non-b3a2 mutation existed. Patients with non-b3a2 mutation might present worse clinical feature leading to patient exclusion.

In conclusion, we saw a trend to achieve earlier CHR in subjects with b3a2 *BCR-ABL* mutation type, although statisticalsignificance was not reached. Group of cases with non-b3a2 mutation might present with worse clinical and laboratory profile. Moreover, non-b3a2 *BCR-ABL* mutation concurrent with high EUTOS scores was perceived to have a longer time in achieving CHR.

Authors Disclosures of Potential Conflicts of Interest

No potential conflicts of interest were reported to this article.

Acknowledgments

The authors express their gratitude to Syahru Agung Setiawan and Miraz Radhea Bagaskoro to draft the study and assist the submission process.

References

1. Kantarjian H, Cortes J. Chronic Myeloid Leukemia. In: Kasper D, Fauci A, Hauser S, Longo D, Jameson JL, Loscalzo J, editors. Harrison's Principles of Internal Medicine. 19th ed. USA: McGraw-Hill; 2015. p. 687.
2. Baccarani M, Pileri S, Steegmann J-L, Muller M, Soverini S, Dreyling M. Chronic myeloid leukemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2012 Oct 1;23(suppl 7):vii72–7.
3. Langabeer SE. Is the BCR-ABL1 transcript type in chronic myeloid leukaemia relevant? Med Oncol. 2013 Jun 21;30(2):508.
4. Lucas CM, Harris RJ, Giannoudis A, et al. Chronic myeloid leukemia patients with the e13a2 BCR-ABL fusion transcript have inferior responses to imatinib compared to patients with the e14a2 transcript. Haematologica. 2009 Oct 1;94(10):1362–7.
5. Jain P, Kantarjian H, Patel KP, et al. Impact of BCR-ABL transcript type on outcome in patients with chronic-phase CML treated with tyrosine kinase inhibitors. Blood. 2016 Mar 10;127(10):1269–75.
6. Lin H-X, Sjaarda J, Dyck J, et al. Gender and BCR-ABL transcript type are correlated with molecular response to imatinib treatment in patients with chronic myeloid leukemia. Eur J Haematol. 2016 Apr;96(4):360–6.
7. Arora A, Scholar EM. Role of Tyrosine Kinase Inhibitors in Cancer Therapy. J Pharmacol Exp Ther. 2005 Dec;315(3):971–9.
8. Reksodiputro AH, Tadjoedin H, Rinaldi I. Preliminary Report: Clinical Characteristic, Hematologic Response and Gene Mutation of Patients with Chronic Phase Chronic Myeloid Leukemia (CML) to Imatinib at Cipto Mangunkusumo National Hospital (RSUPN CM). Indones J Cancer. 2011 Dec 31;5(4).
9. Deininger M, O'Brien S, Guilhot F, et al. International Randomized Study of Interferon Vs STI571 (IRIS) 8-Year Follow up: Sustained Survival and Low Risk for Progression or Events in Patients with Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Treated with Imatinib. In: 51st Annual Meeting of the American Society of Hematology. 2009.
10. Hanfstein B, Lauseker M, Hehlmann R, et al. Distinct characteristics of e13a2 versus e14a2 BCR-ABL1 driven chronic myeloid leukemia under first-line therapy with imatinib. Haematologica. 2014 Sep 1;99(9):1441–7.
11. Putra IMA, Rena R, Suega K. Respon hematologi pasien leukemia mieloid kronik yang mendapat pengobatan tyrosine kinase inhibitor selama setahun di RSUP Sanglah Denpasar. E-Jurnal Med Udayana [Internet]. 2015; Available from: https://ojs.unud.ac.id/index.php/eum/article/view/20920
12. Bilen Y, Erdem F. Hematologic, cytogenetic, and molecular responses to imatinib therapy for chronic myeloid leukemia: A single-center experience in Turkey. Turkish J Med Sci. 2012;42(1).
13. O'Brien SG, Guilhot F, Larson RA, et al. Imatinib Compared with Interferon and Low-Dose Cytarabine for Newly Diagnosed Chronic-Phase Chronic Myeloid Leukemia. N Engl J Med. 2003 Mar 13;348(11):994–1004.

14. Kantarjian H, Sawyers C, Hochhaus A, et al. Hematologic and Cytogenetic Responses to Imatinib Mesylate in Chronic Myelogenous Leukemia. N Engl J Med. 2002 Feb 28;346(9):645–52.
15. Balatzenko G, Vundinti BR, Margarita G. Correlation between the type of bcr-abl transcripts and blood cell counts in chronic myeloid leukemia – a possible influence of mdr1 gene expression. Hematol Rep. 2011 Mar 23;3(1):3.
16. Sharma P, Kumar L, Mohanty S, Kochupillai V. Response to Imatinib mesylate in chronic myeloid leukemia patients with variant BCR-ABL fusion transcripts. Ann Hematol. 2010 Mar 29;89(3):241–7.

Table 1. Basic characteristics of patients with CML

Parameter	n	%	mean	median (range)
Age (year)	72		47.0	50.0 (18-77)
Sex				
Male	36	50.0		
Female	36	50.0		
Mutation				
b3a2	48	66.7		
Non b3a2	24	33.3		
Hematological response in 6 months				
Complete	49	68.1		
Not complete	23	31.9		
Hemoglobin (g/dl)			10.9	10.2 (5-19.9)
Leukocyte ($x10^3/mm^3$)			160	65 (3.7-741)
Platelet ($x10^3/mm^3$)			597	500 (59-2204)
Spleen				
Palpable	64	88.9		
Not palpable	8	11.1		
Spleen size (cm)			11.7	12.0 (0-32)
Spleen size > 10 cm	40	55.0		
Spleen size ≤ 10 cm	24	33.3		

Table 2. Basic characteristics based on the *BCR-ABL* mutation types

	BCR-ABL mutation type								*p*
	b3a2				non b3a2				
Parameter	n	%	mean	Median	n	%	mean	median	
Age (year)			46.0	49.5			49.2	52	0.396^
Sex									
Male	28	58.3			8	33.3			0.046*

Female	20	41.7			16	66.7		
Hemoglobin (g/dl)			11.3	10.3			10.2	9.8	0.423^
Platelet($x10^3/mm^3$)			614.6	504.0			564.1	443.0	0.558^
Leukocyte ($x10^3/mm^3$)			143.9	53.0			194.4	120.0	0.181^
Spleen									
Palpable	41	85.4			23	95.8			0.185*
Not palpable	7	14.6			1	4.2			
Spleen size (cm)			10.3	8.0			14.5	16.0	0.024^
Spleen size									
>10cm	22	53.6			18	82.6			0.045*
≤10cm	19	46.4			5	17.4			
Basophil (%)[#]			2.72	2.0			3.5	1.0	0.917^
Score EUTOS[#]			59.79	52.2			82.4	86.3	0.146^
>87	7	22.6			9	50			0.048*
≤87	24	77.4			9	50			

*Chi-Square ^Mann-Whitney [#]subjects with EUTOS scores (49 subjects)

Table 3. Number of patients achieving the CHR in 6 months based on the *BCR-ABL* mutation types

	Response status		*Time response rate* (month)		*P*
Mutation	Complete	Non complete	Mean	Median	
b3a2	33 (68.8%)	15 (31.3%)	3.71	3.0	0.908
non b3a2	16 (66.7%)	8 (33.3%)	3.71	3.0	

Table 4. Number of patients achieving the CHR in 6 months based on the EUTOS scores

	Response status		*Time response rate* (month)		*p*
EUTOS Score	Complete	Non complete	Mean	Median	
>87 (high)	10 (62.5%)	6 (37.5%)	4.0	3.0	0.578
≤87 (low)	22 (66.7%)	11 (33.3%)	3.6	3.0	

Table 5. Mean and median time to response in 6 months based on the mutation types and EUTOS scores (in month)

Parameter	Mean	Median
Score EUTOS >87* (over all)	4.0	3.0
With b3a2 mutation	3.4	3.0
With non-b3a2 mutation	4.4	5.0
Score EUTOS ≤87 (over all)	3.6	3.0
With b3a2 mutation	3.9	3.0
With non-b3a2 mutation	3.1	3.0

* $p = 0.781$

ISSN 2515-8260 Volume 08, Issue 02, 2021

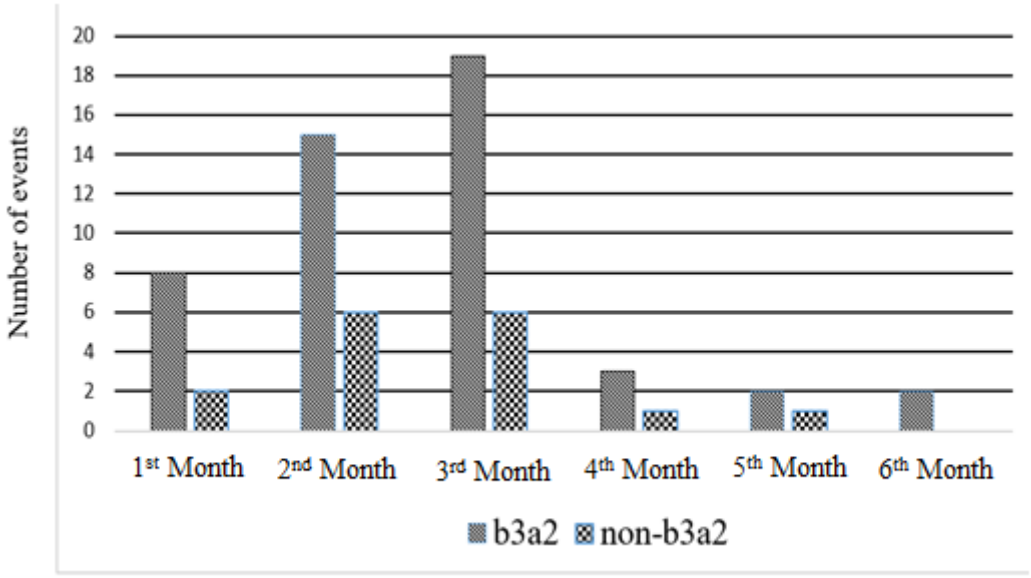

Figure 1. Complete hematological response (CHR) events within 6 months.

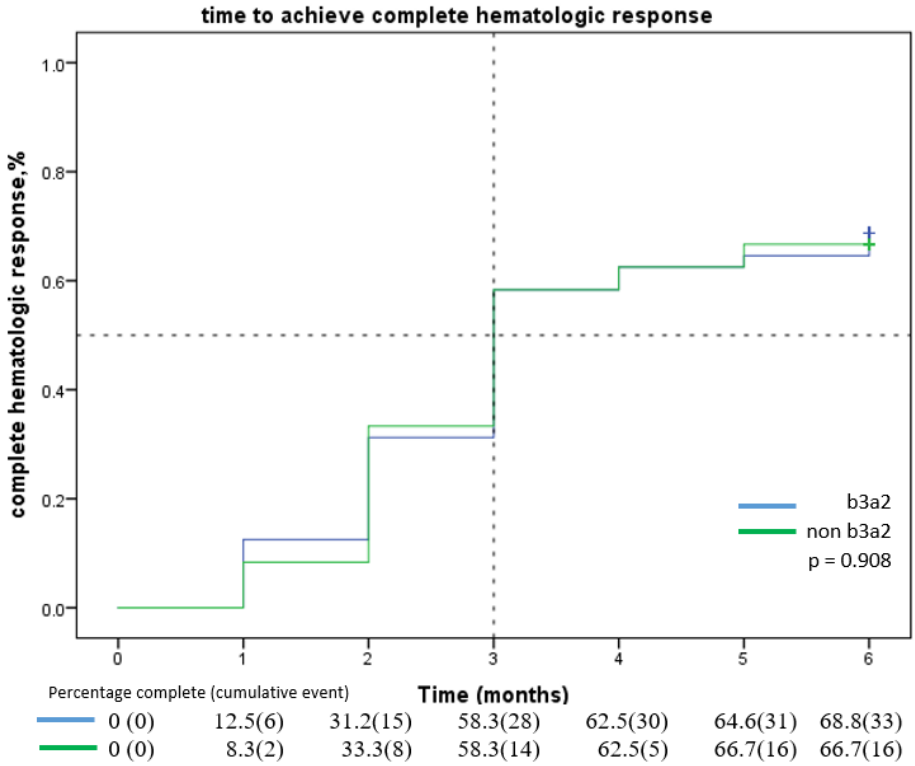

Figure 2. Kaplan-Meier curve of the complete hematological response (CHR) based on the mutation types. There were 49 subjects from b3a2 (33 subjects) and non b3a2 (16 subjects), as listed in Table 3.

ISSN 2515-8260 Volume 08, Issue 02, 2021

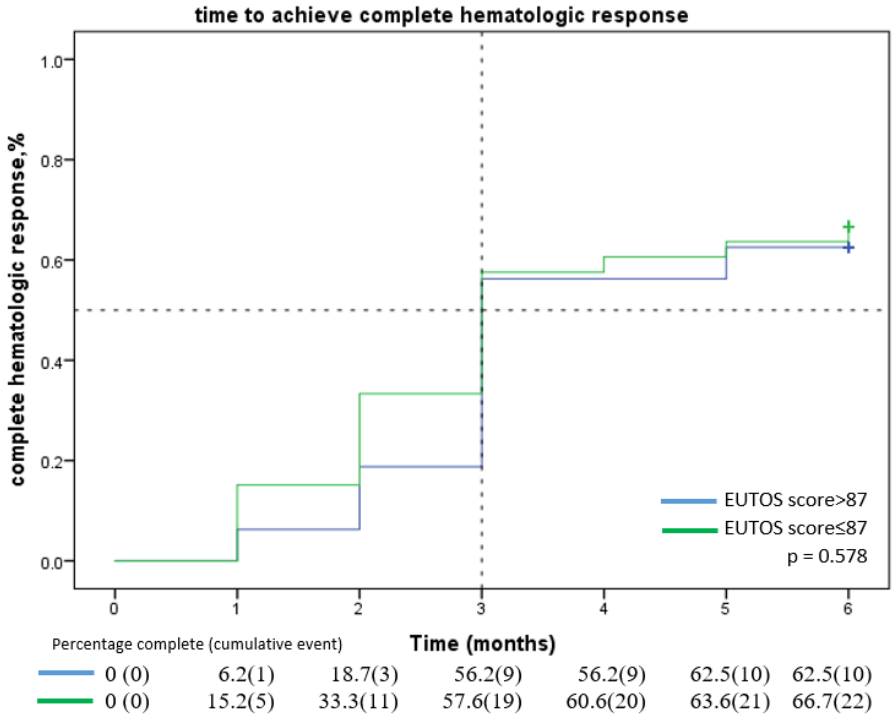

Figure 3. Kaplan-Meier curve of the complete hematological response (CHR) based on the EUTOS scores.There were 32 subjects from EUTOS score of >87 (10 subjects) and score of ≤ 87 (22 subjects), aslisted in Table 4.

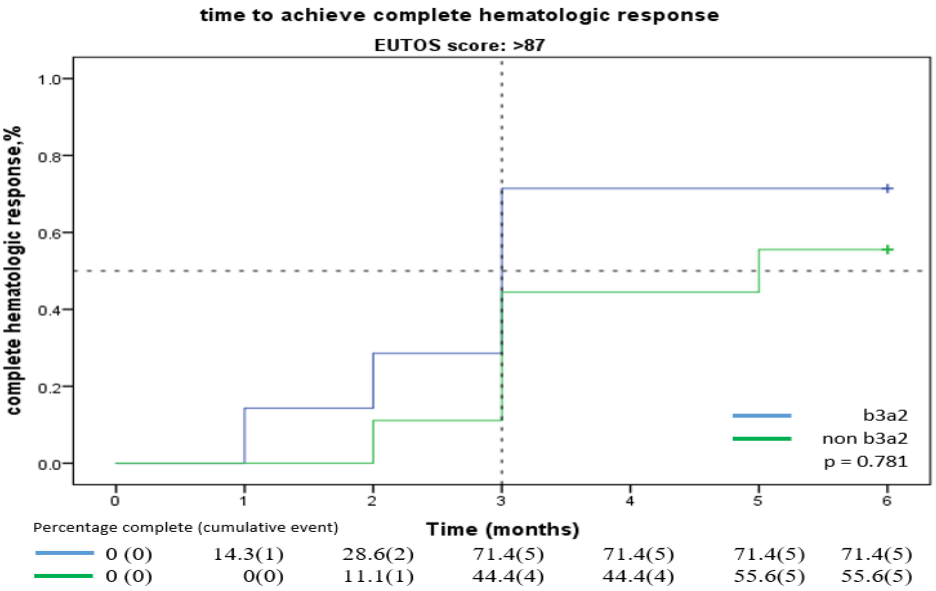

Figure 4. Kaplan-Meier curve of the complete hematological response (CHR) in patients with high EUTOS scores. There were 10 subjects from b3a2 (5 subjects) and non b3a2 (5 subjects), as listed in Table 4.