



# High STAT5A Expression is Associated with Major Molecular Response Achievement Failure of Chronic Phase Chronic Myeloid Leukemia Patients Receiving Hydroxyurea before Imatinib: A Cross-sectional Study

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## Abstract

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**BACKGROUND:** STAT5 is a transcriptional factor which when highly expressed in chronic myeloid leukemia (CML) cells stimulate proliferation and mediate resistance from tyrosine kinase inhibitors, resulting in major molecular response (MMR) failure. STAT5 has two isoforms, STAT5A and STAT5B. However, prolonged use of imatinib appears to only upregulate STAT5A pathway. In addition, the resistance conferred by STAT5A does not extend to other drugs such as hydroxyurea. Hence, STAT5A and STAT5B might have different functions in CML cells.

**AIM:** The objective of the study was to determine the association of STAT5A and STAT5B expression with MMR failure in CML patients.

**METHODS:** This was a cross-sectional study of CML patients in chronic phase with age  $\geq 18$  years old, receiving IM therapy  $\geq 12$  months, and previously given hydroxyurea. MMR status was evaluated and patients were categorized as achieved or failed to achieve MMR. Expression levels of STAT5A and STAT5B were conducted using RT-PCR methods. Associations between STAT5A expression, STAT5B expression, hydroxyurea duration, and imatinib duration with MMR achievement were calculated using logistic regression.

**RESULTS:** A total of 118 patients were analyzed; 71.1% failed to achieve MMR. Multivariate logistic regression analysis showed statistically significant association between high STAT5A expression (odds ratio [OR]: 3.852; 95% confidence interval [CI]: 1.420–10.452;  $p = 0.008$ ), STAT5A/STAT5B interaction (OR: 0.150; 95% CI: 0.038–0.593;  $p = 0.007$ ), longer hydroxyurea administration (OR: 3.882; 95% CI: 1.023–14.733;  $p = 0.046$ ), and shorter imatinib administration (OR: 0.333; 95% CI: 0.132–0.840;  $p = 0.020$ ) with MMR achievement failure. After adjusting STAT5A expression with STAT5A/STAT5B interaction, high STAT5A expression independently increased the likelihood of MMR achievement failure only in high expression STAT5B patients (OR: 3.852; 95% CI: 1.420–10.452;  $p = 0.008$ ).

**CONCLUSION:** High STAT5A expression which is induced by high STAT5B is associated with MMR achievement failure of chronic phase CML patients who received hydroxyurea before imatinib. Longer duration of hydroxyurea and shorter duration of IM confound of STAT5A expression to MMR achievement failure.

## Introduction

Chronic myeloid leukemia (CML) is one of myeloproliferative neoplasms that usually affect older adults [1]. In CML, there is a fusion of the breakpoint cluster region (BCR) gene on chromosome 22 with Abelson murine leukemia (ABL1) gene on chromosome 9 resulting in BCR-ABL1 oncogene [2]. The BCR-ABL1 oncogene has numerous signaling pathways such as RAS, RAF, JUN kinase, MYC, JAK, and STAT which can activate tyrosine kinase continuously [3]. Tyrosine kinase activation has many purposes such as maintaining survival of the cells, increasing cells proliferation, and increasing resistance against apoptosis [4]. In 1996, imatinib mesylate (IM)

was discovered as the first tyrosine kinase inhibitor (TKI) and then become the first-line therapy for CML [5]. In Indonesia, IM is used for CML patients who were previously given hydroxyurea both less and more than 6 months [6].

The molecular response of IM treatment needs to be evaluated after 12 months [7]. Poor responses of IM such as failure to achieve major molecular response (MMR) are a sign of IM resistance which led to the discovery of new generations of TKIs of BCR-ABL such as dasatinib, nilotinib, radotinib, ponatinib, and bosutinib or even different class of treatment [8], [9]. Several causes of IM resistance have been known such as point mutation of T315I [9], [10].

Previously, we have conducted analysis of P-glycoprotein expressions, malondialdehyde, and

hypoxia-inducible factor 1- $\alpha$  concentrations with MMR achievement in chronic CML patients who were previously given hydroxyurea [6]. Although the study showed the lack of association between those three markers with MMR achievement, the study revealed that long-term use of hydroxyurea before IM for at least 6 months can influence MMR achievement failure through IM resistance [6]. According to a study by Warsch *et al.*, STAT5 activation protects leukemic cells from TKIs but does not prevent cytotoxicity effect from hydroxyurea treatment which may suggest potential different pathways of hydroxyurea-induced MMR achievement failure [11].

There are many studies showing that the consequence of longer duration of IM treatment will result in the activation of STAT5A in leukemic stem cells and progenitor cells which correlated with BCR-ABL point mutation and increase of reactive oxygen species production [11]. In a study by Zhang *et al.*, there are increases in STAT5A and RUNX3 expression in IM resistance [11], [12], [13]. An *in vitro* study by Warsch *et al.* showed that IM treatment for 9 days can induce increase of STAT5A expression [11]. Another *in vitro* study observed that STAT5B expression is also increased in IM-resistant CML [13]. Meanwhile, the effect of long-term IM use on STAT5B is still unclear. However, the study done by Zhang *et al.* showed STAT5A but not STAT5B as a predictor of secondary IM resistance [14]. Nevertheless, STAT5 is a potential therapeutic target as shown by higher MMR achievement from downregulation of STAT5 using STAT5 inhibitors [15], [16], [17].

To the best of our current knowledge, there are no literatures that describe effect of long-term hydroxyurea use with STAT5 activation. Hence, the role of hydroxyurea on STAT5 expression is still unknown. Therefore, this study aimed to identify the relationship between STAT5A expression and STAT5B with MMR in chronic phase CML patient on IM treatment and to analyze the effect of hydroxyurea and IM with STAT5 expression and MMR achievement failure.

## Materials and Methods

### Study site and population

This study is a cross-sectional study that analyzed whole blood samples obtained from previous study [6]. Patients were categorized as either achieved the MMR and did not achieve the MMR. MMR achievement is defined as the ratio of break cluster region-Abelson murine leukemia/Abelson murine leukemia (BCR-ABL/ABL) <0.1% which is detected by using a quantitative RT-PCR method. The expressions of STAT5A and STAT5B were examined using quantitative RT-PCR LightCycler 480 Roche™ using Thunderbird SYBR qPCR Mix (Toyobo, Japan) kit. Quantification cycle

(Cq) was calculated automatically and the level of expression was processed with Livak's method [18]. The target gene and housekeeping gene are shown in Table 1.

**Table 1: Primer sequences used in this study**

Gene	Primer	Sequence
$\beta$ -actin (housekeeping gene)	Forward	5'-GCTGGAAGGTGGACAGCGA-3'
	Reverse	5'-GGCATCGTGATGGACTCCG-3'
STAT5A (target gene)	Forward	5'-CATCGGTCCCTGGCTGAC-3'
	Reverse	5'-AGAGGTGAAAAGACCGGCAG-3'
STAT5B (target gene)	Forward	5'-TCCCTGCGAGTCTGCTACT-3'
	Reverse	5'-CCATTGTCTCCTCCAGATCGAA-3'

### Sample size calculation

Sample size calculation was performed using formula for difference of proportion test. This research was never conducted in Indonesia before, hence, it is estimated that the difference in the proportion of high expression of STAT5 genes in the group that did not reach MMR and those who reached MMR was 60% and 40%, respectively. As a result, the total sample size calculated was 95 patients.

### Inclusion and exclusion criteria

The inclusion criteria are CML patients in chronic phase with a range of age  $\geq 18$  years old, on the treatment of IM for more than 12 months, previously given hydroxyurea and MMR status available. However, patients in an acceleration or blast crisis or having IM more than 400 mg/day and the blood sample is not appropriate for the test were excluded from this study.

### Statistical analysis

Statistical analysis was conducted by using SPSS version 20.0. Continuous data were presented as mean with standard deviation (for normally distributed data) or median with minimum to maximum range (for non-normally distributed data). Categorical data were presented as numbers and percentages. Chi-square test or Fisher's exact test were used to compare categorical data and  $p < 0.05$  is considered to be statistically significant. Regression logistic was used for multivariate analysis. Variables chosen for regression logistic are variables which have  $p < 0.25$ .

## Results

Total 118 subjects met our inclusion and exclusion criteria, then were analyzed. There were 84 (71.1%) subjects that failed to achieve the MMR and only 34 (28.9%) subjects that achieved MMR. The male-to-female ratio was 1.2:1, with mean age  $42.7 \pm 13.2$  years. Characteristics of all patients and subgroup analysis of patients who achieved the MMR

**Table 2: Baseline characteristics**

Variables	Total	MMR	
	n: 118	Failed to achieve n: 84, median (min-max)	Achieved n: 34, median (min-max)
Male Sex (%)	53 (44.9%)	39 (46.4%)	14 (41.1%)
Age (years) (SD)	42.77 ± 13.207*	41.89 ± 13.146	44.94 ± 13.301
Duration of Imatinib Administration (months) (Median)	18.5 (12–72)	17 (12–72)	26 (12–72)
Duration of Hydroxyurea Administration (months) (Median)	2 (0–168)	2 (0–168)	1 (0–24)
Imatinib Dose (mg) (Median)	400 (200–400)	400 (200–400)	400 (300–400)
Hemoglobin (g/dl) (SD)	12.311 ± 2.41*	12.34 ± 2.63	12.24 ± 1.82
Platelet, (10 <sup>3</sup> /μl) (Median)	234.5 (14.4–1,232.0)	240.0 (14.4–1,232.0)	217.5 (143–543)
WBC (10 <sup>3</sup> /μl) (Median)	6700 (2.3–103.7)	7.4 (2.3–103.7)	6.1 (3.4–28.2)
Basophil, (%) (Median)	0.00 (0.00–47)	0.00 (0–47)	0.00 (0–5)
Bands neutrophil, (%) (Median)	0.00 (0–3)	0.00 (0–3)	0.00 (0–0)
Segmented neutrophil, (%) (Median)	65.25 ± 14.972*	63.85 ± 15.446	68.71 ± 13.320
Lymphocyte, (%) (Median)	25.75 ± 12.99*	25.63 ± 13.495	26.06 ± 11.835
Blast, (%) (Median)	0 (0–9)	0 (0–9)	0 (0–0)
STAT5A expression (Fold over control) (Median)	0.7374 (0–867.0672)	0.8873 (0–867.0672)	0.5284 (0–59.5075)
STAT5B Expression (Fold over control) (Median)	0.3511 (0–26.3549)	0.2052 (0–19.1597)	0.4429 (0–26.3549)

and did not achieved the MMR were shown in Table 2.

The overall median expression of STAT5A was 0.7374 (0.000–867.0672). The median then becomes the cutoff point to determine the subject into two groups, high expression STAT5A for subjects who have expression level more than 0.7374 and low expression in subjects who have expression level equal to less than 0.7374.

The overall median expression of STAT5B was 0.3511 (0.000–26.3549). Similar with STAT5A, the median of STAT5B then becomes the cutoff point to determine the subject into two groups, high expression STAT5B for subjects who have expression level more than 0.3511 and low expression in subjects who have expression level equal to less than 0.3511.

### Univariate analysis of association between STAT5A and STAT5B expression with MMR achievement

Based on univariate analysis which was conducted to analyze the association between STAT5A expression with MMR achievement, no statistically significant association was observed between STAT5A expression and MMR achievement (prevalence ratio: 1.265; 95% confidence interval [CI]: 0.995–1.608;  $p = 0.076$ ), as shown in Table 3. Meanwhile, there was a statistically significant association between STAT5B expression and MMR achievement. Patients with high expression of STAT5B have lower prevalence ratio of failed to achieve MMR than patients with low expression of STAT5B (prevalence ratio: 0.728; 95% CI: 0.570–0.929;  $p = 0.013$ ), as shown in Table 3.

**Table 3: Univariate analysis of STAT5A, STAT5B, and confounding factors**

Variable	Category	MMR status (%)		p-value (continuity correction)	Prevalence ratio (95% CI)
		Failed to achieve	Achieved		
STAT5A Expression	High	49 (79)	13 (21)	0.076	1.265 (0.995–1.608)
	Low	35 (62.5)	21 (37.5)		
STAT5B Expression	High	34 (59.6)	23 (40.4)	0.013	0.728 (0.570–0.929)
	Low	50 (82.0)	11 (18)		
Duration of Hydroxyurea Administration	>6 months	28 (90.3)	3 (9.7)	0.012	1.403 (1.156–1.704)
	≤6 months	56 (64.4)	31 (35.6)		
Duration of IM Administration	>24 months	24 (57.1)	18 (42.9)	0.022	0.724 (0.544–0.964)
	≤24 months	60 (78.9)	16 (21.1)		

CI: Confidence interval.

We also included duration of hydroxyurea and

IM administrations as variables due to being confounding factors in this study. Both of them are significant factors associated with MMR achievement based on univariate analysis (Table 3). The group that administered hydroxyurea more than 6 months has higher prevalence ratio of failed to achieve of MMR (prevalence ratio: 1.403; 95% CI: 1.156–1.704;  $p = 0.012$ ) (Table 3). In contrast, patients that administered IM more than 24 months have lower prevalence ratio of failed to achieve MMR when compared with IM duration equal to or less than 24 months (prevalence ratio: 0.724; 95% CI: 0.544–0.964;  $p = 0.022$ ) (Table 3).

Univariate analysis between STAT5A expression, STAT5B expression, and confounding factors (duration of hydroxyurea and IM administrations) with MMR achievement revealed significance for all variables ( $p < 0.25$ ) (Table 3). All variables with  $p < 0.25$  were then subjected to multivariate analysis.

STAT5A expression, STAT5B expression, STAT5A/STAT5B interaction, STAT5A/hydroxyurea interaction, STAT5A/imatinib interaction, duration of hydroxyurea administration, and duration of imatinib administration were included for multivariate analysis. Result of multivariate analysis showed significant association between STAT5A expression (odds ratio [OR]: 3.852; 95% CI: 1.420–10.452;  $p = 0.008$ ), STAT5A/STAT5B interaction (OR: 0.150; 95% CI: 0.038–0.593;  $p = 0.007$ ), duration of hydroxyurea administration more (OR: 3.882; 95% CI: 1.023–14.733;  $p = 0.046$ ), and duration of imatinib administration (OR: 0.333; 95% CI: 0.132–0.840;  $p = 0.020$ ) with MMR achievement (Table 4). STAT5B, STAT5A/hydroxyurea interaction, and STAT5A/imatinib interaction failed to gain statistical significance in the multivariate analysis.

**Table 4: Model 1 of association between variables with MMR achievement failure**

Independent variable	Category	OR	95% CI	p-value
STAT5A expression	High	3.852	1.420–10.452	0.008
	Low	Reference		
Duration of hydroxyurea administration	>6 months	3.882	1.023–14.733	0.046
	≤6 months	Reference		
Duration of IM administration	>24 months	0.333	0.132–0.840	0.020
	≤24 months	Reference		
STAT5A/STAT5B interaction	-	0.150	0.038–0.593	0.007
	Constant	0.191		

OR: Odds ratio, CI: Confidence interval.

Due to the significant presence of STAT5A and STAT5B interaction (OR: 0.150; 95% CI: 0.038–0.593;



$p = 0.007$ ) (Table 5), we conducted adjustment of STAT5A expression with STAT5B expression. The result is high STAT5A expression independently increased the likelihood of MMR achievement failure in high expression of STAT5B (OR: 3.852; 95% CI: 1.420–10.452;  $p = 0.008$ ). However, high STAT5A expression in low STAT5B expression had no association with MMR achievement failure (OR: 0.578; 95% CI: 0.054–6.196;  $p = 0.008$ ), as shown in Table 5.

**Table 5: Adjusted STAT5A odds ratio based on STAT5B expression level**

Variable	Adjusted OR	95% CI
High STAT5A expression in high STAT5B	3.852	1.420–10.452
High STAT5A expression in low STAT5B	0.578	0.054–6.196

OR: Odds ratio, CI: Confidence interval.

As duration of hydroxyurea and IM administrations in the first model have potential to be confounder, we analyzed several variations of multivariate analysis models to determine the best fit model. Four models were analyzed and compared based on OR differences to model 1 and level of precision to obtain the best fit regression logistic model.

The first model contains STAT5A expression, duration of hydroxyurea administration, duration of IM administration, and STAT5A/STAT5B interaction variables (Table 4). The second model contains STAT5A expression, duration of hydroxyurea administration, and STAT5A/STAT5B interaction variables (Table 6).

**Table 6: Model 2 of multivariate analysis without duration of imatinib administration variable**

Independent variable	Category	OR	95% CI	p-value
STAT5A expression	High	4.222	1.604–11.108	0.004
	Low	Reference		
Duration of hydroxyurea administration	>24 months	3.842	1.036–14.243	0.044
	≤24 months	Reference		
STAT5A by STAT5B interaction	-	0.178	0.048–0.665	0.010
Constant	-	0.092		≤0.001

OR: Odds ratio, CI: Confidence interval.

The third model contains STAT5A expression, duration of IM administration, and STAT5A/STAT5B interaction variables (Table 7). The fourth model contains STAT5A expression and STAT5A/STAT5B interaction variables (Table 8). The fifth model only contains STAT5A expression variable (Table 9).

**Table 7: Model 3 of multivariate analysis without the duration of hydroxyurea administration variable**

Independent variable	Category	OR	95% CI	p-value
STAT5A expression	High	4.538	1.712–12.033	0.002
	Low	Reference		
Duration of IM administration	>24 months	0.337	0.137–0.831	0.018
	≤24 months	Reference		
STAT5A by STAT5B interaction	-	0.119	0.031–0.454	0.002
Constant	-	0.546	0.153	

OR: Odds ratio, CI: Confidence interval.

**Table 8: Model 4 of multivariate analysis without the duration of imatinib and hydroxyurea administration variables**

Independent variable	Category	OR	95% CI	p-value
STAT5A expression	High	4.929	1.913–12.697	0.001
	Low	Reference		
STAT5A by STAT5B interaction	-	0.139	0.038–0.504	0.003
Constant	-	0.265		≤0.001

OR: Odds ratio, CI: Confidence interval.

The OR differences when compared with model 1 (original model) from model 2, model 3, model 4, and model 5 are 9.6%, 17.8%, 27.95%, and 41.27%, respectively (Table 10). Differences of 17.8%, 27.95%, and 41.27% are considered significant, hence model 3, model 4, and model 5 are rejected. Model 2 provides OR value which is relatively close with the OR value of model 1 (original model). Hence, both model 1 and model 2 are valid models. To determine the best fit model between model 1 and model 2, the precision of model 1 and model 2 was compared and the model with the highest precision is chosen as the final model. Based from Table 10, since model 2 has lower precision than the model 1 (9.505 vs. 9.033), model 1 is chosen as the final model.

**Table 9: Model 5 of multivariate analysis without the duration of imatinib duration administration, duration of hydroxyurea administration, and STAT5A/STAT5B interaction variables**

Independent variable	Category	OR	95% CI	p-value
STAT5A Expression	High	2.262	1.000–5.117	0.050
	Low	Reference		
Constant	-	0.265	-	≤0.001

OR: Odds ratio, CI: Confidence interval.

### Hosmer and Lemeshow test

Hosmer and Lemeshow test was conducted for the best fit model (Table 4) which provided  $p = 0.369$  (Table 11). Therefore, there is no statistically significant difference between observed MMR achievement status and expected MMR achievement status from model 1. Contingency table for Hosmer and Lemeshow test was then calculated (Table 12).

### Receiver operating characteristic (ROC) curve

After obtaining the best model from multivariate analysis, ROC curve was generated (Figure 1). The ROC analysis of the curve showed that the area under the curve was 79% (95% CI: 69.8–88.2) with  $p \leq 0.001$  (Table 13).

## Discussion

The characteristics of our subject were different in the matter of age when compared with patients from western countries [19]. For example, the median age in US, France, and Germany is 66, 56, and 57 years, respectively [19]. Hence, our subjects were younger compared to the patients from western countries, as shown in Table 2. Of the total 118 subjects, the mean age was  $42,77 \pm 13.207$  with the youngest age were 19 years old and the oldest were 79 years old. The previous study in Indonesia done by Reksodiputro

**Table 10: Summary of different models**

Model	Variable (s)	OR	Min	Max	Changes of OR from Model 1 (%)	Precision
1	STAT5A+Hydroxyurea+Imatinib+STAT5A*STAT5B	3.852	1.420	10.453	-	9.033
2	STAT5A+Hydroxyurea+STAT5A*STAT5B interaction	4.222	1.604	11.108	0.370 (9.6%)	9.505
3	STAT5A+Imatinib+STAT5A*STAT5B interaction	4.538	1.721	12.033	0.686 (17.8%)	10.312
4	STAT5A+STAT5A*STAT5B interaction	4.929	1.913	12.697	1.077 (27.95%)	10.784
5	STAT5A	2.262	1	5.117	-1.590 (41.27%)	4.117

OD: Odds ratio.

**Table 11: Hosmer and Lemeshow test**

Step	Chi-square	df	p-value
1	7.602	7	0.369

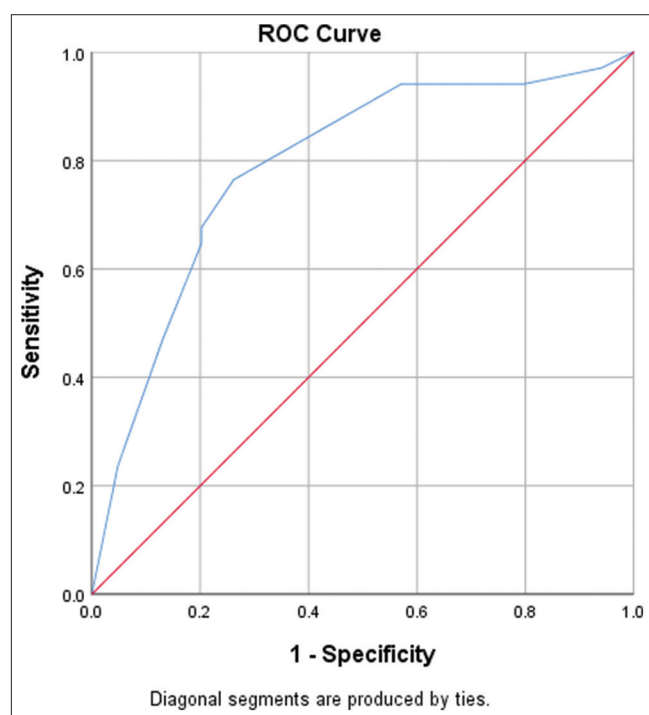
**Table 12: Contingency table for Hosmer and Lemeshow test**

Step 1	MMR not achieved		MMR achieved		Total
	Observed	Expected	Observed	Expected	
1	5	5.788	1	0.212	6
2	12	12.225	1	0.775	13
3	12	10.607	0	1.393	12
4	7	5.807	0	1.193	7
5	26	25.678	6	6.322	32
6	5	6.182	4	2.818	9
7	6	6.898	6	5.102	12
8	7	7.698	8	7.302	15
9	4	3.117	8	8.883	12

**Table 13: ROC analysis of logistic regression model and MMR achievement failure**

Area	Standard error	p-value	95% CI
0.790	0.047	≤0.001	0.698–0.882

CI: Confidence interval, ROC: Receiver operating characteristic.

**Figure 1: Receiver operating characteristic analysis of logistic regression model and major molecular response achievement failure**

*et al.*, which was conducted in 2003–2008 showed younger median of age 37 (16–67) years [20]. The ratio of female to male in our study was 1:1.2, which are similar with other literatures [19], [20].

The level of hemoglobin and platelet in our study was in the normal range, and it was  $12.31 \pm 2.41$  mg/dl and  $234.5 (14.4–1.232.0) \times 10^3/\text{ml}$ , respectively. This result was same with previous study done by Kantarjian *et al.* and other

study by Reksodiputro, it was around 10.0–12.5 mg/dl and  $303.0–424.0 \times 10^3/\text{ml}$ , respectively [21], [22]. However, the median of leukocyte in this study was higher than normal value, it was 11,845 (2.300–103.700)/ml, with 26.3% subject having leukocyte more than 10,000/ml after more than 12 months of IM treatment. This result was different than International Randomized Study of Interferon and STI571 (IRIS) study, which has leukocyte lower than 10,000/ml after 1 month of IM treatment [22], [23]. It means our population was in poorer condition compare to IRIS study population, possibly because of administration of hydroxyurea was more than 6 months before IM administration.

Our study used molecular response as a monitor for response after 12 months of IM therapy that has been known as (MMR; BCR-ABL1  $\leq 0.1\%$  on the International Scale) [24]. This study reveals that after 12-month treatment of IM, 28.8% (n: 34) subjects achieve the MMR. The result was in the range of previous study, between 18% and 58% [25]. The highest proportion of patients who achieve the MMR was observed in IRIS study, after 8 years, 86% patients achieve the MMR [22], [23]. The difference between the proportion of our study and IRIS study can be possibly explained from the use of hydroxyurea administration before IM administration more than 6 months in our study which translated to higher IM resistance in our population than IRIS study.

The mechanism of resistance to IM based on molecular basis was differentiated into three major mechanisms. Two of the mechanisms affect the BCR-ABL gene such as mutation in tyrosine kinase domain and overexpression of BCR-ABL protein while the third mechanism is independent to BCR-ABL gene, which also includes overexpression of STAT5 [26], [27]. The overexpression of STAT5 shown a role of IM resistance by activation of ABL-XL as antiapoptotic, gain of uncontrolled proliferation, and angiogenesis ability [26], [28].

The aim of our study is to identify the association between expression levels of STAT5A and STAT5B with MMR achievement in chronic phase CML patients on IM treatment. In the univariate analysis, we observe that no statistically significant association was between STAT5A expression and MMR achievement failure (prevalence ratio: 1.265; 95% CI: 0.995–1.608;  $p = 0.076$ ). Meanwhile, high expression of STAT5B is as a protective factor to MMR achievement failure (prevalence ratio: 0.728; 95% CI: 0.570–0.929;  $p = 0.013$ ).

This study also identifies the role of hydroxyurea

and IM administration in MMR achievement failure. In the univariate analysis, longer hydroxyurea (>6 months) administration was associated with MMR achievement failure (prevalence ratio: 1.403; 95% CI: 1.156–1.704;  $p = 0.012$ ) and longer IM (>24 months) administration was associated with successful MMR achievement (prevalence ratio: 0.724; 95% CI: 0.544–0.964;  $p = 0.022$ ), as shown in Table 3. This result is inherent with previous study that showed hydroxyurea more than 6 months which was associated with MMR achievement failure as well [6].

In the final model of multivariate analysis, STAT5A expression becomes associated with MMR achievement failure (OR: 3.852; 95% CI: 1.420–10.452;  $p = 0.008$ ) and STAT5B has no contribution to MMR achievement failure (Table 4). We also measured the interaction between STAT5A and STAT5B. The result of the interaction showed that STAT5A is only associated with MMR achievement failure when in high STAT5B expression patients (OR: 3.852; 95% CI: 1.420–10.452). If the patients have low STAT5B expression, then STAT5A is not associated with MMR achievement (OR: 0.578; 95% CI: 0.054–6.196). Variations of the models were analyzed to obtain the best multivariate analysis model (Table 10). The result showed that the original model is the best model for MMR achievement failure.

The result of multivariate analysis is also supported by Hosmer and Lemeshow test which showed no significant difference between the model prediction and observed events (Table 11). Finally, ROC analysis has AUC value of 79% (95% CI: 69.8–88.2) which is moderately accurate in differentiating between patients that achieve MMR and those that fail to achieve MMR.

This finding also supports the hypothesis that high expression STAT5A has a role in CML IM resistance and is in concordance with other studies such as the study done by Warsch *et al.*, which found higher mean of STAT5A expression in the IM resistance subjects than in IM sensitive subjects [11], [14]. However, expression in STAT5B shows opposite result with STAT5A. Again, this is similar with other studies [14], [29].

There are several differences of functions between STAT5A and STAT5B such as differences in responses to stress and additionally, in v-ABL expressed cells STAT5B can induce STAT5A expression but STAT5A cannot induce STAT5B expression [30], [31], [32]. Study by Schönitz *et al.* showed that in the presence of BCR-ABL, cytoplasmic retention of STAT5A but not STAT5B is observed [29]. The same study also showed that the interaction of STAT5B-BCR-ABL was required for human cells proliferation [29].

The hypothesis for the cause of lower STAT5B expression in CML patients who failed to achieve MMR compared to STAT5A is due to the major role of STAT5B

in primitive hematopoietic cells while STAT5A has more role in TKI resistance CML [14]. The other hypothesis is the presence of STAT5B mutation which makes it difficult to detect the gene expression of STAT5B.

In the final model, hydroxyurea use of more than 6 months is associated with failure of MMR achievement, but IM use of more than 24 months associated with successful MMR achievement. Therefore, in our study, failure of MMR achievement was caused by administration of hydroxyurea, not by IM.

The exact mechanism of how longer duration of hydroxyurea administration can cause MMR achievement failure might be caused by reactive oxygen species production during hydroxyurea metabolism in the body, however, our previous study showed no differences in P-glycoprotein expressions, malondialdehyde, and hypoxia-inducible factor 1- $\alpha$  concentrations after hydroxyurea use [6]. Elimination of IM duration variables from final models causes an increase of STAT5A OR of more than 10%. It means that longer duration of hydroxyurea administration can increase STAT5A and affects MMR achievement failure. Elimination of hydroxyurea duration variables from final models also causes an increase of STAT5A OR of more than 10%, similar with hydroxyurea. This also means that longer duration of IM administration can increase STAT5A and affects MMR achievement failure. It can be inferred that both hydroxyurea and IM duration are confounding factors.

Finally, the duration of hydroxyurea and IM administration were included in the final model of MMR achievement failure. In clinical practice, hydroxyurea administration is almost always given before IM. Administration of hydroxyurea was causing an increase of STAT5A, then effect to MMR achievement failure. After IM administration, the effect of STAT5A to MMR achievement failure was decreased (OR = 4222 vs. 3852). This final model showed that the duration of hydroxyurea can cause MMR achievement failure through STAT5A expression.

### Study limitation

There were several limitations in this study. The first limitation was lack of IM duration and hydroxyurea duration restriction in our subjects. Second, there were differences in IM and hydroxyurea treatment duration. Hence, it is possible that there may be a confounding in the analysis. In addition, the time of MMR measurement conducted was different between each patient, which may cause heterogeneity in outcome measurement. Finally, this study has the usual limitations of a cross-sectional study.

## Conclusion

In conclusion, the higher expression of STAT5A may be used as a potential marker of MMR achievement failure in chronic phase CML patient who received hydroxyurea before IM administration more than 12 months. High expression of STAT5B is shown to induce high STAT5A expression. An inhibitor of STAT5B might be beneficial as it may indirectly inhibit STAT5A to overcome of IM resistance in our study. Longer duration of hydroxyurea and IM confound STAT5A expression to MMR achievement failure. Research with cohort study design is needed to confirm our findings of the role of STAT5A and STAT5B in CML.

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## Ethical Statements

This study protocol as well as the written informed consent form has been reviewed and approved by the Ethical Committee of the Faculty of Medicine, Universitas Indonesia (Certificate of ethical approval 1053/UN2.F1/ETIK/2018). All patients were given explanation about the study and provided written informed consent before the interview.

## Availability of Data and Material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Author Contributions

Anastasia Putri, Ikhwan Rinaldi, Melva Lousia, and Sukamto Koesno conceived of the presented idea, developed the theory, performed the computations, and verified the analytical methods. Anastasia Putri and

Ikhwan Rinaldi wrote the manuscript with support and collaboration from Melva Lousia and Sukamto Koesno. All authors discussed the results and contributed to the final manuscript. Ikhwan Rinaldi is the corresponding author.

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