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BCR-ABL1 Fusion Patterns Identified by Fluorescence *in situ* Hybridization (FISH) in Chronic Myeloid Leukemia (CML)

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ABSTRACT

BCR-ABL1 gene rearrangement is believed to be the most common molecular genetic abnormality in chronic myeloid leukemia (CML). This fusion has been assessed using the fluorescence *in situ* hybridization (FISH) technique. This study aimed to identify the FISH signal patterns observed in a large cohort of patients in order to provide the necessary information and a guideline for CML treatment. Blood or bone marrow samples from 431 patients diagnosed as suspected CML were processed by standard FISH procedures for identifying *BCR-ABL1* translocation using XL BCR-ABL1 Plus Translocation/Dual Fusion Probe. One hundred forty-seven samples (34.11%) were positive for *BCR-ABL1* fusion, while 284 cases (65.89%) were negative. For positive *BCR-ABL1* fusion, the typical signal pattern was seen in 103 patients (70%). Atypical signal patterns were found in the remaining 44 cases (30%) which included 8.84% of multiple patterns. Besides, we also found 0.68% deletion of *BCR* locus, 1.36% three copies of *ABL1*, and 0.68% four copies of *BCR* with four copies of *AML1*. For CML, the FISH assay has an advantage to support diagnosis, especially in the laboratory that cannot have the facility to perform karyotyping. Therefore, monitoring the *BCR-ABL1* signal pattern identified by FISH is an effective way to provide prognostic guidance and treatment options for CML patients and can be used to predict disease progression and relapse.

Keywords: chronic myeloid leukemia; *BCR-ABL1*; fluorescence *in situ* hybridization

INTRODUCTION

Chronic Myeloid Leukemia (CML) is characterized by the presence of a balanced reciprocal translocation between the *ABL1* gene on chromosome 9 and the *BCR* locus on chromosome 22 or t(9;22) (q34.1;q11.2), resulting in the formation of a *BCR-ABL1* fusion gene called Philadelphia (Ph) chromosome. This fusion gene causes abnormal proliferation and disrupts hematopoietic stem cell (HSCs) differentiation into leukemia progenitor cells (Lim *et al.*, 2005). This chromosome abnormality can also be found in acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL) with some variations in the breakpoint regions.

Currently, the first-line treatment of CML is imatinib mesylate (imatinib), a tyrosine kinase inhibitor (TKI) that explicitly targets *BCR-ABL1* fusion protein. Imatinib works directly on cancer cells and has less side effects than bone marrow transplantation and chemotherapy (Druker *et al.*, 2006; O'Brien *et al.*, 2003; Savona *et al.*, 2008). However, up to 30% of patients with the *BCR-ABL1* fusion gene were resistant to imatinib (Jabbour *et al.*, 2013; Parker *et al.*, 2011). Therefore, improved detection and understanding of *BCR-ABL1* kinase mutation variants will affect CML patients, improving the diagnosis's accuracy. It also helps to evaluate the treatment outcome for CML patients. Various techniques can be used to detect gene mutation, such as conventional cytogenetic analysis of the bone marrow cells, which is the most commonly used method for confirming the presence of the t(9;22). However, this technique requires a cell culture facility and is labor-intensive (Furukawa *et al.*, 2011; Jain *et al.*, 2012).

Fluorescence *in situ* hybridization (FISH) is the technique developed to detect genetic abnormalities of leukemia (Lee *et al.*, 2002) using the commercially available locus-specific dual-color; a dual fusion probe to confirm t(9;22). FISH can ensure t(9;22) whether the signal patterns are typical or atypical. FISH-specific DNA probe as a high-performance tool can be a convenient assay for interphase screening of the *BCR-ABL1* fusion gene in CML patients. Only a few studies describe the patterns of *BCR-ABL1* fusion FISH signals in an unselected patient with CML. Our study described FISH signal patterns observed in a large cohort of patients for necessary information and a guideline for adjusting CML patient treatment.

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MATERIALS AND METHODS

Specimen Collection

Four hundred thirty-one patients were obtained from clinically diagnosed patients as suspected CML between January 2016 to December 2020. Peripheral blood or bone marrow samples were collected on sodium heparin or EDTA approximately 3-5 mL. Clotted blood specimens were excluded.

Ethics

Approval for this study was obtained from the Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University, based on the Declaration of Helsinki (COA. MURA2021/22).

Study Design

The fixed cell suspension was performed with a standard FISH protocol XL BCR-ABL1 Plus Translocation/Dual Fusion Probe (Meta System, GmBH, Altlussheim, Germany) (Figure 1). At least 200 interphase cells from each sample at a 1000x fluorescence microscope were evaluated using a Meta System microscope. Isis software (Meta System, GmBH, Altlussheim, Germany) was used for counting the number of signals within the nucleus and interpreted according to the International System for Human Cytogenetic Nomenclature (ISCN) 2020.

Interpretation of FISH signal patterns

BCR-ABL1 probe consists of a red-labeled probe hybridizing to the *ABL1* gene region at 9q34.1 and a green-labeled probe hybridizing to the *BCR* gene region at 22q11.2. In normal individuals, two green (2G) and two red signals (2R) are seen on normal chromosomes 9 and 22. This pattern is altered when *BCR-ABL1* translocation, a red and green probe are juxtaposed to produce a yellow fluorescent signal (red/green fusion signal) (Kardinal *et al.*, 1976; Sawyers, 1999).

RESULTS

In this study, 431 cases of suspected CML were analyzed with the FISH technique. The age of CML patients ranged from 1 to 96 years with the median age of 40 years, and the most common age range is between 30 – 39 years. (Table 1). The majority of patients, 100 cases, aged less than 50 years (68.03%), while only 47 cases were older than 50 (31.97%). One hundred forty-seven cases (34.11%) showed positive *BCR-ABL1* fusion, while 284 patients (65.89%) were negative. For 147 samples of *BCR-ABL1* fusion. These positive *BCR-ABL1* fusion samples included 75 peripheral blood and 72 bone marrow samples. There were 106 male (72.11%) and 41 female (27.89%) samples (Table 1).

When the t(9;22) was present, the typical *BCR-ABL1* fusion pattern (3G 3R 2Y) consists of three green (3G), three red (3R), and two yellow (2Y) signals. Typical *BCR-ABL1* fusion signals were seen on chromosomes 9 and 22 derived from the balanced translocation or t(9;22), while the red and the green signals were seen on the non-rearranged chromosomes 9 and 22, respectively. Moreover, FISH can detect atypical *BCR-ABL1* fusion patterns. There were five atypical *BCR-ABL1* fusion patterns; 2G2R1Y, 2G3R1Y, 3G3R1Y, 4G4R3Y, and more than one signal pattern (Multiple Patterns). The interpretation of FISH signal patterns was summarized in Table 2.

The typical signal pattern (3G 3R 2Y) was found in 103 cases (70%), while the atypical signal patterns were found in the remaining 44 cases (30%) (Figure 2). The two most common atypical signal patterns were 10 cases (6.8%) of the t(9;22) with deletion of the derivative chromosome 9 involving only the chromosome 22 sequences 3' of *BCR* breakpoint (2G 3R 1Y), and 9 cases (6.1%) of t(9;22) with deletion of the derivative chromosome 9 involving sequence 5' of *ABL* breakpoint as well the chromosome 22 sequence, 3' of the *BCR* breakpoint (2G 2R 1Y).

The appearance of an additional Ph chromosome (4G 4R 3Y) was found in 1 patient (0.68%). While the diversity of the variant Ph chromosomes involvement of another chromosome (3G 3R 1Y) was found in 7 patients (4.76%). Thirteen patients had more than one type of signal pattern. Seven of them had typical dual fusions associated with single atypical fusion (cases 1-6 and 13). Two types of single atypical fusion (2G 3R 1Y with 2G 2R 1Y) (case 7) and two to three kinds of typical dual fusion (3G 3R 2Y with 3G 2R 2Y) (3G 3R 2Y with 3G 2R 2Y and 2G 3R 2Y) were also found in case 8 and 9 respectively. One patient had dual and single fusion associated with gain(s) of the *ABL1* and *BCR* loci (case 10). These gains could represent tetrasomy of these chromosomes (4G 4R 1Y, 4G 4R 2Y, 3G 3R 1Y), and the remaining 2 patients (cases 11 and 12) had typical dual fusions associated with additional Ph chromosome (3G 3R 2Y with 4G 4R 3Y); detail of these 13 patients was described in Table 3. We also found the deletion of *BCR* locus in some cells (1G 2R), three copies of *AML1* (2G 3R), and four copies of *BCR* and *ABL1* (4G 4R).

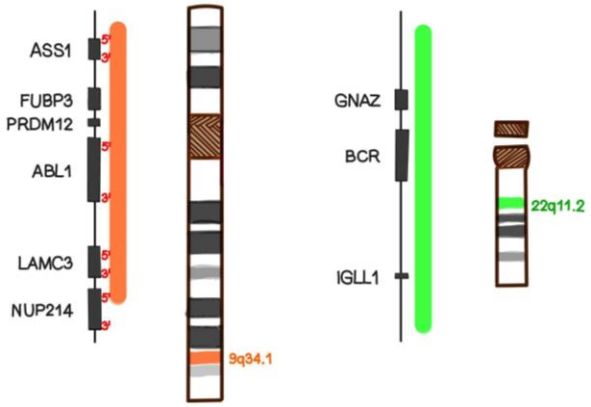

Figure 1. XL *BCR-ABL1* Plus Translocation/Dual Fusion Probe. The orange-labeled probe spans the breakpoint at 9q34.1 (*ABL1*) and the green-labeled probe spans the breakpoint at 22q11.2 (*BCR*).

Table 1. Numbers of CML Patients in this study from January 2016 – December 2020.

Age group year(s)	Male	Female	Total	Percentage (%)
< 20	24	4	28	68.03%
20 - 29	13	1	14	
30 - 39	23	7	30	
40 - 49	19	9	28	
50 - 59	10	10	20	31.97%
60 - 69	10	4	14	
≥ 70	7	6	13	
Total	106 (72.11%)	41 (27.89%)	147	

Table 2. *BCR-ABL1* FISH signal patterns and interpretation.

Signal pattern			Interpretation	PB	BM	Number of patients	
						Total	(%)
A	2G 2R		Normal	*	*	*	*
B	3G 3R 2Y	Typical	t(9;22)	57	46	103	70.07
C	2G 3R 1Y	Atypical	t(9;22) with deletion of the derivative chromosome 9 involving only the sequence 3' of *BCR* breakpoint.	6	4	10	6.81
D	2G 2R 1Y		t(9;22) with deletion of the *ABL1-BCR* fusion on one derivative chromosome.	7	2	9	6.12
E	4G 4R 3Y		t(9;22) with additional Ph chromosome	1	0	1	0.68
F	3G 3R 1Y		Variant (three-or-four way) t(9;22)	2	5	7	4.76
			More than one signal pattern (Multiple Patterns)	9	3	13	8.84
G	1G 2R	Other	Deletion of 9q34 (*BCR* locus)	1	0	1	0.68
H	2G 3R		Three copies of *ABL1* locus	0	2	2	1.36
I	4G 4R		Four copies of *BCR* and *ABL1* locus	0	1	1	0.68
			Total	83	63	147	

PB-peripheral blood
BM-bone marrow

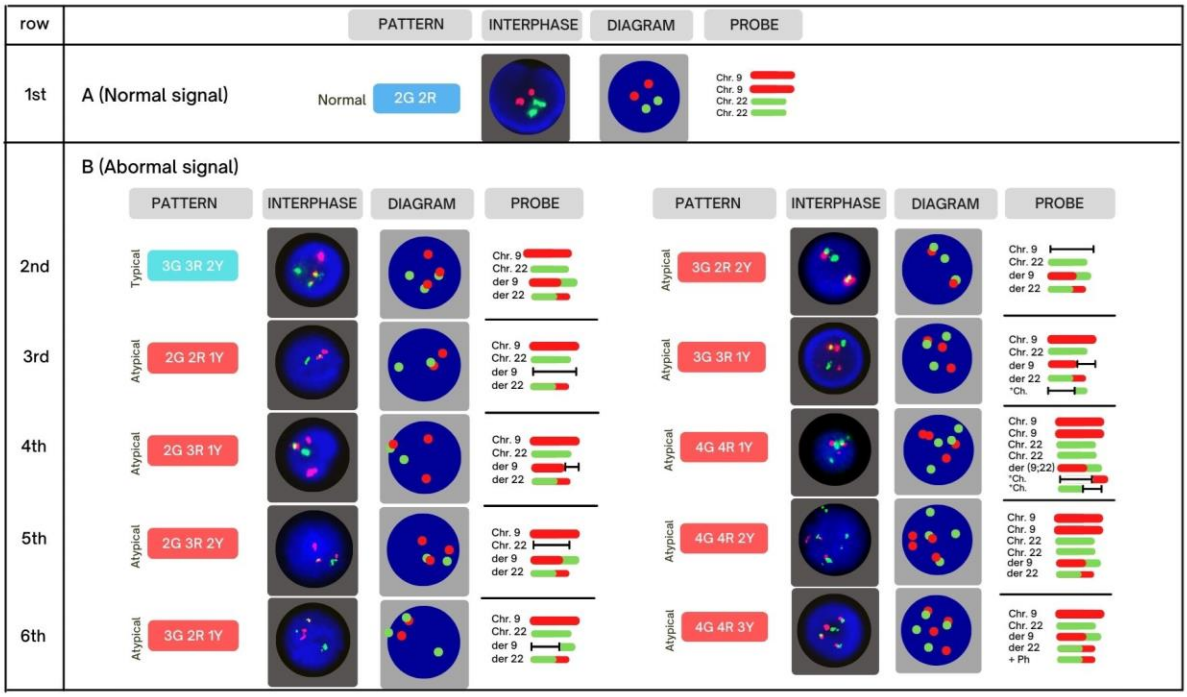

Figure 2. *BCR-ABL1* fusion signal patterns detected by FISH analysis. Part A, the first row: Normal pattern (2G 2R). Part B: ten types of abnormal patterns, the second row (left): Typical (3G 3R 2Y) / (right): Atypical (3G 2R 2Y), third row: (left): Atypical (2G 2R 1Y) / (right): Atypical (3G 3R 1Y), fourth row: (left): Atypical (2G 3R 1Y) / (right): Atypical (4G 4R 1Y), fifth row (left): Atypical (2G 3R 2Y) / (right): Atypical (4G 4R 2Y), sixth row: (left): Atypical (3G 2R 1Y) / (right): Atypical (4G 4R 3Y) *variable includes chromosome other than 9 and 22.

Table 3. Multiple *BCR-ABL1* fusion signal patterns detected by FISH.

Signal pattern	%Dual Fusion (DF)				%Single Fusion (SF)			%DF + SF + Gain(s)*		%DF + add Ph**
	2G3R2Y	3G2R2Y	3G3R2Y	2G2R1Y	2G3R1Y	3G3R1Y	3G2R1Y	4G4R2Y	4G4R1Y	4G4R3Y
Case 1			3				97			
Case 2			29			71				
Case 3			97	3						
Case 4			85			15				
Case 5			5		43	52				
Case 6	5		91	4						
Case 7				19	81					
Case 8		96	4							
Case 9	5	17	78							
Case 10						13		22.5	64.5	
Case 11			69							31
Case 12			87							13
Case 13			1	99						

* dual and single fusion associated with gain(s) of the *ABL1* and *BCR* loci

** typical dual fusions associated with additional Ph chromosome

DISCUSSION

CML is a disease that occurs in any age group. In this study, CML patients were found from 1 to 96 years old, with most patients aged 30-39 years old. The median age of 40 years old is consistent with Bhatti *et al.* and differed from Chen *et al.* that the average age was 66 years old, which was higher than in other studies as shown in Table 4 (Bhatti *et al.*, 2012; Chen *et al.*, 2013). In other words, in the incidence of CML patients living in the United States of America, the majority are Caucasian (85% of the population) (Chen *et al.*, 2013). In addition, it was reported to have a different race from the other studies in Table 4 that are most Asian population. Therefore, it may be concluded that the average age of Caucasians is higher than Asians (Kim *et al.*, 2010). In addition, the incidence of CML in Caucasian populations is mainly found in over 50 years old but in Asians is found in patients younger than 50 years old consistent with this study.

The previous report on the relationship between the age of CML patients and prognosis or progression, indicated that older patients had been recognized as an adverse prognostic factor in CML. The patient's age is a poor prognostic factor due to that the older patients have comorbidities and poor/non-compliance issues; for example, they tend to be less likely to follow up, not take the total amount of medication, or are not likely to control the underlying disease (Ghimire *et al.*, 2012).

Our results indicated that about 70% of Ph-positive patients displayed the typical *BCR-ABL1* dual fusion (3G3R2Y) signal pattern, similar to the previous report (Lim *et al.*, 2005). In contrary to another study, 90-95% incidence was reported (Rowley *et al.*, 1982). We found 13 patients had a similar number of two patterns (2G3R1Y and 2G2R1Y) (Row C, D in Table 2). This abnormality represented deletions on the derivative chromosome 9 which were reported in patients with rapid disease progression (Huntly *et al.*, 2003; Primo *et al.*, 2003). Moreover, recent studies showed that the derivative chromosome 9 deletions were associated with a poor prognosis in all disease phases of CML (Primo *et al.*, 2003; Kim *et al.*, 2010). However, the adverse effect of derivative chromosome 9 deletions can be mitigated by Imatinib therapy at least in early chronic CML (Quintás-Cardama *et al.*, 2011).

The appearance of additional chromosome abnormalities (ACAs) (4G4R3Y) has been reported in 5% of patients (Luatti *et al.*, 2012) while our data was approximately 1% of patients. A recent study showed that ACAs in CML patients during treatment with a tyrosine kinase inhibitor regime were generally associated with resistance to treatment and a sign of disease progression to accelerated phase (AP) or blast phase. (Siti-Mariam *et al.*, 2022). Moreover, the European LeukemiaNet stated that patients with additional Ph chromosomes should be closely monitored because patients may develop symptoms from the AP to early chronic phase (CP) faster than usual and became an adverse prognostic factor in CML patients treated with imatinib (Quintás-Cardama *et al.*, 2011).

Usually, variant Ph translocations are present in 5-10% of CML cases (Mysorekar *et al.*, 2015). In our samples, we also analyzed 7 patients (4.76%) with CML variant translations. The variant translocation with der (9) deletion was found in the same as a previous study of 10-15% CML patients in the CP. These deletions are thought to occur during Ph translocations associated with worsened survival (Sinclair *et al.*, 2000). However, it was also reported that patients with variant translocation had a prognosis similar to those with classical Ph translocation when treated with imatinib mesylate (El-Zimaity *et al.*, 2004). On the other hand, Imatinib was reported to overcome the poor prognostic significance of derivative chromosome 9 deletions in CML patients (Quintas-Cardama *et al.*, 2005).

According to FISH methods, using Dual Color Dual Fusion probe to detect *BCR-ABL1* is able to confirm the existence of a common t(9;22) or atypical signal patterns which could establish the complex *BCR-ABL1* signal patterns (more than one signal pattern; multiple patterns). In this study, we found that 13 patients had more than one type of signal pattern. They are usually insensitive to TKIs, indicating worse clinical outcomes in these patients (Short *et al.*, 2017). More importantly, patients with complex *BCR-ABL1* signal patterns had poorer overall survival (OS) time than those with single patterns (Zhang *et al.*, 2019). Therefore, treatment with hematopoietic stem cell transplantation or the next generation of TKI as soon as possible might overcome the poor prognostic.

CONCLUSION

Monitoring the *BCR-ABL1* signal pattern identified by FISH is an effective way to provide prognostic guidance and treatment options for CML patients, and can be used to predict disease progression, relapse, and OS. A minimum of 50 total cells should be scored per probe (Mascarello *et al.*, 2011).

However, we estimated 200 cells which were able to detect acquired abnormalities. It led FISH to detect additional small clones or gain of a Ph chromosome which provides evidence of disease progression. As a property of the FISH probe, it cannot reveal abnormalities that involves other chromosomes. Therefore, in selected cases, a combination of karyotyping and FISH analysis would give the maximum information in a particular case. In the further study, we plan to explore CML patient incidence and clinical significance in a larger sample size of patients.

Table 4. Age and sex of CML patients

	The current study	**Bhatti *et al.*, 2012**	**Chen *et al.*, 2013**
Average age (years)	40	42	66
<50 years old (%)	68	71.1	20.3
Male: Female Ratio	2.6: 1	2: 1	1.7: 1

REFERENCES

Bhatti FA, Ahmed S, Ali N. Clinical and hematological features of 335 Patients of chronic myelogenous leukemia diagnosed at single centre in Northern Pakistan. Clinical Medicine Insights: Blood Disorders. 2012;5:15-24.

Chen Y, Wang H, Kantarjian H, Cortes J. Trends in chronic myeloid leukemia incidence and survival in the United States from 1975 to 2009. Leuk Lymphoma. 2013;54(7):1411-1417.

Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N, Deininger MW, Silver RT, Goldman JM, Stone RM, *et al.* Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med. 2006;355(23):2408-2417.

El-Zimaity MM, Kantarjian H, Talpaz M, O'Brien S, Giles F, Garcia-Manero G, Verstovsek S, Thomas D, Ferrajoli A, Hayes K, *et al.* Results of imatinib mesylate therapy in chronic myelogenous leukaemia with variant Ph chromosome. Br J Haematol. 2004;125(2):187-195.

Furukawa T, Narita M, Koike T, Takai K, Nagai K, Kobayashi M, Koyama S, Seki Y, Takahashi H, Fujiwara M, *et al.* Clinical value of assessing the response to imatinib monitored by interphase FISH and RQ-PCR for *BCR-ABL* in peripheral blood for long-term survival of chronic phase CML patients: results of the Niigata CML-multi-institutional co-operative clinical study. Int J Hematol. 2011;93(3):336-343.

Ghimire KB, Shah BK. Chronic myeloid leukemia survival in older population in pre- and post-imatinib era in the United States. Blood. 2012;120(21):4237.

Huntly BJ, Bench AJ, Delabesse E, Reid AG, Li J, Scott MA, Campbell L, Byrne J, Pinto E, Brizard A, *et al.* Derivative chromosome 9 deletions in chronic myeloid leukemia: poor prognosis is not associated with loss of *ABL-BCR* expression, elevated *BCR-ABL* levels, or karyotypic instability. Blood. 2002;99(12):4547-4553.

Huntly BJ, Guilhot F, Reid AG, Vassiliou G, Hennig E, Franke C, Byrne J, Brizard A, Niederwieser D, Freeman-Edward J, *et al.* Imatinib improves but may not fully reverse the poor prognosis of patients with CML with derivative chromosome 9 deletions. Blood. 2003;102(6):2205-2212.

Jabbour EJ, Cortes JE, Kantarjian HM. Resistance to tyrosine kinase inhibition therapy for chronic myelogenous leukemia: A clinical perspective and emerging treatment options. Clin Lymphoma Myeloma Leuk. 2013;13(5):515-529.

Jain PP, Parihar M, Ahmed R, Abraham A, Vishwabandya A, George B, Mathews V, Srivastava A, Srivastava VM. Fluorescence *in situ* hybridization patterns of *BCR-ABL1* fusion in chronic myelogenous leukemia at diagnosis. Indian J Pathol Microbiol. 2012;55(3):347-351.

Kardinal CG, Bateman JR, Weiner J. Chronic granulocytic leukemia. Review of 536 cases. Arch Intern Med. 1976;136(3):305-313.

Kim DW, Banavali SD, Bunworasate U, Goh YT, Ganly P, Huang H, Irving I, Jootar S, Goh HG, Koh LP, *et al.* Chronic myeloid leukemia in the Asia-Pacific region: current practice, challenges and opportunities in the targeted therapy era. Leuk Res. 2010;34(11):1459-1471.

Lee, YK, Lee DW, Kim YL, Lee S, Min CK, Kim YJ, Oh IH, Kim TG, Kim CC, Kim DW. Detection of the BCR-ABL gene by interphase fluorescence *in situ* hybridization (iFISH) in chronic myelogenous leukemia patients after hemopoietic stem cell transplantation. Int J Hematol. 2002;(2):180-185

Lim TH, Tien SL, Lim P, Lim AS. The incidence and patterns of *BCR/ABL* rearrangements in chronic

myeloid leukaemia (CML) using fluorescence *in situ* hybridisation (FISH). Ann Acad Med Singap. 2005;34(9):533-538.

Luatti S, Castagnetti F, Marzocchi G, Baldazzi C, Gugliotta G, Iacobucci I, Specchia G, Zanatta L, Rege-Cambrin G, Mancini M, *et al.* Additional chromosomal abnormalities in Ph-positive clone: Adverse prognostic influence on frontline imatinib therapy: a GIMEMA Working Party on CML analysis. Blood. 2012;120(4):761-767.

Mascarello JT, Hirsch B, Kearney HM. Section E9 of the American College of Medical Genetics technical standards and guidelines: fluorescence *in situ* hybridization. Genet Med. 2011;13(7):667-675.

Mysorekar VV, Subramanian M, Kilara N, Sundareshan TS. Variant Philadelphia translocations in chronic myeloid leukemia: A report of five cases. J Cancer Res Ther. 2015;11(3):654.

O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, Cornelissen JJ, Fischer T, Hochhaus A, Hughes T, *et al.* Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2003;348(11):994-1004.

Parker WT, Lawrence RM, Ho M, Irwin DL, Scott HS, Hughes TP, Branford S. Sensitive detection of *BCR-ABL1* mutations in patients with chronic myeloid leukemia after imatinib resistance is predictive of outcome during subsequent therapy. J Clin Oncol. 2011;29(32):4250-4259.

Primo D, Tabernero MD, Rasillo A, Sayagués JM, Espinosa AB, Chillón MC, Garcia-Sanz R, Gutierrez N, Giralt M, Hagemeijer A, *et al.* Patterns of *BCR/ABL* gene rearrangements by interphase fluorescence *in situ* hybridization (FISH) in *BCR/ABL*+ leukemias: incidence and underlying genetic abnormalities. Leukemia. 2003;17(6):1124-1129.

Quintas-Cardama A, Kantarjian H, Talpaz M, O'Brien S, Garcia-Manero G, Verstovsek S, Rios MB, Hayes K, Glassman A, Bekele BN, *et al.* Imatinib mesylate therapy may overcome the poor prognostic significance of deletions of derivative chromosome 9 in patients with chronic myelogenous leukemia. Blood. 2005;105(6):2281-2286.

Quintás-Cardama A, Kantarjian H, Shan J, Jabbour E, Abruzzo LV, Verstovsek S, Garcia-Manero G, O'Brien S, Cortes J. Prognostic impact of deletions of derivative chromosome 9 in patients with chronic myelogenous leukemia treated with nilotinib or dasatinib. Cancer. 2011;117(22):5085-5093.

Ratan ZA, Zaman SB, Mehta V, Haidere MF, Runa NJ, Akter N. Application of Fluorescence *in situ* hybridization (FISH) technique for the detection of genetic aberration in medical science. Cureus. 2017;9(6):e1325-e1325

Rowley JD, Testa JR. Chromosome abnormalities in malignant hematologic diseases. Adv Cancer Res. 1982;36:103-148.

Savona M, Talpaz M. Getting to the stem of chronic myeloid leukaemia. Nat Rev Cancer. 2008;8(5):341-350.

Sawyers CL. Chronic myeloid leukemia. N Engl J Med. 1999;340(17):1330-1340.

Short NJ, Kantarjian HM, Sasaki K, Ravandi F, Ko H, Cameron Yin C, Garcia-Manero G, Cortes JE, Garris R, O'Brien SM, *et al.* Poor outcomes associated with +der(22)t(9;22) and -9/9p in patients with Ph chromosome-positive acute lymphoblastic leukemia receiving chemotherapy plus a tyrosine kinase inhibitor. Am J Hematol. 2017;92(3):238-243.

Sinclair PB, Nacheva EP, Leversha M, Telford N, Chang J, Reid A, Bench A, Champion K, Huntly B, Green AR. Large deletions at the t(9;22) breakpoint are common and may identify a poor-prognosis subgroup of patients with chronic myeloid leukemia. Blood. 2000;95(3):738-743.

Siti Mariam I, Norhidayah R, Zulaikha AB. Differential prognostic impact of stratified additional chromosome abnormalities on disease progression among Malaysian chronic myeloid leukemia patients undergoing treatment with imatinib mesylate. Front Oncol. 2022;12:720845.

Zhang Z, Chen Z, Jiang M, Liu S, Guo Y, Wan L, Li F. Heterogeneous *BCR-ABL1* signal patterns identified by fluorescence *in situ* hybridization are associated with leukemic clonal evolution and poorer prognosis in *BCR-ABL1* positive leukemia. BMC Cancer. 2019;19(1):935.