CASE SERIES

A Case Series of Atypical BCR:: ABL1 Signal Patterns in Chronic Myeloid Leukaemia (CML) Patients

Lin Hui Wong[1], Raja Zahratul Azma Raja Sabudin[1], Sabariah Md Noor[2], Nor Rafeah Tumian[3], Guang Yong Chong[2], Mohd Fikri Mustapa[4], Reena Rahayu Md Zain[1], Ahmed Maseh Haidary[5]

[1] Department of Pathology and Laboratory Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Selangor, Malaysia.
[2] Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia.
[3] Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Selangor, Malaysia.
[4] Department of Laboratory Diagnostic Services, Hospital Canselor Tunku Muhriz, Kuala Lumpur, Malaysia,
[5] Department of Pathology and Clinical Laboratory, French Medical Institute for Mothers and Children (FMIC), Kabul, Afghanistan.

ABSTRACT

Introduction: Chronic Myeloid Leukaemia (CML) is a myeloproliferative neoplasm defined by the BCR::ABL1 fusion gene resulting from the Philadelphia chromosome t(9;22)(q34.1;q11.2). Fluorescence in situ hybridization (FISH) is helpful in detecting both typical and atypical BCR::ABL1 rearrangement patterns. This case series highlights the clinical, cytogenetic and molecular implications of atypical signal patterns in CML patients. **Methods**: Four newly diagnosed CML patients exhibiting atypical BCR::ABL1 FISH signal patterns were included in this case series. Clinical features, cytogenetic findings, and molecular responses to frontline tyrosine kinase inhibitor (TKI) therapy were documented. **Results**: Each patient demonstrated distinct atypical signal patterns: Patient 1 had a deletion on derivative chromosome 9; Patients 2 and 3 showed deletions on derivative chromosome 22; Patient 4 exhibited BCR::ABL1 fusion with concurrent ABL1 and BCR deletions. Patients 1–3 failed to achieve major molecular remission during follow-up. Patient 4, although initially responsive, required a switch from imatinib to nilotinib due to loss of complete cytogenetic remission. **Conclusion**: Atypical BCR::ABL1 FISH signal patterns may correlate with suboptimal therapeutic responses to frontline TKIs. While their prognostic significance remains uncertain, these patterns offer valuable cytogenetic insights that may aid in early identification of patients at risk for treatment resistance or poor major cytogenetic response.
Malaysian Journal of Medicine and Health Sciences (2026) 22(3): 1-5.doi:10.47836/mjmhs.v22i3.1598

Keywords: Chronic Myeloid Leukaemia (CML), Atypical FISH Signal Patterns, BCR::ABL1 Fusion Gene, Fluorescence in Situ Hybridization (FISH), Cytogenetic Abnormalities, Tyrosine Kinase Inhibitors (TKIs)

Corresponding Author:
Dr Raja Zahratul Azma Raja Sabudin, MBBS (UM)
Email: zahratul@hctm.ukm.edu.my
Tel: +603 91455780

INTRODUCTION

Chronic Myeloid Leukaemia (CML) is cytogenetically defined by the reciprocal translocation t(9;22)(q34.1;q11.2), resulting in the formation of the Philadelphia chromosome (Ph) (1). This rearrangement fuses the ABL1 gene on chromosome 9 with the BCR gene on chromosome 22, resulting in the BCR::ABL1 fusion gene that drives the leukaemogenic process in CML (2). This means that there is an exchange of genetic material between the long arm of chromosome 22 (22q−) and the long arm of chromosome 9 (9q+), producing a derivative chromosome 22 (3). Together with to conventional cytogenetic analysis (CCA), fluorescence in situ hybridization (FISH) serves as a widely employed diagnostic tool in CML patients (4). CCA confirms the Philadelphia chromosome in approximately 90% of CML cases, although subtle changes such as microdeletion may be missed (5). A US research team reported that FISH were able to identify submicroscopic deletions on derivative chromosome 9 in 9–15% of patients, localised near the translocation breakpoints. The study also found that these deletions, often present at diagnosis with the Philadelphia chromosome, may result in loss of sequences from chromosome 9, chromosome 22, or both when encompassing the ABL1-BCR junction. (6). FISH with dual-colour, dual-fusion probes which are usually commercially available detects BCR::ABL1 and differentiates typical from

atypical signal patterns. Atypical FISH patterns may indicate deletions on derivative chromosome 9, complex variant translocations, gain of additional Philadelphia chromosomes, or other abnormalities. (5).

Here, we present four cases of CML with atypical BCR::ABL1 rearrangement FISH patterns and describe the clinicopathological characteristics of each case.

CASE SERIES

Patient 1: A 32-year-old male with underlying young hypertension, primary hyperaldosteronism and polycythaemia presented to emergency department with complaints of lethargy, reduced oral intake and weight loss (12 kg in past 2 months). On examination, he had hepatosplenomegaly. His full blood count (FBC) revealed haemoglobin (Hb) of 8.0g/dL, total white cell (TWC) of 166 x10^9/L and platelet (PLT) count of 504 x10^9/L. Peripheral blood film (PBF) findings showed features of CML in chronic phase, as well as leucoerythroblastic blood picture with less than 1% blast cells seen. A diagnostic bone marrow aspiration and biopsy (BMAT) showed features consistent with CML in chronic phase. Conventional karyotyping showed a result of 46, XY, t(9;22) (q34;q11.2) [5] and a BCR::ABL1 rearrangement was identified in the FISH study, with a derivative 9 chromosome deletion (Figure 1C). His peripheral blood was positive for major fragments of BCR::ABL1 gene, while negative for JAK2 V617F mutation. He was started initially with hydroxyurea 500mg once daily (OD) then changed to imatinib 400mg OD. He achieved complete haematological response initially at 5 months post-diagnosis and cytogenetic remission (CCyR) at 12 months post-diagnosis. However, he failed to achieve major molecular remission (MMR) at 12 months, and the latest BCR::ABL1 fusion gene transcript level was 1.69% IS at 21 months post-diagnosis.

Patient 2: A 38-year-old male presented with early satiety for 7 years, reduced effort tolerance for 2 years, associated with vomiting recently. On physical examination, he had an enlarged spleen measuring 21cm. A computed tomographic (CT) scan of the thorax, abdomen and pelvis (CT TAP) showed diffuse splenomegaly measuring 21.2cm. His full blood count reported Hb of 8.9g/dL, TWC count of 235 x10^9/L and PLT count of 293 x10^9/L. PBF and BMAT showed features of CML in chronic phase. His conventional karyotyping study reported a normal male karyotype (46, XY [20]), but FISH findings were consistent with BCR::ABL1 rearrangement (translocation) with a deletion on the derivative chromosome 22 (Figure 1D). Reverse transcriptase polymerase chain reaction (RT-PCR) of his peripheral blood sample detected major fragments of BCR::ABL1 gene. He was initially treated with hydroxyurea 1g OD for about 2 weeks, followed by imatinib 400mg OD. The patient achieved a complete haematological response at 4 months post-diagnosis but only achieved partial cytogenetic remission (PCyR) at 6 months after diagnosis. He failed to achieve MMR with BCR-ABL fusion gene transcripts of 3.92% IS at 10 months post-diagnosis.

Patient 3: A 59-year-old male with underlying hypertension presented to a private hospital with body weakness, lethargy, weight loss and loss of appetite for the past two months. On examination, he had an enlarged spleen measuring 12cm. His FBC showed Hb of 11.5g/dL, TWC count of 195 x10^9/L and PLT count of 893 x10^9/L. PBF reported hyperleukocytosis with a leucoerythroblastic picture. Diagnostic BMAT findings were consistent with CML in chronic phase. His conventional karyotyping was suboptimal due to poor metaphase spread. FISH study was positive for BCR::ABL1 rearrangement (translocation) with a deletion on the derivative 22 (Figure 1E). Molecular analysis using RT-PCR reported the presence of major fragments of the BCR::ABL1 gene. The Amplification refractory mutation system - PCR (ARMS-PCR) analysis of his peripheral blood sample was negative for the JAK2 V617F mutation. He was started on imatinib 400mg OD. He achieved a complete haematological response in the 5 month post-diagnosis and CCyR 3 months after diagnosis. However, he failed to achieve MMR with BCR::ABL1 fusion gene transcripts of 16.44% IS at 11 months and >55% IS at 22-month post-diagnosis.

Patient 4: A 70-year-old male, with underlying hypertension, dyslipidaemia and ischaemic heart disease presented with intermittent fever, weight loss and lethargy. On examination, he had an enlarged spleen measuring 15cm. His full blood count reported Hb of 14.5g/dL, TWC count of 18.7 x10^9/L and PLT count of 544 x10^9/L. PBF showed polycythaemia, leucocytosis, thrombocytosis with a leucoerythroblastic picture but no blast cell. BMAT findings suggested of myeloproliferative neoplasm (MPN) with grade 3 fibrosis. Conventional karyotyping revealed a normal male karyotype (46, XY) [1]. FISH identified findings consistent with a BCR::ABL1 translocation, including an atypical FISH signal (Figure 1F), comprising one (1) green, one (1) orange and one (1) fused green-orange (yellow) signal pattern. Molecular analysis of his peripheral blood sample was negative for major fragments of BCR::ABL1 gene in but positive for JAK2 V617F mutation. Treatment was initiated with imatinib 400mg OD and T. hydroxyurea 500mg OD. He achieved complete haematological response 18 months after diagnosis and a CCyR 9 months post-diagnosis. Molecular monitoring using BCR::ABL1 quantification was not performed as patient was suspected to have the p230 variant. 17 months post-diagnosis, the patient experienced loss of CCyR (i.e No Ph-positive metaphases) and was switched to nilotinib 200mg BD. He subsequently achieved CCyR again 8 months after initiating nilotinib.

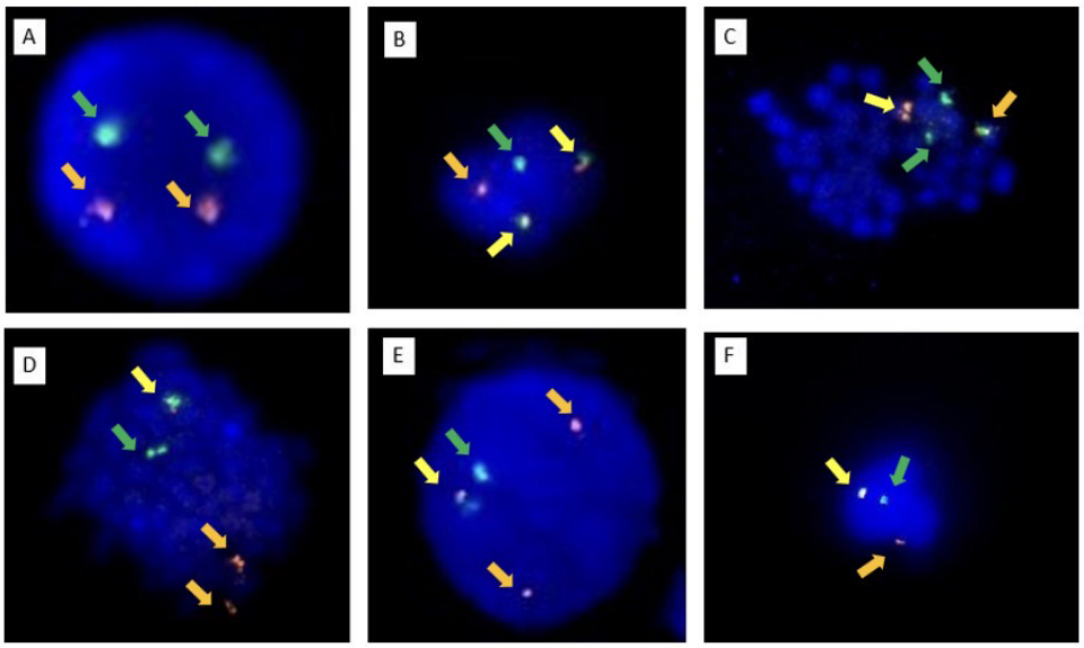

Figure 1: BCR::ABL1 signal patterns and their interpretations (O = orange indicated by orange arrow / ABL1 signal; G = green indicated by green arrow / BCR signal; F = fusion signal consisting of one ABL1 signal and one BCR signal, indicated by yellow arrow), (A) Interphase nuclei, negative, 2O2G; (B) Typical signal pattern, 1O1G2F; (C) Patient 1, metaphase cells, ABL1 deletion, 1F1O2G; (D) Patient 2 and (E) Patient 3, BCR deletion, 1F2O1G, (F) Patient 4, ABL1 and BCR fusion deletion, 1F1O1G.

DISCUSSION

CML is a myeloproliferative neoplasm marked by a neoplastic clonal proliferation of haemopoietic stem cells, predominantly involving granulocytic lineage expansion (7). The hallmark of CML is the chromosomal translocation t(9;22)(q34.1;q11.2), resulting in the Philadelphia (Ph) chromosome that harbours the BCR::ABL1 fusion gene. (4). The fusion gene encodes a constitutively active tyrosine kinase that drives uncontrolled proliferation of haematopoietic stem cells (8). In CML, one of the main diagnostic methods for detecting the Ph chromosome is conventional cytogenetic analysis (CCA). This technique identifies the hallmark chromosomal translocation by analysing the karyotype of bone marrow metaphases and detecting additional structural and numerical chromosomal abnormalities beyond the Ph chromosome (9). Another widely used molecular cytogenetic method is fluorescence in situ hybridization (FISH). This technique uses a commercially available locus-specific dual-colour, dual-fusion probe to identify the BCR::ABL1 fusion gene in interphase nuclei or metaphase cells of bone marrow samples.

In our centre, the FISH probes for CML are designed by using an orange (O) probe for ABL1 and a green (G) probe for BCR. When the BCR::ABL1 fusion gene is present, a fused green-orange signal, producing yellow signal (F) can be observed (Figure 1B). In the absence of the Ph chromosome translocation, FISH signal patterns demonstrate two orange signals corresponding to normal chromosome 9 copies and two green signals representing normal chromosome 22 copies (2O2G pattern) (Figure 1A). In contrast, a diagnosis of CML is established when the FISH pattern demonstrates two fusion (F) signals which reflects the derivative chromosomes 9 and 22, along with one orange and one green signal corresponding to the remaining normal chromosomes 9 and 22, respectively (2F1O1G pattern). In Patient 1, an atypical FISH signal pattern of 1F1O2G was observed, comprising one fusion signal (derivative chromosome 9), one orange signal (normal chromosome 9), and two green signals, arising from a normal chromosome 22 and the reciprocally translocated chromosomes 9 with 22. This atypical pattern suggests ABL1 deletion and is likely indicative of sub-microscopic deletions on the derivative chromosome 9 (6). Patient 1 exhibited this atypical FISH pattern during the diagnostic cytogenetic test and failed to achieve MMR at 12th month post-diagnosis.

To date, the prognostic implication of chromosome 9 (der(9)) derivative deletion remains inconclusive due to conflicting study reports. In pre-tyrosine kinase inhibitor (TKI) era, early studies suggested that der(9) deletion was linked with poor prognosis across all phases of CML. However more recent studies indicate that imatinib, one of such from the first generation TKIs, may provide CML patients a good opportunity to overcome this poor prognostic impact (6). A research (10) involving 78 CML patients treated with frontline TKIs concluded that variant dual-colour, dual-fusion FISH signal pattern, including deletions of ABL, BCR or BCR::ABL1 on derivative 9 did not correlate with therapy response. In contrast, a larger study from India (11), which involved 489 CML patients found differing outcomes.

Patients with accelerated phase or blast crisis CML harbouring derivative chromosome 9 (der(9)) deletions demonstrated poor response to imatinib, whereas those with der(9) deletions in early-phase CML exhibited favourable responses to imatinib. The researchers further suggested that atypical BCR::ABL1 gene rearrangements may contribute to disease progression in CML. In some patients, large deletions near the ABL breakpoint on derivative chromosome 9 have been associated with the loss of one or more tumour suppressor genes. This loss could be a contributory factor to the poor prognostic impact (3). Thus, further studies are necessary to investigate the role of ABL deletions on der(9) in CML progression.

In Patient 2 and Patient 3, the atypical FISH pattern indicated BCR deletion and was termed 1F2O1G. This pattern consisted of one fusion signal (from derivative chromosome 22), two orange signals (one from the reciprocally translocated chromosomes 9 and 22, and one from normal chromosome 9), and one green signal (normal chromosome 22). In Patient 4, a deletion involving ABL1 and BCR fusion was detected, yielding an atypical 1F1O1G FISH pattern consisting of one fusion signal (derivative chromosome 22), one red signal (normal chromosome 9), and one green signal (normal chromosome 22). While the exact function of the normal BCR gene remains unclear, the BCR region translocated to del/der(9) encodes p21rac protein, which activates a GTP-ase, vital in regulation of growth and proliferation of cell by exerting an inhibitory effect when bound to p21rac. Loss of this protein may result in abnormal cell growth and proliferation (3). However, to date, no established association exists between deletions involving derivative chromosome 22 deletion and prognosis (12). In our case series, Patient 2, 3 and 4 all exhibited the atypical FISH pattern at diagnosis. Patient 2 and Patient 3 failed to achieve MMR at 10 month and 11 month post-diagnosis, respectively. For these patients, closer cytogenetic monitoring to evaluate their treatment response - after excluding issues such as drug resistance and compliance issue - would likely be beneficial these patients in subsequent follow ups.

The utility of metaphase FISH to detect both the presence and/or location of BCR::ABL1 translocation, particularly in cases with variant translocation or cryptic rearrangement, is exemplified in Patient 4. In this case, the patient's BMAT findings were suggestive of an MPN with grade 3 fibrosis. Conventional karyotyping was suboptimal, yielding fewer than 20 metaphases, and molecular analysis failed to detect major fragments of the BCR::ABL1 fusion, despite the patient testing positive for JAK2 V617F mutation. However, the FISH study successfully identified the BCR::ABL1 translocation with an atypical signal pattern. A plausible explanation for this discordant result is that the BCR::ABL1 fusion that arose outside the classically defined major breakpoint cluster region. Without the FISH study or its ability to detect the BCR::ABL1 translocation, a diagnostic dilemma could have arisen, making it challenging to differentiate an MPN in the fibrotic phases and JAK2 mutated primary myelofibrosis. This case highlights the importance of understanding the limitations of various diagnostic assays and their complementary roles in interpreting result and managing CML patient effectively.

In Patient 4, the rare coexistence of a JAK2-mutated MPN and BCR::ABL1 translocation observed in 24 of 85 reported cases in current scientific literature (13). While the clonal origin remains debated, this study favours the presence of two independent clones, based on an increased JAK2 mutation burden after TKI-induced suppression of the BCR::ABL1-positive clone. This dual pathology may also arise sequentially and should be considered in atypical MPN courses before diagnosing treatment failure or resistance.

Our case series highlights the potential of identifying and monitoring BCR::ABL1 signal patterns detected by FISH as an effective tool for providing prognostic insights and predicting disease progression. Previous studies have reported that patients with derivative 9 deletions treated with frontline TKIs exhibited lower haematological response rates and major cytogenetic response compared to those without these deletions (14). Similarly, we observed a lower molecular response in Patient 1, who had derivative 9 deletions. In addition, the emergence of derivative 9 deletion during the treatment course may signify the development of secondary chromosomal aberrations. This suggests disease progression and a potential drug resistance for patients with chronic-phase of CML (11).

CONCLUSION

Identifying atypical BCR::ABL1 signal patterns can help recognise CML patients with a reduced response to frontline TKIs treatment, such as those with additional chromosomal abnormalities. Also, monitoring these atypical signal pattern throughout the treatment course can aid in the early detection of potential drug resistance or identify patients at higher risk of disease progression.

REFERENCES

1. Chen Z, Cortes JE, Jorgensen JL, Wang W, Yin CC, You MJ, et al. Differential impact of additional chromosomal abnormalities in myeloid vs lymphoid blast phase of chronic myelogenous leukemia in the era of tyrosine kinase inhibitor therapy. Leukemia. 2016 Feb 3;30(7):1606–9. doi: 10.1038/leu.2016.6
2. Srivastava V, Jain P, Parihar M, Ahmed R, Abraham A, George B, et al. Fluorescence in situ hybridization patterns of BCR/ABL1 fusion in chronic myelogenous leukemia at diagnosis. Indian Journal of Pathology and Microbiology [Internet]. 2012 [cited 2019 Nov 6];55(3):347. Available from: http://www.ijpmonline.org/text.asp?2012/55/3/347/101742. doi: 10.4103/0377-4929.101742
3. Željka Tkalčić vabek, Josipović M, Horvat I, Zadro R, Sanja Davidović-Mrsić. The incidence of atypical patterns ofBCR-ABL1rearrangement and molecular-cytogenetic response to tyrosine kinase inhibitor therapy in newly diagnosed cases with chronic myeloid leukemia (CML). Blood Research [Internet]. 2018 Jan 1 [cited 2023 Dec 19];53(2):152–2. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6021570/. doi: 10.5045/br.2018.53.2.152
4. Haidary AM, Noor S, Sharif S, Yousufzai AW, Nasir N, Noor S, et al. PB1901: Karyotypic Profile Of Chronic Myeloid Leukaemia Diagnosed At Tertiary Level In Afghanistan. HemaSphere. 2022 Jun;6:1780–1. doi: 10.1097/01.hs9.0000850456.72817.89
5. Zhang Z, Chen Z, Jiang M, Liu S, Guo Y, Wan L, et al. 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 Oct 8;19(1). doi: 10.1186/s12885-019-6137-8
6. Quintás-Cardama A, Kantarjian H, Shan J, Jabbour E, Abruzzo LV, Verstovsek S, et al. Prognostic Impact of Deletions of Derivative Chromosome 9 in Patients With Chronic Myelogenous Leukemia Treated With Nilotinib or Dasatinib. Cancer [Internet]. 2011 Nov 15 [cited 2022 Feb 10];117(22):5085–93. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4324753/. doi: 10.1002/cncr.26147
7. Haidary AM, Azma RZ, Ithnin A, Alauddin H, Tumian NR, Tamil AM, et al. FISH versus real-time quantitative PCR for monitoring of minimal residual disease in chronic myeloid leukaemia patients on tyrosine kinase inhibitor therapy. The Malaysian journal of pathology [Internet]. 2019 Aug [cited 2022 Feb 10];41(2):149–60. Available from: https://pubmed.ncbi.nlm.nih.gov/31427550/
8. Turkina A, Wang J, Mathews V, Saydam G, Jung CW, Al Hashmi HH, et al. TARGET: a survey of real-world management of chronic myeloid leukaemia across 33 countries. British Journal of Haematology. 2020 Mar 30;190(6):869–76. doi: 10.1111/bjh.16599
9. Haidary AM, Ahmed ZA, Abdul-Ghafar J, Rahmani S, Noor S, Erfani F, et al. Philadelphia chromosome positive chronic myeloid leukemia with 5q deletion at diagnosis. Molecular Cytogenetics. 2021 Mar 8;14(1). doi: 10.1186/s13039-021-00539-0
10. Patel BP, Trivedi PJ, Brahmbhatt MM, Gajjar SB, Iyer RR, Dalal EN, et al. Detection of derivative 9 deletion by BCR-ABL fluorescence in-situ hybridization signal pattern to evaluate treatment response in CML patients. Archive of Oncology. 2009 Jan 1;17(1-2):13–8. doi: 10.2298/AOO0902013P
11. Chandran RK, Geetha N, Sakthivel KM, Aswathy CG, Gopinath P, Nair JKrishnaKM, et al. Prognostic Implications of Derivative Chromosome 9 Deletions in Patients with Advanced-Stage Chronic Myelogenous Leukemia. Journal of Environmental Pathology, Toxicology and Oncology. 2018;37(2):117–26. doi: 10.1615/JEnvironPatholToxicolOncol.2018026023
12. Egan D, Radich J. Making the diagnosis, the tools, and risk stratification: More than just BCR-ABL. Best Practice & Research Clinical Haematology. 2016 Sep;29(3):252–63. doi: 10.1016/j.beha.2016.10.015
13. Zanelli M, Bisagni A, Sanguedolce F, Broggi G, Fragliasso V, Zizzo M, et al. Co-occurrence of JAK2-V617 F mutation and BCR::ABL1 translocation in chronic myeloproliferative neoplasms: a potentially confounding genetic combination. Frontiers in Oncology [Internet]. 2024 Jan 12 [cited 2025 Apr 21];13. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10811046/
14. Fernandes A, Shanmuganathan N, Branford S. Genomic Mechanisms Influencing Outcome in Chronic Myeloid Leukemia. Cancers [Internet]. 2022 Jan 26;14(3):620. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8833554/. doi: 10.3390/cancers14030620