results. Additionally, splicing variants are detected by qPCR and incorporated into the Molecular Response (MR) calculation, which may result in poorer MR outcomes, as literature suggests these variants exhibit reduced sensitivity to TKIs. **Methods:** To address these challenges in CML management, we developed a PCR-based technique coupled with fragment analysis to accurately identify these variants prior to sequencing. We validated this test with 30 samples with Δ7 and/or INS35, prior identified by the Sanger sequence. Moreover, we designed a specific qPCR assay to quantify splicing variants exclusively. **Results and conclusion:** We performed in silico modeling of the wild-type versus Δ7 and INS35 *BCR::ABL1* isoforms, showing the KD disruption caused by the early stop codon resulting from the frameshift caused by Δ7 and INS35. Additionally, our findings indicate that this molecular approach enables highly accurate and sensitive identification of splicing variants, even at very low levels. We observed that these events are independent of CML status, also occurring in healthy donor samples. Furthermore, Δ7 and INS35 are present throughout the longitudinal patient follow-up and are not associated with TKI failure. However, Δ7 with a variant allele frequency (VAF) greater than 0.15 is more prevalent in samples with higher *BCR::ABL1* transcript counts, whether at diagnosis, failure, or relapse. This study highlights how Δ7 and INS35 can confound mutational testing results and demonstrates how to overcome these challenges for a more precise analysis of point mutations in CML patients carrying splicing variants.

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PHILADELPHIA CHROMOSOME-NEGATIVE CHRONIC MYELOID LEUKEMIA WITH BCR::ABL1 FUSION

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Introduction: Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm characterized by the chromosome philadelphia (Ph) in 90-95% of cases. The Ph chromosome results from the reciprocal t(9;22)(q34.1;q11.2) and encodes the fusion gene *BCR::ABL1*. The chimeric tyrosine kinase oncoprotein is constitutively activated, leading to continuous cell proliferation and driving leukemogenesis. **Case report:** Herein, we present a rare case of a 36-years-old male patient diagnosed with Ph-negative CML. The patient was admitted to the Hematology Department of Instituto Nacional de Câncer (INCA) in September 2019 presenting persistent fever, splenomegaly, weight loss and night sweats. Blood workup showed significant leukocytosis (229.450/μL), thrombocytosis (639.000/μL) and eosinophilia (16.000/μL, 7%). At diagnosis, the patient exhibited 5% of myeloid blasts in blood and the b2a2 isoform of *BCR::ABL1* was detected by qualitative reverse-transcription polymerase chain reaction (RT-PCR). The patient was classified as high risk according to the Sokal Index (2.1) and his initial treatment with hydroxyurea was followed by Imatinib (600 mg/day). Molecular monitoring was performed by qualitative RT-PCR for p210, because *ABL* control gene failed to amplify in our quantitative RT-PCR (qPCR) assay in several samples, despite the successful amplification of *BCR::ABL1* fusion gene. After 18 months, and again after 42 months, clinical worsening due to the patient's low adherence to treatment led to a change in therapy to Nilotinib (800 mg/dia) and Dasatinib (100 mg/dia), respectively. In 2023, the patient was hospitalized with accelerated phase CML (AP-CML) characterized by basophilia and no detectable myeloid blast. Conventional cytogenetic analysis was performed in bone marrow sample from relapse and revealed a normal karyotype (46, XY[20]), however, FISH using LSI *BCR::ABL1* Dual Color/Dual Fusion Probe identified one *BCR* signal; two *ABL* signals and a single fusion signal in 86/100 nuclei analyzed, consistent with the *BCR::ABL1* detection by RT-PCR. Additionally, the F317L mutation was detected by the *BCR::ABL1* mutational testing realized at relapse. The mutation in AP-CML scenario led to the recommendation for hematopoietic stem cell transplantation. Three months post-transplantation, the patient rejected the graft, showing complete autologous recovery. Nilotinib treatment (800 mg/dia) was reintroduced with satisfactory clinical response. **Discussion:** This case describes a rare instance of CML with a normal karyotype but positive for *BCR::ABL1*, often referred to as Ph-negative CML. Literature suggests that those cases may arise from complex chromosomal abnormalities, including three-way or multi-way translocations involving chromosomes 9, 22, and additional chromosomes. In this case, the normal karyotype by conventional cytogenetics together to the FISH pattern implies the possibility of a cryptic insertion between chromosomes 9 and 22, where a partial *ABL1* segment from chromosome 9 may have inserted into the *BCR* locus on chromosome 22, thus hindering detection by conventional cytogenetics. This highlights the importance of integrating molecular cytogenetics and molecular biology techniques with conventional cytogenetics for comprehensive CML diagnosis, as cases with a normal karyotype may still harbor complex chromosomal abnormalities only detectable by FISH and PCR testing.

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DIFFERENTIAL GENE EXPRESSION AND PATHWAYS ENRICHMENT ANALYSIS IN TWO RNA-SEQ DATASETS OF JAK2V617F POSITIVE CELL LINE, TREATED WITH RUXOLITINIB, INDICATE FOCAL ADHESION PATHWAY MODULATION

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