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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Objective: Myeloproliferative neoplasms (MPNs) are clonal disorders characterized by activation of JAK/STAT and related pathways, leading to excessive proliferation, resistance to apoptosis and inflammation. The JAK2 inhibitor Ruxolitinib (Ruxo) is indicated for some groups of patients with MPN, reducing symptoms and improving overall survival. However, the factors that influence its effectiveness in reducing malignant clones and, consequently, the emergence of resistance, are not fully understood. Besides, the Focal Adhesion Kinase (FAK), a kinase involved in adhesion, migration, proliferation and apoptosis, participates in JAK2 V617F-positive cells'viability and apoptosis, and Ruxo seems to modulate genes related to the Focal Adhesion pathway in a public RNAseq dataset of SET-2 cells, as previously shown by this research group. The present work aimed to investigate whether the Focal Adhesion pathway is modulated after Ruxo treatment in JAK2 V617F-positive cells HEL cells, by analyzing two publicly available RNAseq datasets. **Materials and methods:** GSE184850 and GSE229712 were selected for analysis from the "Gene Expression Omnibus" (www.ncbi.nlm.nih.gov/geo/). Both datasets are from RNAseq in HEL cells (JAK2 V617F-positive) treated with DMSO and Ruxo 0.5 uM for 48h. The datasets were first analyzed separately for differentially expressed genes (DEGs) using DESeq2 package in R and Rstudio. Then, the common DEGs among top 250 DEGs were determined using ggVennDiagram package and analyzed in the STRING database (string-db.org) for KEGG pathways enrichment. **Results:** There were 153 common DEGs. Enrichment analysis in STRING database showed that Focal Adhesion pathway was among the 10 KEGG relevant pathways, with 10 DEGs: *RELN, SHC1, ITGA5, IGFR1, FLT4, VASP, CCDN2, ACTN1, KDR, ZYX* (strength 0.82, FDR: 0.00060). *RELN* and *IGF1R* were upregulated in Ruxo compared to DMSO, in both datasets, while *SHC1, ITGA5, FLT4, VASP, CCDN2, ACTN1, KDR, ZYX* were downregulated. **Discussion:** The downregulated genes are directly involved in migration and invasion processes. They are associated with cytoskeleton remodeling, which is important for disease progression. In addition, their expression is dysregulated in other types of cancers and leukemias. Upregulated genes *RELN* and *IGF1R* are genes involved in the activation of signaling pathways related to disease progression, such as those associated with proliferation and survival. **Conclusions:** The present results indicate a possible modulation of Focal Adhesion pathway after Ruxo treatment, which may be involved in treatment effectiveness and, therefore, further investigations regarding involvement of these genes in MPN may contribute to elucidate the influence of this pathway on patient treatment and prognosis.

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EFFICACY OF OLVEREMBATINIB IN THE TREATMENT OF REFRACTORY CHRONIC MYELOID LEUKEMIA: A SYSTEMATIC REVIEW AND SINGLE-ARM META-ANALYSIS

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Objective: To perform a systematic review and meta-analysis to evaluate the efficacy of Olverembatinib, a novel BCR::ABL1 tyrosine kinase inhibitor (TKI), in refractory chronic myeloid leukemia (CML). **Material and methods:** PubMed, Embase and Cochrane database were searched, until June 2024, for randomized clinical trials (RCTs) that assess the efficacy of Olverembatinib in patients with CML, with or without *T315I* mutation, who have failed at least two prior TKI therapy. The primary outcomes were Major Molecular Response (MMR), Complete Cytogenetic Response (CCyR) and Major Cytogenetic Response (MCyR). Statistical analysis was performed using R-4.4.1. Heterogeneity was examined with I^2 statistics. **Results:** 238 patients were included from 3 RCTs, with follow-up ranging from 0 to 5 years. The pooled analysis revealed an MMR of 0,4205 (95% CI [0,2789 - 0,5765]; $p < 0,01$); a CCyR of 0,5501 (95% CI [0,3529 - 0,7328]; $p < 0,01$) and an MCyR of 0,6548 (95% CI [0,4886 - 0,7902]. Heterogeneity was high across all outcomes, with I^2 values of 85% for MMR, 88% for CCyR and 85% for MCyR. **Discussion:** The results demonstrate that Olverembatinib has significant efficacy in achieving high rates of MMR, CCyR and MCyR in refractory CML patients. However, the high heterogeneity observed in all three outcomes suggests notable variability across studies. This variability likely stems from differences in baseline characteristics, including the number of prior TKI treatment lines and median treatment duration among study populations. The small number of studies included also contributes to this variability. **Conclusion:** Olverembatinib shows promise as an effective treatment option for refractory CML. However, further research is warranted to elucidate the significant heterogeneity observed across studies, optimize treatment strategies, and provide more robust evidence of its efficacy.

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MASTOCITOSE SISTÊMICA COM MUTAÇÃO C-KIT EXON 17 (D816V): RELATO DE CASO

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Introdução: A mastocitose é uma doença rara com uma incidência de 1 em 10.000 pessoas e predominância em mulheres adultas. Ela se caracteriza pelo acúmulo anormal de mastócitos, que podem se limitar à pele (mastocitose cutânea) ou afetar outros tecidos (mastocitose sistêmica). Os sintomas incluem máculas na pele, rubor, taquicardia, diarreia, anafilaxias e pancitopenias. O diagnóstico de mastocitose sistêmica segue os critérios da OMS de 2016, que incluem infiltração densa de mastócitos em órgãos e medula óssea