consolidação com Citarabina, resultando em menor tempo de tratamento. Conforme literatura atualizada, pacientes com mutação NPM1 tratados com FLAG-Ida e GO tiveram resposta mais rápida e profunda, além de maior sobrevida livre de progressão e menor risco de recidiva, independente da mutação FLT3. Além disso, esse esquema terapêutico está associado a níveis menores de DRM, possibilitando redução na indicação de TCTH. Todavia, no Brasil o resultado de NGS demanda maior tempo para liberação, o que impossibilitou início de QT de indução com FLAG-Ida e GO, como preconizado. O caso relatado demonstra a importância do estudo citogenético e molecular para escolha do regime de quimioterapia de indução e programação do tratamento. O atraso no acesso aos resultados pode implicar tratamento mais prolongados e intensivos, que demandam indicação de TCTH.

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## INTEGRATION OF SINGLE-CELL RNA SEQUENCING AND MACHINE LEARNING MODELING IN THE IDENTIFICATION OF STEMNESS FEATURES IN ACUTE MYELOID LEUKEMIA PATIENTS

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**Introduction:** Human hematopoiesis is a dynamic process initiated during embryonic development in distinct anatomical niches, culminating in the formation of hematopoietic stem cells (HSCs), responsible for lifelong blood cell production. With aging, HSCs undergo functional decline, contributing to hematological disorders such as acute myeloid leukemia (AML). In these conditions, altered stem cells may drive disease progression and resist treatment. Advances in single-cell transcriptomics (scRNA-seq) have enabled high-resolution analysis of cellular heterogeneity in both healthy and diseased tissues. To manage the complexity of such data, machine learning (ML) algorithms have been increasingly employed to uncover gene expression patterns, differentiation states, and pathological features. These models outperform traditional marker-based methods by classifying cell types, estimating dedifferentiation levels, and detecting resistant subpopulations. In this context, algorithms such as One-Class Logistic Regression (OCLR) and decision tree-based methods have shown promise in identifying molecular stem cell signatures across biological settings. Once trained, these models can be applied to new datasets to recognize cells with similar profiles, aiding in the functional annotation of both normal and malignant samples. **Objectives:** The aim of this study was to train machine learning models on public bone marrow scRNA-seq datasets to identify cells with a stemness profile, apply these models to transcriptomic data to classify samples, and evaluate their clinical impact. **Material and methods:** ML models including OCLR, Random Forest, and linear-kernel Support Vector Machine (SVM), were used to train classifiers on public bone marrow scRNA-seq datasets. The models were applied using Spearman correlation on normalized and scaled raw counts from transcriptomic data of the TCGA AML cohort (n = 151) and two public cohorts with healthy samples (n = 101). A z-score was calculated as: z = sample score − mean (healthy)/ SD (healthy). Scores above 1.96 were considered indicative of high stemness. Hazard ratios were calculated using Cox proportional hazards models. **Results:** All models achieved comparable performance in metrics such as AUC and accuracy, with Random Forest showing higher Area Under Precision Recall Curve (AUPRC) in external validation and statistically outperforming SVM (p = 0.0380, Nemenyi post-test). Survival analysis revealed that the Random Forest model was significantly associated with overall survival. Patients in the high-stemness group (z-score > 1.96) had a hazard ratio (HR) of 1.73 (95% CI: 1.03−2.89, Logrank p value = 0.0344) compared to the low-stemness group. The median survival was 0.75 years for the high group and 1.59 years for the low group. In contrast, no significant association was observed for the OCLR (HR = 1.02, 95% CI: 0.59 −1.76, p = 0.922) or SVM (HR = 1.15, 95% CI: 0.66−2.02, p = 0.600) models. **Discussion and conclusion:** In conclusion, although all models demonstrated similar discriminative performance, the Random Forest approach not only achieved superior AUPRC in external validation but also showed a significant prognostic association with overall survival. These findings suggest that, among the tested stemness scoring methods, the Random Forest−derived HSCsi model may provide greater clinical utility by integrating predictive accuracy with prognostic relevance. **Funding:** This work was supported by the National Council for Scientific and Technological Development (CNPq), Brazil.

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## INTESTINAL PRIMARY MYELOID SARCOMA AS AN ISOLATED PRESENTATION WITHOUT BONE MARROW INVOLVEMENT: CASE REPORT AND NARRATIVE REVIEW.

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**Introduction:** Myeloid sarcoma (MS) is a rare extramedullary manifestation of immature myeloid cell proliferation, and its occurrence preceding acute myeloid leukemia (AML) is uncommon. MS can affect several organs and tissues, as the infiltration of gastrointestinal (GI) tract being particularly rare. A standard treatment for isolated MS has not yet been established; however, due to the high risk of progression to AML, intensive chemotherapy is generally recommended and may be combined with allogeneic stem cell transplantation (allo-SCT), radiotherapy (RDT), and surgery. Therefore, this study aims to report a case of an isolated MS and compare it

with what is available in the literature as a narrative review. **Methods:** We conducted a review of similar cases of MS published in the last 10 years by searching the PUBMED database. Our search identified 28 articles, 18 of which were included in this review. Results: (1) Case report: a 35-year-old male presented with lower abdominal pain associated with alternating episodes of diarrhea and constipation in January 2024. In April, he was hospitalized due to an intestinal obstruction, as the abdominal CT scan revealed a small intestine wall obstruction mass by the mesogastric region. He underwent emergency enterectomy with the resected material consistent with MS by immunohistochemistry (IHC): positive for MPO, CD71, CD34, c- KIT; negative for CD61. The patient was kept without any other intervention until his first consultation at our service in May 2024. His blood counts showed no alterations, and a bone marrow biopsy revealed no signs of infiltration. PET-CT demonstrated an area of abnormal uptake (SUV max of 7.3) in the pelvic region corresponding to a heterogeneous pre-sacral mass. He underwent a CT guided biopsy that confirmed the persistence of the MS. The patient started induction chemotherapy in May 2024 with daunorubicin and cytarabine (3+7), achieving a complete remission (CR) by the PET-CT performed in June 2024. The patient followed treatment with 4 cycles of consolidation with high dose cytarabine until October 2024 and additionally, he underwent RDT with 24 Gy in December 2024. A new PET-CT was performed in January 2025 still maintaining CR. (2) In our literature review, 18 cases of primary gastrointestinal MS were identified. The male-to-female ratio was 2:1, and the mean age was 45 years. The most affected site in the GI tract was the small intestine (50% of cases). IHC showed positivity for MPO, CD34, and CD117 in nearly all cases, along with high Ki-67 indices ranging from 30% to 95%. Most patients responded well to intensive chemotherapy regimens based on cytarabine and anthracyclines, with or without allo-SCT (only 3 paients underwent consolidation with all- SCT), whereas surgical treatment alone was insufficient for disease control. **Conclusion:** MS is a rare entity with a challenging diagnosis and no established standard of care. A transplant-free approach may be viable depending on patient-specific factors, with sustained complete responses achieved through chemotherapy and radiotherapy alone. Understanding the complex interplay of genetic, environmental, and immunological factors in the pathogenesis of isolated MS is essential to identifying effective treatment strategies and improving patient outcomes.

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## INVESTIGAÇÃO TRANSLACIONAL DO TOLINAPANT (ASTX660) NA LEUCEMIA MIELOIDE AGUDA UTILIZANDO ABORDAGENS INTEGRADAS CLÍNICAS, BIOINFORMÁTICAS E FARMACOLÓGICAS

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**Introdução:** O tolinapant (ASTX660) é um antagonista das proteínas inibidoras da apoptose (IAPs) cIAP1 (BIRC2), cIAP2 (BIRC3) e XIAP. Essas IAPs atuam inibindo a atividade das caspases, suprimindo a apoptose. Recentemente, o tolinapant foi introduzido no tratamento de neoplasias hematológicas e demonstrou resultados promissores em modelos pré-clínicos de linfoma de células T (TCL), com efeitos imunomodulatórios benéficos confirmados em um pequeno grupo de pacientes com TCL. Na leucemia mieloide aguda (LMA), um estudo avaliando o tolinapant como agente único e em combinação com a cedazuridina (ASTX727) em pacientes com LMA recidivada/refratária foi encerrado precocemente (NCT04155580). **Objetivos:** Investigar a base farmacológica e molecular da atividade do tolinapant, bem como o papel de seus alvos, no contexto da LMA. **Material e métodos:** Investigamos os alvos do tolinapant (BIRC2, BIRC3, XIAP) quanto à expressão, impacto prognóstico e relevância funcional em LMA. Foram utilizados dados de pacientes da coorte TCGA (n = 121), dados ex vivo da coorte BeatAML (n = 570) e dados do DepMap com deleção de BIRC2, BIRC3 e XIAP em 25 linhagens celulares. Avaliamos a citotoxicidade do tolinapant (0–100 $\mu$M) em 14 linhagens de LMA por ensaio MTT. Testes combinatórios com venetoclax foram realizados em linhagens resistentes (Kasumi-1 e OCI-AML3), usando MTT (25 combinações), citometria de fluxo (Annexina V/PI) e Western blot (PARP1 clivado, $\gamma$H2AX). Sobrevida foi analisada por Kaplan– Meier, e associações por testes estatísticos apropriados (ANOVA, Spearman, log-rank). Valores de IC50 foram obtidos por regressão não linear, e escores ZIP de sinergia foram gerados com SynergyFinder. **Resultados:** Alta expressão de BIRC2 (p = 0,04) foi associada à pior sobrevida global, enquanto BIRC3 e XIAP não mostraram essa associação. BIRC3 foi mais expresso em pacientes de risco intermediário. Citogeneticamente, BIRC2 esteve aumentado em del(5), t(9;11)+outros e del(7q); BIRC3 em del(7q)+outros e del(5q)+outros; XIAP foi reduzido em del(5). Em análises ex vivo, BIRC2 associou-se com resistência a 20 agentes, BIRC3 a 28 (e sensibilidade a 7), e XIAP a 12 (e sensibilidade a 20), todos com $p < 0,05$. No DepMap, observou-se dependência moderada de BIRC2 (−0,334) e mínima para BIRC3 e XIAP. O tolinapant isolado apresentou citotoxicidade modesta (IC50 mensurável em 5/14 linhagens; faixa: 29,8–77,2 $\mu$M; eficácia de 13,6% a 99,9%). Combinações com venetoclax mostraram sinergia em Kasumi-1 (ZIP = 13,8) e efeito aditivo em OCI-AML3 (ZIP = 8,4), com aumento de apoptose ($p < 0,05$). Análises moleculares revelaram maior clivagem de PARP1 e acúmulo de $\gamma$H2AX com a combinação. **Discussão e conclusão:** Nosso estudo revela que a alta expressão de BIRC2 está associada a pior prognóstico e resistência a múltiplos agentes em LMA, sugerindo seu potencial como biomarcador e alvo terapêutico. Embora o tolinapant tenha eficácia