avaliar o potencial antineoplásico do THZ-P1-2 em modelos celulares de LMA e LLA. **Métodos:** Um painel contendo 12 linhagens celulares de LMA e 8 de LLA foi utilizado. A viabilidade celular foi avaliada por ensaio de MTT, apoptose por anexina V/PI e citometria de fluxo (CF), a formação de organelas vesiculares ácidas (OVAs) por marcação com laranja de acridina e CF, o ciclo celular por marcação de iodeto de propídeo por CF e a sinalização celular por PCR quantitativo (PCRq) e Western blot. Para as comparações, foi utilizado o teste ANOVA e o pós-teste de Bonferroni. Um valor de $p < 0,05$ foi considerado significativo. **Resultados:** Em modelos de LMA e LLA, o tratamento com THZ-P1-2 foi capaz de reduzir a viabilidade celular de forma dependente de concentração, sendo as células Jurkat as mais sensíveis (IC_{50} 2,9 μM) e as células SET2 as mais resistentes (IC_{50} >100 μM). Duas linhagens de LMA (OCI-AML3 e MV4-11) e duas de LLA (Jurkat e NALM6) foram selecionadas para caracterização dos efeitos celulares e moleculares mais detalhados. O tratamento com THZ-P1-2 induziu apoptose e a formação de OVAs de forma dependente de concentração (todos $p < 0,05$). A análise do ciclo celular indicou o aumento de células em subG1 e redução de células em G2/M (todos $p < 0,05$). A análise molecular indicou que THZ-P1-2 apresenta um papel paradoxal na autofagia, em um primeiro momento o composto induziu as etapas iniciadoras da autofagia (aumento de LC3II) e depois inibiu as etapas finais do fluxo autofágico (redução do consumo de p62/SQSTM1), o que pode aprisionar a célula em um estado de inércia. Além disso, marcadores de dano em DNA (γH2AX) e apoptose (clivagem de PARP1) foram induzidos pelo THZ-P1-2. A análise do perfil de expressão gênica indicou que processos como a morte celular por via mitocondrial, autofagia, sinalização de via intrínseca de apoptose induzida por dano em DNA e a resposta celular à privação de nutrientes estão envolvidas no mecanismo de ação do composto. **Discussão e conclusão:** A contribuição dos genes que compõe a família PIP4K2 para as leucemias agudas tem sido revelada nos últimos anos, sendo desde marcadores de prognóstico até a associação com a suscetibilidade genética. A identificação de inibidores farmacológicos das PIP4K2 pode representar novas oportunidades para intervenções terapêuticas nessas doenças. Nesse sentido, o THZ-P1-2 parece apresentar uma propriedade farmacológica singular, atuando paradoxalmente como indutor e inibidor da autofagia, o que está de acordo com estudos genéticos relacionados às funções das PIP4K2. Diante das evidências crescentes da importância da autofagia na biologia das neoplasias hematológicas, o THZ-P1-2 pode emergir como uma classe de fármacos inovadora nesse cenário. **Financiamento:** FAPESP, CAPES e CNPq.

https://doi.org/10.1016/j.htct.2021.10.253

ABSENT CD33 IN AML PATIENT MIMICS MRD POSITIVE: A SINGLE CASE REPORT

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Background: Diagnosis and treatment of acute myeloid leukemia (AML) remains difficult to this day, cure rates remain low and measurable residual disease (MRD) assessment is still challenging. Early detection of residual leukemic cells is essential to predict therapeutic success and Multiparametric Flow Cytometry (MFC) has been an effective tool for it. Here, we focus on the importance in caution when interpreting MDR in AML. **Methods:** Bone marrow (BM) sample was assessed to *FLT3* and *NPM1* mutation, *RUNX1/RUNX1T1* and *CBFB-MYH11* rearrangements, karyotype (KT) and immunophenotyping. Using an 8-colour antibody panel (CD45, CD34, CD117, CD 33, HLA-DR, CD38, CD123, CD13, CD19, CD7, CD11b, CD4, CD56, CD64, CD11c, CD15, CD133, CD41a and NG2), we applied a gating strategy with fixed gates comparing normal to AML BM, settled to catch LAIPs (Leukemia Associated-Immunophenotypes), abnormal cell maturation pattern and Leukemic Stem Cell, that were detected on diagnosis and evaluated for MRD. Assays were performed at the Hematology Laboratory of the Medical School of Ribeirão Preto, University of São Paulo, in accordance to Local Ethical Boards. **Results:** A 21-year-old male patient with AML without maturation, diagnosed after 60 days of symptoms, presented anemia (7.9 g/dL), thrombocytopenia (26 000/μL) and white blood cell count of 5710/μL with 73% blasts in peripheral blood and 84% in BM. MFC revealed CD45dim and low side scatter; positive staining for CD117, CD34, HLA-DR, CD38, CD123, CD11c, CD64, CD15 and CD133; negative staining for CD33, CD14, CD7, CD11b, CD4, CD56, CD19, CD41a and NG2. We found no metaphases (KT), no molecular abnormality and he failed risk stratification. Treatment consisted of 2 induction cycles (cycle 1: 3 days of Daunorubicin 60 mg/m2 and 7 days of cytarabine 200 mg/m2; cycle 2: 6 days of cytarabine 1 g/m2 twice a day) and 1 or 2 consolidation cycles (6 days of cytarabine 1 g/m2 twice a day). BM was obtained at diagnosis, 30 and 33 days after 1st and 2nd induction, and 3, 9, 12 and 15 months after consolidation of chemotherapy. The positivity of a specific phenotype (CD117+/CD34+/CD33wk/CD13+ $\geq$ 0.1%) was preserved at diagnosis and follow-ups: 0,506%, 0,681%, 0,402%, 0,101%, 0,158% and 0,122%. Complete remission was achieved by day 30 after 1st induction and no change in the outcome was reported. Absent CD33 was observed in diagnosis and follow-ups, during and after treatment. **Discussion:** Post-analytical phase of MFC is the most vulnerable to wrong interpretations. CD33 is a common myeloid antigen expressed on malignant blasts in AML and had been reported

as a potential target therapy. CD33^{low} blasts are associated to a more mature AML and its high expression is related to adverse landscapes, highlighting the importance of CD33 evaluation. Standard care for AML enrolls MRD monitoring and failure to achieve an MRD-negative, CR or detected MRD during or after therapy is associated with relapse or poor outcomes. Still, MRD analysis must embrace a set of standards in order to prevent post-analytic errors. Here we present a case report of AML with absent CD33 maintained from diagnosis to MRD follow-ups, mimicking MRD positivity. This data emphasizes the importance of caution in MFC analysis and correlation of diagnostic and follow-ups results interpretation, as well as constant training of the team.

https://doi.org/10.1016/j.htct.2021.10.254

ACUTE UNDIFFERENTIATED LEUKEMIA WITH BCR-ABL TRANSLOCATION: CASE REPORT AND LITERATURE REVIEW

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The 2016 WHO classification defines acute undifferentiated leukemia (AUL) as a rare type of acute leukemia (AL) without defining markers for either lymphoid or myeloid lineage commitment. Literature suggests AUL does not harbour the genetic mutations commonly seen in lymphoblastic leukemia or acute myeloid leukemia (AML) including FLT3, WT1, rearrangements of KMT2A or BCR-ABL1 and it highly expresses genes associated with poor prognosis in AML (BAALC, ERG, and MN1); therefore, AUL is often associated with dismal outcome (Arber et al. 2016). This case report depicts an unusual genetic presentation of an AUL with BCR-ABL fusion gene. A 54-year-old female patient presented to our hospital in July 2021 with fatigue and adynamia one month before. She was also found with deep vein thrombosis in the left leg and segmental pulmonary thromboembolism. Laboratory exams showed Hb 9,7 g/dL, WBC 75.77×10^9/L (77% blasts), platelets 175×10^9/L. Coagulogram and fibrinogen were normal. Bone marrow (BM) aspiration was hypercellular, showing 86% of agranular blasts. Flow cytometry (FC) from BM blasts exhibited expression of CD34, CD45, HLA-DR, CD33, CD38, and partial expression of CD10 and CD123. The blasts were negative for MPO, CD79a, CD19, CD7, CD3, CD13, C117, CD16, CD11b, CD64, CD15, CD14, CD300e, CD61, NG2, CD71, CD4, CD36, CD22, CD42b. Conventional cytogenetics revealed a Ph+ disease (46,XX,t(9;22)(q34q11.2)[7]/46, idem, add(4)(q35)[7]/46,XX [6]). Real-time quantitative PCR showed positive BCR-ABL with p190 transcripts. FLT3 and NPM1 were found negative. Diagnosis of AUL Ph+ was made. AML chemotherapy was started with 3+7 standard doses regimen plus imatinib 600 mg/day. On day 14th BM evaluation found 89% of blasts with the same immunophenotype as in the diagnosis. The patient presented neutropenic colitis with the need to delay rescue therapy. We searched articles in the Pubmed database using the following strategy: "Bcr-Abl Tyrosine Kinase"AND "undifferentiated leukemia", "Philadelphia chromosome"AND "undifferentiated leukemia"between 1976 and 2021. We found two case reports that described patients with AUL and positive BCR-ABL gene. In 1983, Smadja, N et al. reported a patient with AL considered at diagnosis to be undifferentiated. Neither the diagnosis cytogenetic nor FC data was provided by the authors, and the AL classification was based only on morphological characteristics. After 17 months, the patient developed Ph+ chronic myelogenous leukemia with a complex translocation (2; 9; 22). Hattori, M et al., in 1995, described a patient that following treatment to non-Hodgkin lymphoma developed myelodysplastic syndrome (MDS) which progressed to AUL, both found with the presence of chimeric BCR-ABL transcripts. The FC showed blasts that expressed CD7 and HLA-DR, and were negative for lineage markers: CD14,CD33,CD2,CD3,CD4,CD5,CD8,CDl0,CD19,CD20 and CD41. Qasrawi, A. et al., in 2019 and 2020 performed two studies analyzing data from 24 and 1444 cases of AUL, respectively. The most common pathogenic mutations were PHF6, SRSF2, RUNX1, ASXL1, and BCOR. No BCR-ABL mutation was described. We believe that with the advent of target therapies, the prognosis of AUL can improve. The patient described has the BCR-ABL gene as a potential target to a tyrosine kinase inhibitor. Considering this is not a usual finding, we hope this report can further help treatment decisions.

https://doi.org/10.1016/j.htct.2021.10.255

ARHGAP6 EXPRESSION IS ASSOCIATED WITH MOLECULAR RISK AND IMPACTS CLINICAL OUTCOMES IN ACUTE MYELOID LEUKEMIA

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Objectives: Despite the advances in the large-scale generation of genomic data from acute myeloid leukemia (AML) patients, much effort is still needed to convert these data into biological functions, which may pave avenues for the discovery of new prognostic markers and therapeutic targets. In a previous study, four cytoskeleton-related genes (ARHGAP6, EZR, MAP3K11 and PAK1) were identified as a prognostic factor in AML. In the present study, we detail the prognostic impact, as well as its functional genomic associations, of the ARHGAP6 gene in AML patients. **Material and methods:** Clinical and genomic data were obtained from the TCGA AML cohort (n = 173). The clinical impact (overall survival [OS] and disease-free survival [DFS]) for ARHGAP6 expression was investigated by the proportional hazards Cox regression model. Functional genomic analyzes were performed by gene set