

requires complete standardization of processes to ensure optimal results. In addition, clinical validation of the MRD assessment strategy is essential. This study aimed to retrospectively evaluate the clinical effectiveness of a previously analytically validated 10-color MFC protocol to detect AML MRD. **Material, patients and methods:** Databases from the flow cytometry laboratory and from the bone marrow transplantation service as well as information from the electronic medical records of AML patients were accessed. Inclusion criteria: patients with MRD evaluated by the validated 10-color MFC protocol, with at least four months of follow-up after the last MRD assessment or who died due to leukemia relapse before the end of this 4-months follow-up. The samples included should have at least 500,000 evaluable CD45+ cells. All levels of MRD positivity were considered for survival analysis. Eighty-two patients with non-promyelocytic AML with a median age of 38 (10-69) years were included in the cohort; 43 were female. Seventy-six patients underwent allogeneic hematopoietic stem cell transplantation (alloHSCT) and six were treated with intensive chemotherapy. The strategy to validate the effectiveness of the 10-color MFC protocol in detecting AML MRD was the concordance of MRD results with patients' expected outcomes. Clinical sensitivity and specificity, positive and negative predictive values, and diagnostic concordance were calculated even for patients with MRD evaluations post-alloHSCT and after the second cycle of induction chemotherapy. Overall survival (OS) and relapse free survival (RFS) were estimated using the Kaplan-Meier method and the cumulative incidence method was used to calculate the incidence of relapse (CIR). p -values ≤ 0.05 were considered statistically significant. The results showed high sensitivity (87%), specificity (97%), positive predictive value (78%), negative predictive value (98%) and diagnostic concordance (95%) of the 10-color MFC protocol. The two-year OS, RFS, and CIR of pre-HSCT patients ($N=68$) according to MRD $< 0.1\%$ vs $> 0.1\%$ were 76.4% vs 51.5% ($p=0.117$), 79.8% vs 5.3% ($p=0.006$), and 53% vs 2% ($p < 0.001$), respectively. Regarding post-HSCT and after 2nd cycle of intensive chemotherapy patients ($N=74$), OS and RFS were higher in patients with MRD $< 0.1\%$ vs $> 0.1\%$ (75.5% vs 50% $p=0.008\%$) and (78.4% vs 14.3% $p < 0.006$) respectively, with smaller CIR (3% vs 6% $p < 0.001$). In conclusion, objective parameters were used in this study to calculate the clinical sensitivity and specificity of the MRD method. However, the aspects such as the clonal evolution, the MRD kinetics and the therapeutic interventions have impact on sensitivity of MRD results and should be considered for the interpretation of AML relapse. Despite the small cohort and short follow-up time, the 10-color MFC protocol for AML MRD showed high correlation of levels of MRD with clinical outcome and proved to be useful for clinical decision-making.

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IMMUNOPHENOTYPIC SIGNATURE OF LEUKEMIA ARREST STAGE IS ASSOCIATED WITH NPM1/FLT3 MUTATIONAL PROFILE AND PROGNOSIS IN ACUTE MYELOID LEUKEMIA

AG Carvalho ^{a,b}, LO Marani ^{a,b}, CAB Garcia ^{a,b}, M Medeiros ^{a,b}, LS Binelli ^{a,b}, AFO Costa ^c,

EVB Ferreira ^a, JLS Schiavinato ^a, PS Scheucher ^a, LL Figueiredo-Pontes ^{a,b}

^a Hematology, Hemotherapy and Cell Therapy Division, Department of Medical Imaging, Hematology, and Clinical Oncology, Faculdade de Medicina de Ribeirão Preto (FMRP), Universidade de São Paulo (USP), Ribeirão Preto, Brazil

^b Fundação Hemocentro de Ribeirão Preto (FUNDHERP), Faculdade de Medicina de Ribeirão Preto (FMRP), Universidade de São Paulo (USP), Ribeirão Preto, Brazil

^c Division of Hematology and Oncology, Department of Medicine, University of Alabama at Birmingham, Birmingham, United States

Acute Myeloid Leukemia (AML) is a severe hematopoietic disorder marked by uncontrolled proliferation of immature myeloid progenitors due to differentiation arrest, with significant immunophenotypic diversity. *FLT3*-ITD mutations' prognostic impact varies with *NPM1* mutation presence and the *FLT3* allelic ratio. While cytogenetic and molecular features are well-established for risk stratification by European LeukemiaNet (2017 ELN), the role of leukemia arrest stages in prognosis remains unclear. This study classified the maturation arrest stages of leukemic blasts in a Brazilian AML cohort, analyzing its association with genetic risk and clinical data. Bone marrow from 148 *de novo* AML patients (Sep-2015-Jul-2024) was evaluated for *FLT3* and *NPM1* mutations, *FLT3* allelic ratio, and immunophenotyping. MPO cytochemistry stains and phenotypic markers by flow cytometry defined six stages of leukemia arrest (SLA): Hematopoietic Stem Cells-like (HSC-L:CD34⁺CD117⁺CD13⁺CD33⁺HLA-DR⁺MPO⁺); Multipotent Progenitors-like (MPP-L:CD34⁺CD117⁺CD13⁺CD33⁺HLA-DR⁺MPO⁺); Common Myeloid Progenitors-like (CMP-L:CD34⁺CD117⁺CD13⁺CD33⁺HLA-DR⁺MPO⁺); Granulocyte-Monocyte Progenitors-like (GMP-L:CD34⁺CD117⁺CD13⁺CD33⁺HLA-DR⁺MPO⁺); Monocyte Progenitors-like (MP-L:CD34⁺CD117⁺CD13⁺CD33⁺HLA-DR⁺MPO⁺); and Granulocyte Progenitors-like (GP-L:CD34⁺CD117⁺CD13⁺CD33⁺HLA-DR⁺MPO⁺). SLA showed highest frequency in MP-L ($n=57$, 38.5%) and GP-L ($n=53$, 35.8%), followed by GMP-L ($n=23$, 15.5%), CMP-L ($n=7$, 4.7%), MPP-L ($n=6$, 4.1%), and HSC-L ($n=2$, 1.4%). Predominance of *FLT3*^{wt}/*NPM1*^{mut} patients ($n=80$, 54.1%) was observed, with similar distribution among *FLT3*^{mut} patients (*FLT3*^{mut}/*NPM1*^{mut}: $n=32$, 21.6%; *FLT3*^{mut}/*NPM1*^{wt}: $n=36$, 24.3%). CMP-L and GMP-L frequencies were increased in *FLT3*^{low}/*NPM1*^{wt} and *FLT3*^{high}/*NPM1*^{wt} vs. *FLT3*^{wt}/*NPM1*^{mut} (CMP-L:13.33%, 14.28% vs 1.25%, $p < 0.05$; GMP-L:40%, 42.85% vs 7.5%, $p < 0.05$). GP-L frequency was decreased in *FLT3*^{low}/*NPM1*^{wt} and *FLT3*^{high}/*NPM1*^{wt} vs. *FLT3*^{wt}/*NPM1*^{mut} (0%, 9.52% vs 43.75%, $p < 0.05$). Response to treatment (CR/CRi, CR/CRiMRD⁺ and PD–Persistence disease) was evaluated after 1st and 2nd cycles of Induction of Remission (1IR, 2IR). GMP-L was increased in PD after 1IR (OR:6.09, 95% CI:1.22–30.19, $p < 0.05$). Immature (HSC+MPP+GMP) SLA was higher in MRD+ patients compared to MRD- (OR:6.81, 95% CI:0.86–47.05, $p < 0.05$). Overall survival ($p=0.81$) and relapse-free survival ($p=0.27$) analyses showed that GMP-L patients relapsed sooner. In 2017 ELN risk assessment, *FLT3*-ITD stratification was based on allelic ratio and *NPM1* mutation status. A high *FLT3*-ITD allelic ratio with *NPM1* mutation

indicated intermediate risk, and with wild-type *NPM1*, it indicated unfavorable risk. The 2022 ELN guidelines classify *FLT3-ITD* alone as intermediate risk, regardless of *NPM1* status. Mutated *NPM1* is favorable only when *FLT3-ITD* is absent. Supporting these findings, *FLT3* mutation, regardless of allelic ratio, in the absence of *NPM1*, led to immature SLA and increased GMP-L frequency. This SLA was linked to PD after the 1IR. Immature SLA significantly impacts prognosis with lower survival rates, higher relapse risks, and increased post-induction remission failure. Our findings enhance the understanding of the cell of origin in AML, and further investigation of the SLA-dependent mechanisms that contribute to leukemia resistance are warranted.

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INVESTIGATION OF THE GENETIC ALTERATIONS IN *CDKN2A/B* AND *IL7R* IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

RMB Lemos^a, ES Costa^b, T Ferraz^c, MT Schramm^{a,d}, MM Lins^e, MCN Fagundes^a, ALT Maciel^f, M Emerenciano^a

^a Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil

^b Instituto de Puericultura e Pediatria Martagão Gesteira (IPPMG), Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil

^c Instituto Estadual de Hematologia Arthur de Siqueira Cavalcanti (Hemorio), Rio de Janeiro, Brazil

^d Prontobaby Hospital da Criança Ltda, Rio de Janeiro, Brazil

^e Instituto de Medicina Integral Professor Fernando Figueira (IMIP), Recife, Brazil

^f Universidade Federal do Paraná (UFPR), Curitiba, Brazil

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematopoietic disorder triggered by the acquisition of molecular alterations that affect thymocytes in early stages of its development. Deletions in *CDKN2A/B* are found in 65-70% of cases. However, their prognostic value is still undefined. Loss of *CDKN2A*, in addition to *IL7R* activation, contributes to the development of B-cell precursor ALL (B-ALL). The activation of *IL7R*, either due to the presence of gain-of-function mutations (10% of cases) or its upregulation, is a recurrent event in T-ALL associated with disease relapse. Thus, *IL7R* activation is a crucial event in T-ALL initiation, but its transcriptional profile and prognostic role are still unclear. We aimed to investigate the presence of molecular alterations in *CDKN2A/B* and in *IL7R* in samples from pediatric and adult patients diagnosed with T-ALL. Samples from patients with a morphological and immunophenotypic diagnosis of T-ALL between the years 2018-2023 from the INCA Hematology Service and different cancer treatment centers in Brazil were included. The SALSA MLPA P335-C1 kit (MRC Holland), multiplex ligation-dependent probe amplification (MLPA) technique, was used to evaluate the presence of copy number alterations (CNA) in genes related to lymphopoiesis and commonly

altered in ALLs, such as *CDKN2A/B*. The presence of mutations/single nucleotide polymorphism (SNP) in *IL7R* was assessed by polymerase chain reaction (PCR) for amplification of exon 6 followed by ABI3500 sanger sequencing. The transcriptional levels of *IL7R* were investigated using real-time quantitative PCR (RT-qPCR). 38 T-ALL samples were included (13 female and 25 male individuals), whose median age was 11.5 years. *CDKN2A/B* deletions were observed in 16 pediatric (44.4%, being 8 monoallelic and 8 biallelic deletions) and 2 adults (5.6%, 1 monoallelic and 1 biallelic deletions). Activating indel mutations in *IL7R*, which affect the juxtamembrane region of the protein, were found in 2 pediatric patients. Both patients had high leukocyte counts ($> 100 \times 10^9$ cells/mL) at diagnosis and presented concomitant *CDKN2A/B* biallelic deletions. Besides, we found that 36.8% of our cohort presented a single nucleotide polymorphism (SNP) rs6897932 in exon 6 of *IL7R*. Among these samples, 12/14 were pediatric and 2/14 adults. In the pediatric subset, 7 samples presented heterozygous (TC) and 5 homozygous variants (TT). Both adult samples had heterozygous genotype (TC). Our data corroborates the literature showing that *CDKN2A/B* genetic alterations are frequently found in T-ALL patients. The analysis of the mutation or SNP on the *IL7R* expression is undergoing. However, the two patients harboring *IL7R* activating mutations did not present transcriptional deregulation in this gene. Since it's still unclear whether the mutations lead to increased gene expression in our series of cases, we aim to explore other transcriptional pathways that might play a role in *IL7R* deregulation scenario in T-ALL. Of note, to the best of our knowledge, this is the first study describing a high frequency of the SNP rs6897932 in T-ALL.

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RHOA KNOCKDOWN AFFECTS P53 SUBCELLULAR LOCALIZATION IN LEUKEMIA CELLS IRRADIATED WITH UVC

AD Ferreira^a, SSC Sampaio^a, PSSM Ferrari^a, ASS Duarte^b, CRR Rocha^a, STO Saad^b, M Lazarini^a

^a Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil

^b Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil

Introduction and aims: Acute myeloid leukemia (AML) is a severe hematological malignancy with low rates of survival. Mutations in the tumor suppressor gene *TP53* are present in around 10% of AML cases and are associated with a worse prognosis. Evidence suggests that the Rho GTPase proteins RhoA and RhoC are involved in the regulation of p53 activity. In this study, we aimed to investigate the relation between RhoA and RhoC expression and p53 expression and subcellular localization in leukemia cells. **Methods:** OCI-AML3 cell line (wild type *TP53*) was transduced with lentiviral particles containing specific shRNA for the silencing of RhoA (shRhoA) or RhoC (shRhoC) or non-specific shRNA (shCTRL). Cells were irradiated with UVC and nucleus (DAPI) and p53 were