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Introduction: Because IKZF1 deletions are associated with an increased risk of relapse in patients with B-cell precursor acute lymphoblastic leukemia (B-ALL), the development of methods for rapid and accurate detection of this alteration has a great clinical impact. Although multiplex ligation-dependent probe amplification (MLPA) has been widely used for the evaluation of IKZF1 deletions, multiplex (M)-PCR is a valuable tool for the validation of those IKZF1 deletions. However, this method is still limited to detect recurrent intragenic deletions (Δ2-3, Δ2-7, Δ2-8, Δ4-7, Δ4-8). Therefore, here we evaluate the breakpoint map of IKZF1 deletions with a special focus on non-recurrent deletions to update methodologies for the diagnosis of this genetic alteration. Moreover, we explore the genetic features underlying those IKZF1 deletions to infer its molecular pathogenesis. **Methodology:** This study included four international cohorts of pediatric and adult patients with B-ALL. IKZF1 deletions were identified by MLPA (P335 and P202 assays) and M-PCR. Non-recurrent deletions were verified by next-generation sequencing using the Illumina DNA Prep with Enrichment assay. We also revised previously published data regarding IKZF1 deletions in B-ALL for compiling breakpoint sequence information of these deletions. Sequence data from our cohorts and literature were used in further analyses. After identifying the breakpoint clusters, we performed an agnostic motif search using MEME and annotated the presence of filler DNA or microhomologies at the junctions of IKZF1 deletions. **Results:** A total of 1,472 B-ALL samples were screened, and IKZF1 deletions were found in 16% of these patients. Although non-recurrent deletions were associated with 1% of the total cohort, it represents 8% of all IKZF1 deletions. The analysis of breakpoint sequences at DNA level revealed that non-recurrent IKZF1 deletions comprises miscellaneous alterations, but the majority of them encompases Δ1 and Δ1-3. Considering that IKZF1 exon 1 deletions are more difficult to reliably detect by MLPA due to the high GC content (>50%) of this region, we mapped several exon 1 deletions at DNA level and identified one commonly deleted region which is ideal for designing MLPA probes. Next, we searched for motifs, additional nucleotides/microhomologies associated with IKZF1 deletion sequences. We verified an enrichment of the 5'-CACACAGTG-3'sequence within 21 out of 24 breakpoint clusters of IKZF1 deletion (E-value: 2.9e-008). Additionally, the filler DNA within the junctions of IKZF1 deletions presented a higher GC nucleotide content at these clusters as compared to the deletions outside of them (71% vs. 56%). **Conclusions:** This study provides the genetic landscape of IKZF1 deletions in B-ALL. Although rare types of deletion may be identified at the routine diagnosis, they should be considered after using adapted MLPA protocols, including additional probes within the common deleted region nearby IKZF1 exon 1. We also verified the occurrence of the heptamer sequence and an enrichment of GC nucleotides exclusively within breakpoint clusters, suggesting that RAG recombination and TdT activity may promote most IKZF1 deletions, although rare types of alterations may be associated with another molecular mechanism of leukemogenesis.

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PURINE-DERIVED PHENYLHYDROXAMATE COMPOUNDS INDUCES CELL DEATH IN HEMATOLOGICAL NEOPLASM MODELS

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Objective: The histone acetylation process is the best-studied type of post-translational modification, playing a key role in chromatin remodeling regulated by the activity of two enzymes: histone acetyltransferases and histone deacetylases (HDAC). High levels of HDAC have been observed in patients with hematological and solid cancers and are associated with poor prognosis. The present study aimed to evaluate the cellular effects of three novel potential HDAC inhibitors in hematological neoplasm models. **Material and methods:** A panel containing myeloid (OCI-AML3, Kasumi 1, HL60, THP1, MOLM13, MV4-11, U937, NB4, NB4-R2, K562, KU812, SET2, and HEL) and lymphoid (Jurkat, CEM, Namalwa, NALM6, Daudi, Raji, SUP-B15, REH, U266, MM1.S, MM1.R, and Karpas 422) neoplasm cells were used. Peripheral blood mononuclear cells (normal leukocytes) from healthy donors were used for toxicity evaluation (n = 4). Three novel purine-derived phenylhydroxamate with potential HDAC inhibitor activity (named here as 82, 83, and 84) were used. Vorinostat was used as a reference drug. Cell viability was assessed by MTT assay, apoptosis by annexin V/propidium iodide labeling and flow cytometry (FC), clonogenicity by autonomous colony formation, cell cycle by propidium iodide and FC, and protein expression by Western Blot. IC_{50} values were obtained by non-linear regression. Statistical analysis was performed by ANOVA and Bonferroni post-test. A $p < 0.05$ was considered significant. **Results:** All compounds tested reduced cell viability at submicromolar concentrations being compound 82 more potent than the others exhibiting less concentration

necessary to reduce cell viability by 50% (IC_{50} ranged from 0.09 to 2.04 μM). THP1, MOLM13, NB4-R2, and HEL leukemia cells were more sensitive to these compounds and were selected for the next experiments. In normal leukocytes, vorinostat and compound 82 did not reduce cell viability at the same extension as observed for leukemia cells, indicating a selective antineoplastic activity. All compounds induced time- and dose-dependent manner apoptosis in THP1, MOLM13, NB4-R2, and HEL cells (all $p < 0.05$). Vorinostat and compound 82 strongly reduced colony formation, being compound 82 more efficient, especially in MOLM13 and NB4-R2 cells (all $p < 0.05$). Low concentration of vorinostat and compound 82 lead to reduced cell cycle progression (all $p < 0.05$). THP1, MOLM13, NB4-R2, and HEL cells were exposed to vorinostat or compound 82 and the expression of proteins involved in apoptosis and HDAC activity was modulated. **Discussion and conclusion:** Our results indicate that the three purine-derived phenylhydroxamate tested presented antineoplastic effects being a future candidate to compose the list of HDAC inhibitors in the antineoplastic arsenal. **Funding:** CNPq, CAPES, and FAPESP.

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TERRA R-LOOPS ARE INCREASED IN ACUTE MYELOID LEUKEMIA

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Introduction: Telomeric repeat-containing RNA (TERRA) are long non-coding RNAs transcribed from subtelomeric regions towards telomere ends. TERRA binds to subtelomeric and telomeric regions, forming R-loops, structures composed of DNA-RNA hybrids and displaced single-stranded DNA. TERRA R-loops protect telomeres from DNA damage and promote telomere elongation in short telomeres by enabling telomerase or by activating alternative telomere lengthening. However, high or low TERRA levels are associated with DNA damage and telomere dysfunction. RNaseH1 and RNaseH2 cleave R-loops at telomeres, and regulate R-loops levels. The role of TERRA R-loops in telomere maintenance in human cells is not well understood, since conflicting results derive from experiments using diverse organisms and commercial cell lines, with few studies applying primary human cells. Here we describe for the first time the TERRA physiology in hematopoietic cells of patients with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), telomeropathies (TEL), and healthy subjects. **Methods:** We initially screened 17 patients with AML, 5 with ALL, 9 with TEL, and 20 healthy subjects. Additionally, 88 AML patients were studied. HeLa, H1, THP1, NB4, and VA13 cell lines were used for functional experiments, and lentivirus vectors containing GFP or RNaseH1-GFP motifs were employed. ATRX, TRF2, TERT, RNaseH1, RNaseH2, TERRA 10q, 15q, 20q, XqYq, XpYp, and 17p expression levels were measured by RT-qPCR. Telomere length was determined by qPCR. R-loops were assessed by immunofluorescence using the S9.6 antibody. Cell line viability was defined by MTT assay. NGS panel was designed to evaluate variants in splicing factors and epigenetic regulators. **Results:** In the screening population, TERRA expression (10q, 15q, 20q, and XqYq) was significantly increased in AML samples ($p < 0.05$) in comparison to healthy controls and VA-13 cells. Telomeres were very short in AML samples and TEL patients compared to controls. R-loop formation was significantly increased in AML samples compared to controls ($p < 0.01$). To examine the TERRA physiology in AML, we further eveluated 88 AML samples. Hierarchical cluster algorithm based on TERRA expression defined two AML groups, one with high and the other with low TERRA expression. Both groups dysplayed short telomeres, but the high TERRA AML group exhibited low *TERT* and *RNaseH2* expression levels ($p < 0.05$). There was no difference between groups on somatic variants or *RNaseH1*, *TRF2*, *ATRX*, and *TRF2* expression levels. AML cell lines were transfected RNaseH1-GFP-containing or empty vector lentiviruses and treated with cytarabine. AML cells with exogenous RNAseH1 expression showed more sensitivity to cytarabine in MTT assays ($p < 0.05$) than cells transfected with the empty vector. **Discussion:** These results show for the first time that TERRA is dysregulated in AML. High TERRA levels are unique to a subset of AML cases with low TERT expression. High TERRA correlated with the accumulation of R-loops, which may be therapeutic targets. TERRA upregulation in AML due to short telomeres is unlikely, given the low TERRA levels in TEL patients. TERRA upregulation did not appear to be directly related to neoplastic differentiation, given low TERRA in ALL. **Conclusion:** TERRA expression and R-loops are dysregulated in AML. TERRA R-loops inhibition increases chemotherapy-induced apoptosis in AML cell lines and may serve as a therapeutic target.

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A NOVEL HDAC INHIBITOR POTENTIATES VENETOCLAX-INDUCED APOPTOSIS IN ACUTE MYELOID LEUKEMIA CELLS

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Objective: Venetoclax (ABT-199) is a selective inhibitor of BCL2 that was introduced in clinical practice for patients with acute myeloid leukemia (AML) and the results of clinical trials are encouraging, especially in groups with very limited