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

effective therapeutic options. In most studies, venetoclax was used in therapeutic regimens containing epigenetic modulators. Histone deacetylases (HDAC) are important epigenetic modulators, as they alter the accessibility of chromatin to transcription factors, reducing the expression of genes with tumor suppressor functions. Higher levels of HDAC have been observed in leukemia patients and associated with poor outcomes. The present study aimed to evaluate the cellular effects of a novel HDAC inhibitor in combination with venetoclax in AML models. **Material and methods:** NB4-R2 and HEL cell lines, which are resistant to venetoclax, were used. The purine-derived phenylhydroxamate with potential HDAC inhibitor activity (compound 82) (0, 0.37, 0.5, 0.75, 1, 1.5 μM) was used in combination with venetoclax (0, 0.4, 0.8, 1.6, 3.2, 6.4 μM). Vorinostat (0, 0.37, 0.5, 0.75, 1, 1.5 μM) was used as a reference drug for all assays. Cell viability was assessed by MTT assay and apoptosis by annexin V/propidium iodide labeling and flow cytometry. Statistical analysis was performed by ANOVA and Bonferroni post-test. A $p < 0.05$ was considered significant. **Results:** In HEL and NB4-R2 cells, a synergic effect was observed in the combination of compound 82 plus venetoclax (all $p < 0.05$). Similar results, at less extension, were observed for the vorinostat plus venetoclax combination (all $p < 0.05$), indicating that compound 82 is more potent in this context. Apoptosis assays confirm these findings, the levels of apoptosis in NB4 cells treated with vehicle, 1.6 μM venetoclax, 1 μM compound 82, 1 μM vorinostat, 1.6 μM venetoclax plus 1 μM compound 82, 1.6 μM venetoclax plus 1 μM vorinostat were 9 ± 1%, 17 ± 1%, 29 ± 4%, 22 ± 2%, 78 ± 1% and 57±4%, respectively (all $p < 0.05$). Similarly, the levels of apoptotic HEL cells treated with vehicle, 6.4 μM venetoclax, 1 μM compound 82, 1 μM vorinostat, 6.4 μM venetoclax plus 1 μM compound 82, 6.4 μM venetoclax plus 1 μM vorinostat were 12 ± 2%, 47 ± 2%, 73 ± 4%, 39 ± 3%, 88 ± 1% and 76 ± 5%, respectively (all $p < 0.05$). **Discussion and Conclusion:** Pharmacological inhibition of HDAC sensitizes venetoclax-resistant cells. A novel HDAC inhibitor identified by our research group showed greater potency and efficacy than vorinostat in increasing venetoclax-induced apoptosis in cellular models of AML. Future studies are needed to elucidate the molecular mechanisms involved. **Funding:** CNPq, CAPES, and FAPESP.

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IMBALANCED ACTIVATING AND INHIBITORY RECEPTORS ON CD56DIM NATURAL KILLER CELLS PREDICTS POOR OUTCOMES IN ACUTE MYELOID LEUKEMIA

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Background: Even with advances in understanding the physiopathology of Acute Myeloid Leukemia (AML), most patients still relapse leading to poor long-term prognosis and cure rates. Natural Killer (NK) cells are regulated by opposing signals from receptors that activate and inhibit effector function and are known to mediate anti-leukemic immunosurveillance in AML, but the mechanisms by which they control hematopoietic neoplasms remain unclear. **Aims:** We hypothesize that functionally impaired NK cells can predict poor outcomes in AML. **Methods:** We analyzed the expression of DNAM-1 and NKG2D (activating) and NKG2A and KIR2DL1 (inhibitory) receptors on $CD56^{+}$, $CD56^{bright}$ $CD16^{-}$ and $CD56^{dim}$ $CD16^{+}$NK subsets, by flow cytometry, in 100 *de novo* AML bone marrow (BM), followed up from 02/2016 to 06/2022, 17 healthy BM (HBM) and 6 BM from remission after induction chemotherapy (AML-CR). Correlations with ELN2017 risk, complete remission (CR), relapse (R), measurable residual disease (MRD), overall survival (OS), and relapse-free survival (RFS) were verified. Co-culture of AML-exposed NK cells and K562 cell line was used to assay for killing and degranulation. Proteomics and genomics were accessed by CITE-seq single-cell sequencing. **Results:** All NK cell subsets were decreased in AML ($p < .01$). AML CD56+ NK cells showed decreased NKG2D ($p < .01$) and increased NKG2A ($p = .04$) and KIR2DL1 ($p < .01$), recovering to near healthy values in AML-CR. NKG2D and DNAM-1 activating receptors were decreased ($p < .01$), while inhibitory NKG2A ($p = .02$) and KIR2DL1 ($p < .01$) were increased in $CD56^{dim}$ NK (Most cytotoxic subset). Both activating receptors within $CD56^{dim}$ NK were decreased in ELN2017 adverse risk (NKG2D, $p = .05$; DNAM-1, $p < .01$), in relapse ($p < .01$), and in MRD+ patients after remission induction. Inhibitory receptors were increased in adverse risk ($p < .01$), relapse (NKG2A, $p < .01$; KIRDL1, $p < .05$), and MRD+ (NKG2A, $p = .03$) patients. Degranulation ($p < .01$) and target cell death (5:1, $p = .01$; 10:1, $p = .002$) were decreased in AML. Patients with lower frequency of $CD56^{dim}$ NK expressing low NKG2D and DNAM-1 showed less degranulation and target cell death ($p = .03$), whereas in those with higher inhibitory receptors lower degranulation (NKG2A, $p = .06$) and cytotoxicity (KIR2DL1, $p = .005$) was found. Both OS and RFS were decreased in patients with lower $CD56^{dim}$ NK (OS, $p = .04$; RFS, $p = .03$) and patients with lower expression of NKG2D (OS, $p = .03$; RFS, $p = .008$) and DNAM-1 (OS, $p = .02$; RFS, $p = .11$). Our single cell preliminary analysis showed that NK cells from AML display a distinct pro-inflammatory gene signature compared to HBM, which is strongly correlated to its impaired