

necessary to reduce cell viability by 50% (IC<sub>50</sub> ranged from 0.09 to 2.04  $\mu$ M). 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.

<https://doi.org/10.1016/j.htct.2022.09.265>

#### TERRA R-LOOPS ARE INCREASED IN ACUTE MYELOID LEUKEMIA

LFB Catto<sup>a</sup>, LC Zanelatto<sup>a</sup>, FS Donaires<sup>a</sup>, VS Carvalho<sup>a</sup>, BA Santana<sup>a</sup>, AL Pinto<sup>a</sup>, DMC Fantacini<sup>a</sup>, F Traina<sup>a</sup>, EM Rego<sup>b</sup>, RT Calado<sup>a</sup>

<sup>a</sup>Faculdade de Medicina de Ribeirão Preto (FMRP), Universidade de São Paulo (USP), Ribeirão Preto, SP, Brazil

<sup>b</sup>Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, SP, Brazil

**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 evaluated 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 displayed 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.

<https://doi.org/10.1016/j.htct.2022.09.266>

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

JAEG Carlos<sup>a</sup>, LC Almeida<sup>a</sup>, K Lima<sup>a</sup>, MT Tavares<sup>b</sup>, KB Waitman<sup>b</sup>, LV Costa-Lotufo<sup>a</sup>, R Parise-Filho<sup>b</sup>, JA Machado-Neto<sup>a</sup>

<sup>a</sup>Departamento de Farmacologia, Instituto de Ciências Biomédicas, Universidade de São Paulo (USP), São Paulo, SP, Brazil

<sup>b</sup>Departamento de Farmácia, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo (USP), São Paulo, SP, Brazil

**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