EDITORIAL

Charting a path through resistance: histone deacetylase inhibitors for *TP53*-mutated B-cell acute lymphoblastic leukemia

Eitan Kugler

Department of Leukemia, UT MD Anderson Cancer Center, Houston TX, USA and Rabin Medical Center and Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Correspondence: E. Kugler
ekugler@mdanderson.org

Received: January 26, 2024.
Accepted: February 7, 2024.
Early view: February 15, 2024.

https://doi.org/10.3324/haematol.2023.284796

In this issue of *Haematologica*, Cox *et al.* describe the therapeutic vulnerabilities of relapsed acute lymphoblastic leukemia (ALL) with *TP53* aberration, a disease subset characterized by very poor prognosis. Utilizing a pediatric cancer drug library and high-throughput screening, the authors aimed to map the drug sensitivity profile of *TP53*-mutated B-ALL. They discovered that when combined with cytarabine, the class I histone deacetylase (HDAC) inhibitor romidepsin effectively restored chemotherapy sensitivity in *TP53*-deficient B-ALL.[1]

TP53 is the gene most commonly mutated in cancer.[2] It frequently displays missense mutations that lead to the production of a p53 protein with impaired function, which fails to bind DNA and activate target genes. Mutant p53 can also have a dominant-negative effect on wild-type p53 and harbor gain-of-function activities by interacting with other transcription factors to drive oncogenic gene transcription.[3,4] In the context of ALL, the incidence of *TP53* aberrations at diagnosis differs significantly between pediatric and adult patients. While these aberrations are relatively rare in children, with an occurrence rate of about 2-3%, they are more frequent in adults, affecting 6-19% of all cases.[5,6] These aberrations are especially prevalent in certain subtypes of ALL, particularly low hypodiploid and near triploid ALL, and are more frequently observed during relapse.[6] In essence, *TP53* mutations contribute significantly to the development of treatment-resistant clones in ALL, leading to early relapses and poorer survival outcomes.

TP53 has long been considered undruggable. Since most small molecule drugs inhibit excessive protein activity, reactivating mutant proteins to restore their tumor suppressive properties can be challenging. Several strategies have been developed to target mutant *TP53* in hematologic malignancies, with the aim of restoring a certain level of wild-type activity (PRIMA-1, APR-246, APR-538, arsenic trioxide) or exploiting vulnerabilities caused by mutant *TP53* (immune checkpoint inhibition).[7] While promising in early phase studies, these strategies have not yet demonstrated efficacy in phase III trials, highlighting an urgent unmet need for novel therapeutic approaches.

Cox *et al.* sought to identify therapeutic vulnerabilities in *TP53*-deficient B-ALL. To this end, they employed CRISPR/Cas9 to create isogenic pairs of *TP53* wild-type (WT) and knockout (KO) from the relapsed B-cell precursor ALL cell lines, Nalm6 and RCH-ACV. This was followed by a high-throughput screening of 198 compounds used in standard care. The authors did not identify any compound that selectively targets $TP53^{KO}$ cells; however, romidepsin, a class I HDAC inhibitor, emerged as a potent suppressor of proliferation of both $TP53^{KO}$ and $TP53^{WT}$ cells.

In a series of *in vitro* experiments on $TP53^{KO}$ and $TP53^{WT}$ Nalm6 and RCH-ACV cells, the authors illustrated that romidepsin, when used in conjunction with cytarabine, elicited a synergistic effect. The synergy was consistently observed with other standard B-ALL chemotherapeutic agents, and it remained significant regardless of the *TP53* status of the cells. Further validation of these findings in an expanded panel of B-ALL cell lines that included both *TP53* wild-type and mutant forms strengthened the reliability of the results.

To elucidate the mechanism underlying the synergistic effect observed, the authors performed RNA sequencing on $TP53^{KO}$ and $TP53^{WT}$ Nalm6 and RCH-ACV cells. Notably, while sets of genes associated with apoptosis were enriched in samples treated with romidepsin, there was no observed upregulation of *TP53* target genes, indicating that romidepsin may induce apoptosis through *TP53*-independent pathways.

Additionally, and perhaps counterintuitively, it was found that gene repression rather than de-repression was the

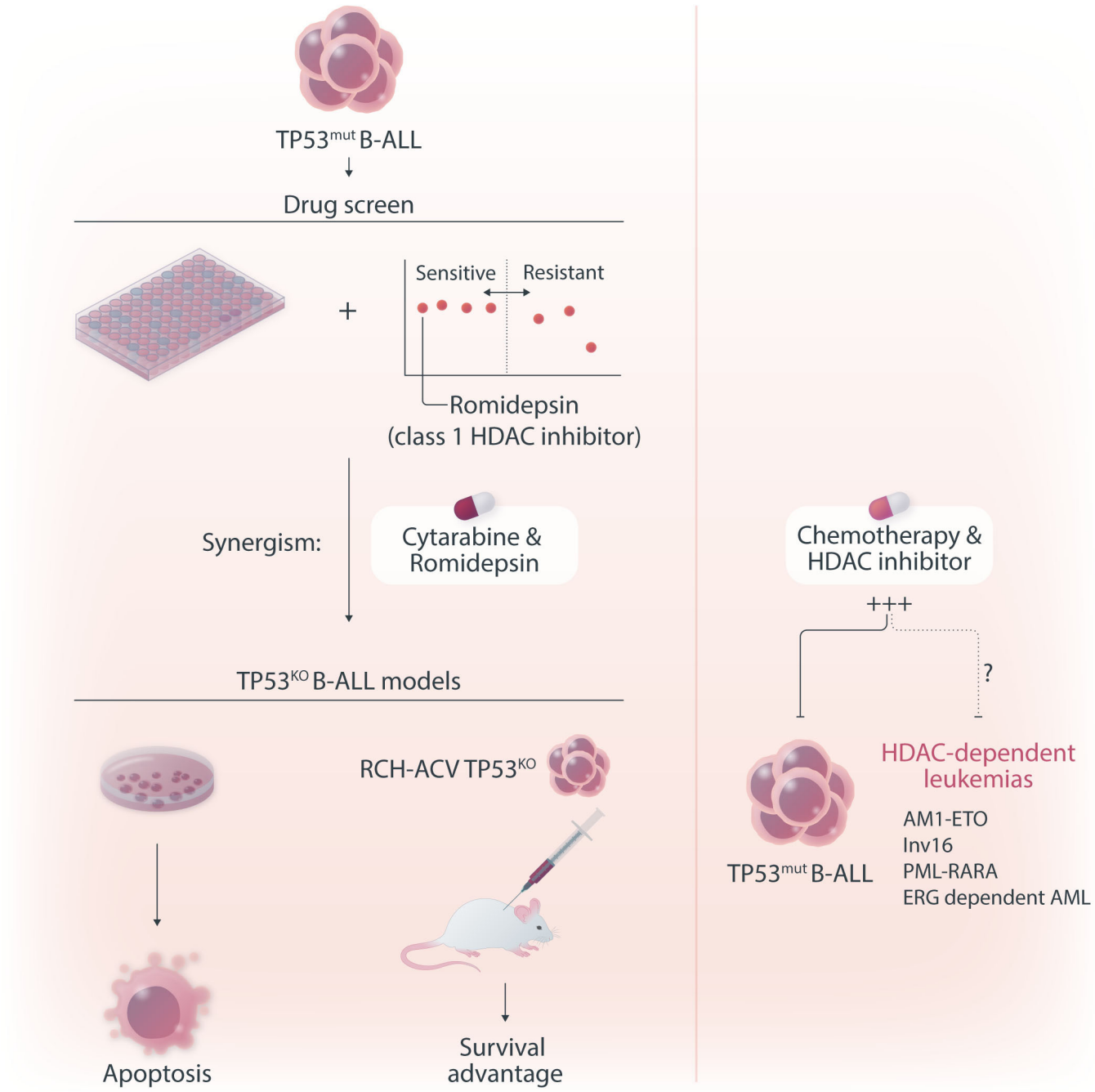

Figure 1. The role of histone deacetylase inhibition in the treatment of acute leukemia. In this issue of *Haematologica*, Cox *et al*. studied the therapeutic vulnerabilities of relapsed acute lymphoblastic leukemia (ALL) with *TP53* aberrations.[1] The high-throughput drug screening they conducted demonstrated that combining the class I histone deacetylase (HDAC) inhibitor romidepsin with cytarabine is synergistic and can overcome the inherent chemoresistance of this high-risk subtype of ALL. Gene expression analysis suggested that romidepsin induces a distinct anti-leukemic effect independent of the *TP53* pathway. The diagram on the right-hand side illustrates additional types of acute leukemia that are modulated by HDAC, highlighting the necessity for more research to determine which classes of HDAC inhibitors could be effective in treating various forms of acute leukemia.

predominant phenomenon in samples treated with romidepsin compared to untreated samples. These genes were associated with ribosome biogenesis and proteasome assembly pathways; however, the effect of romidepsin was not recapitulated upon treatment of B-ALL cell lines with proteasome inhibitors and cytarabine, suggesting an additional, distinct anti-leukemic effect induced by HDAC inhibition.

Finally, the study convincingly demonstrated that the findings extend to *in vivo* models. Immunodeficient mice transplanted with luciferase-expressing RCH-ACV *p53*^{KO} cells had a reduced leukemia burden and survived longer when treated with the romidepsin-cytarabine combination than with cytarabine alone.

HDAC play a pivotal role in multilineage development and hematopoietic stem cell fate.[8] Simultaneous knockdown of HDAC1 and HDAC2 leads to early myeloid differentiation and loss of hematopoietic stem cells.[9] Furthermore, the loss of HDAC1 and HDAC2 is linked to a marked impediment in pre-B-cell development, manifested by G1 arrest and apoptosis, underscoring the importance of HDAC in the early stages of B-cell development and terminal maturation.[8]

The development of HDAC inhibitors for therapy of hematopoietic malignancies originated from the observation that several compounds that induced differentiation of leukemic cell lines were inhibitors of HDAC.

HDAC have been implicated in the pathogenesis of certain subtypes of acute myeloid leukemia with distinct chromosomal translocations, such as *AML1-ETO*, Inv16, *PML-RARA*, and those involving high ERG expression and

dependency.[10,11] The chimeric proteins recruit HDAC and co-repressor complexes to repress genes crucial for myeloid differentiation.[11] Similarly, in the context of ALL, HDAC inhibitors suppress the MLL-AF4 fusion protein and other proto-oncogenes, triggering apoptosis in leukemic cells with *KMT2A* rearrangements.[12]

While the antileukemic mechanisms of HDAC inhibitors are not fully elucidated, they extend beyond histone deacetylation and include a wide array of biological effects on cancer cells. These effects encompass cell cycle arrest, metabolic reprogramming, autophagic cell death induction, generation of reactive oxygen species, and impairment of the DNA damage response.[13]

One strength of the study by Cox *et al*. is that it demonstrated that HDAC inhibitors elicit apoptosis and an anti-leukemic response which overcomes the intrinsic chemoresistance characteristic of *TP53*-mutated B-ALL. Previous observations support these findings, showing that HDAC inhibitors induce apoptosis in cancer cells via both *TP53*-dependent and independent pathways.[14] Whether this effect is specific to an HDAC class, or a cell-intrinsic characteristic remains unknown.

In summary, the data published by Cox *et al*. provide a novel perspective on an established class of drugs. It is important to recognize that despite the convincing anti-cancer potential, the results of several advanced phase clinical trials of HDAC inhibitors in leukemia were disappointing. Notably, these trials predominantly utilized pan-HDAC inhibitors, indicating that future research should focus on determining which specific HDAC inhibitor classes may be effective in treating particular forms of acute leukemia (Figure 1). The work by Cox *et al*. underscores the value of this research direction.

Disclosures

No conflicts of interest to disclose.

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