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POSTER ABSTRACTS

802.CHEMICAL BIOLOGY AND EXPERIMENTAL THERAPEUTICS

Novel Therapeutics Targeting Acute Myeloid Leukemia (AML) Stem Cells Identified through High-Throughput Screening

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Acute myeloid leukemia (AML) is an aggressive form of blood cancer. Despite the use of cytotoxic standard-of-care drugs, patients often succumb to the disease partially due to the inability of medically unfit patients to withstand the cytotoxic treatments, regrowth from minimal residual disease and the chemo-resistant nature of leukemic stem cells (LSCs). Hence, novel therapies should focus on targeting the unique biology of LSCs to eliminate and avoid reoccurrence.

To overcome challenges inherent of screening rare LSCs *in vitro* such as culturing and expanding, we optimized the conditions for a 4-week *in vitro* large-scale expansion (>600 million bulk) and enrichment of the CD34+ LSC-containing fraction (>90% purity) for a primary human AML sample (OCI-AML-8227), functionally validated to be enriched for LSCs in long-term xeno-transplant assays (Eppert *et al.*, 2011). Next, we performed a high-throughput screen of 11,140 chemical molecules in 3 stages. First, the viability of AML CD34+ cells and healthy cord blood (CB) CD34+ cells was read out at 1-2 doses using a CellTiter-Glo® Luminescent assay. 61 compounds had >70% inhibition of 8227 CD34+ cells and <30% inhibition on CB CD34+ cells. Next, we determined the dose response and refined the hits to 33 potent compounds with LC50 < 1 μM, including novel compounds and classes previously shown to target bulk and leukemic stem cells in AML.

We are now presenting the follow up hits identified from the third stage of validation where we determined the LC_{50} specifically in the CD34- (blast) and CD34+CD38- (LSC-enriched) OCI-AML-8227 populations using flow cytometry. We identified 25 novel anti-LSC compounds with high efficacy against CD34+CD38- AML cells (LC_{50} < 500 nM). Venetoclax was among the top hits, a compound that revolutionized treatment for poor prognosis AML patients due to its anti-LSC activities, providing internal validation of the screen.

We then tested these candidates in a second LSC-enriched model with poor prognosis, OCI-AML-20, to ensure the response of compounds on LSCs is not exclusive to OCI-AML-8227 and to investigate the influence of the microenvironment on drug efficiency. A total of 5 hits have significant responses (>50% reduction in LSC enriched populations) and nontoxic to stroma. Furthermore, we refined our top candidates to 7 compounds based on their efficiencies in the two LSC-enriched models (OCI-AML-20 and 8227) and low toxicity on stroma. To gain insights on their mechanisms of action in LSCs, the two models were treated with the 7 candidates and cytarabine as a control for 3 days and apoptosis was measured by flow cytometry. Out of 7 candidates, 5 induced apoptosis in the LSC-enriched fractions, suggesting elimination of LSCs through apoptosis. The remaining compounds may eliminate LSCs through different mechanisms.

From these results, we focused on the three leading compounds and validated them for toxicity on CD34+ hematopoetic stem and progenitor cord blood cells (HSPCs) vs CD34+CD38- (LSC-enriched) OCI-AML-8227 by flow cytometry. Although slight toxicity was observed in HSPCs at higher doses, the LC_{50} for the stem cell vs LSC-enriched populations differ by ~6-40-fold, indicating that a significantly lower concentration can be used to eradicate LSCs while sparing HSPCs (LC_{50} for CD34+ population CB vs 8227; Compound A: 1558 nM vs 83 nM, Compound B: >2000 nM vs 305 nM and Compound C: 612 nM vs 16 nM, respectively). To functionally validate if HSPCs proliferation and differentiation was impaired and if leukemic progenitors were eradicated, a colony forming unit assay was performed for 12 days after 6 days of treatment. Candidates were found to

eliminate leukemic progenitors by 50% or more, reducing leukemia initiating potential and having minimal or no impact on CB progenitor functionality.
Overall, compound A is a potential candidate to move forward due to its ability to target and eliminate LSCs through apoptosis in two LSC-enriched models, its low toxicity on stroma and normal cord blood cells. This candidate is classified as an indole, a class shown by Pabst *et al.*, 2014 to reduce CD34+CD15- AML cells in a drug screen for aryl hydrocarbon modulators, suggesting a potential mechanism for LSC elimination through apoptosis. We now aim to examine LSC eradication in a panel of genetically defined primary AMLs to be able to determine the broad applicability of this compound and translate the preliminary results for clinical use.

Disclosures No relevant conflicts of interest to declare.

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