Venetoclax and dinaciclib elicit synergistic preclinical efficacy against high-risk B-cell leukemia

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BACKGROUND

- Hypodiploid acute lymphoblastic leukemia (ALL) is an aggressive blood cancer with a poor prognosis
- Neither intensive chemotherapy or stem cell transplant improve outcome in this patient population
- Protein profiling identified several dysregulated pathways including BCL-2 family members
- Preclinical studies using Venetoclax indicated a reduction of leukemic burden in peripheral blood, but persistence of blasts in marrow, spleen and liver limiting efficacy of Venetoclax as monotherapy

OBJECTIVE

To identify drugs synergistic with Venetoclax that may provide curative treatments for hypodiploid B-ALL patients and prevent resistance to Venetoclax.

EXPERIMENTAL APPROACH

- Perform an unbiased high-throughput drug screen (HTS), utilizing a library of 1835 small molecules from a Bioactive Compound Library
- Identify mechanisms of action of any synergistic candidate
- Utilize magnetic levitation sorting for selecting pure viable cells in all assays

RESULTS

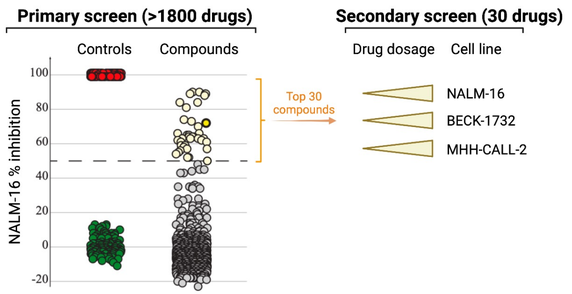

Figure 1. High-throughput screening. Primary drug screening was performed using NALM-16 cells subjected to a library of 1830 bioactive compounds, tested at 0.125 µM. Growth inhibition was measured after 24h using a luminescent cell viability assay and top hits were selected. Negative controls (green circles) represent untreated cells. Positive controls (red circles) represent wells without cells to approximate total inhibition. Hits (yellow circles) were selected from test compounds (grey circles) that exhibited greater than 50% inhibition. The 30 compounds with greatest proliferation inhibition were selected and used for a secondary screening using a range of concentrations in the three hypodiploid cell lines, NALM-16, BECK-1732 and MHH-CALL-2.

RESULTS

A)

Compound name (125 nM)	% inhibition drugs alone	% inhibition + Venetoclax	Bliss score of combination	Targets
Dinaciclib	60	97	0.32	CDK1, 2, 5 and 9
Paclitaxel	35	68	0.19	Microtubules
Quisinostat	48	71	0.1	HDACs
Panobinostat	48	65	0.04	HDACs
Bortezomib	93	98	0.03	Proteasome

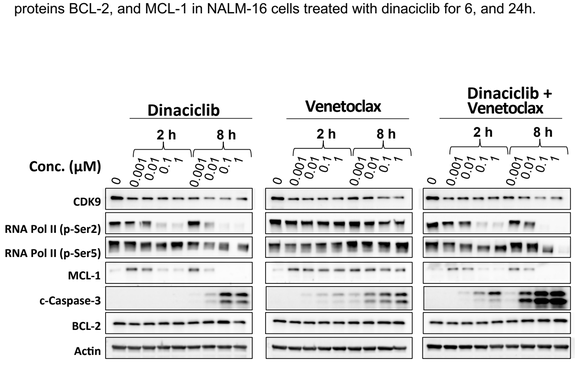

Figure 2. Dinaciclib demonstrates high synergy with venetoclax. (A) Summary of growth inhibition of indicated agents with and without venetoclax (nM) and Bliss score of the combinations in NALM-16 cells. (B) illustration of synergy scores. (C) Synergy scores using an isobologram model of NALM-16 cells treated for 24 h with a combination of dinaciclib and venetoclax at serial dilution doses ranging from 0.008-0.5 µM.

Figure 3: Dinaciclib inhibited CDK-9 mediated transcription of MCL-1. Dinaciclib can inhibit cell cycle regulators as well as CDK9 transcription factor. Western blot data shows levels of CDK1, 2, 9; RNA polymerase II phosphorylation on Ser 2, and prosurvival proteins BCL-2, and MCL-1 in NALM-16 cells treated with dinaciclib for 6, and 24h.

Figure 4. Apoptosis induction by dinaciclib, venetoclax and the combination in isogenic lines. NALM-16 cells were treated with dinaciclib, venetoclax or the combination for 2 hours and 8 hours. Levels of CDK9, RNA polymerase II phosphorylation on Ser 2 or Ser 5, prosurvival proteins MCL-1 and BCL-2, and the apoptosis marker cleaved caspase, are shown via western blotting.

RESULTS

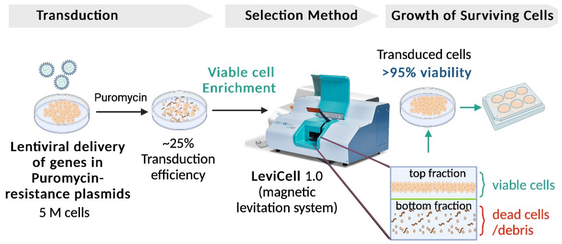

Figure 5: Magnetic levitation sorting of viable cells. Illustration of the LeviCell system (LevitasBio) allowing gentle and fast sorting of viable cells (up to 5M in < 20 min) through magnetic levitation.

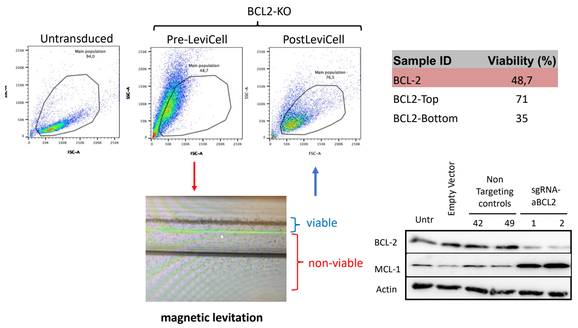

Figure 6: Magnetic levitation sorting of viable cells identified a rare population of BCL-2 ko cells overexpressing MCL-1. Knocking out BCL-2 induced massive cell death, validating sensitivity to Venetoclax. LeviCell sorting identified a rare population of Bcl-2 k.o cells overexpressing MCL-1 validating the Dinaciclib data.

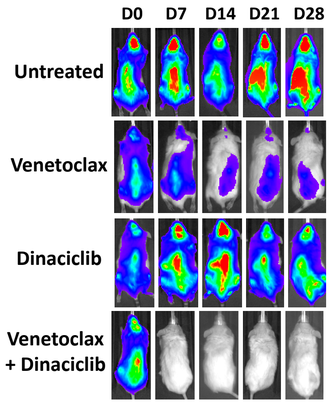

Figure 7. Preclinical study outcomes. (A) All 3 hypodiploid leukemia primary samples (NH1, 2 and 3) were tagged with a luciferase reporter, prior to engraftment, and mice were imaged weekly using bioluminescent imaging before and during treatment. Data displays a single representative subject from each treatment arm, however, reflects the overall trend seen across all treatment groups.

RESULTS

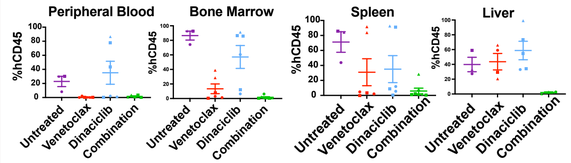

Figure 8. Leukemic burden analysis in various tissues. Percentage of hCD45 in peripheral blood (from cardiac puncture), bone marrow, spleen and liver measured at the end of the trial in all 3 subtypes of hypodiploid ALL PDX mice (NH1 – circle, NH2 – square, NH3 -triangle) and compared across the 4 treatment arms.

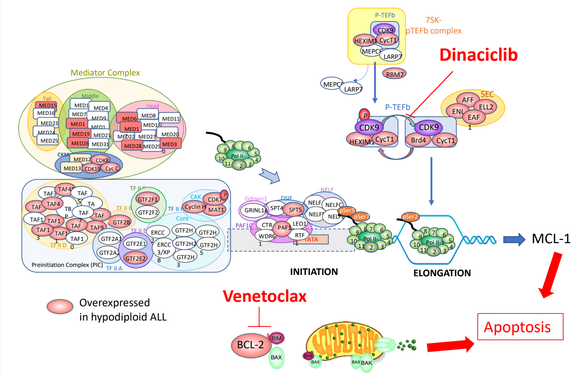

Figure 9. Proposed model for synergy between Venetoclax and Dinaciclib. Venetoclax inhibits Bcl-2 inducing cell death. However, Mcl-1 can rescue cell death in absence of Bcl-2. Concomitant inhibition of CDK9, a transcription factor modulating CDK9 and in turn Mcl-1 prevents such rescue inducing effective cell death.

CONCLUSIONS

- **Hypodiploid cells are highly dependent on BCL-2 for survival**
- **Venetoclax reduces leukemic burden but presents limitations as monotherapy due to cell death rescue mediated by upregulation of MCL-1**
- **Dinaciclib acts synergistically with Venetoclax in inducing cell death in hypodiploid cells**
- **Dinaciclib induces cell death through CDK9 inhibition (rather than CDK1,2 or 5) and subsequent downregulation of MCL-1 levels**
- **Sorting of BCL-2 k.o cells via magnetic levitation demonstrate survival rescue due to MCL-1 upregulation**
- **Dinaciclib + Venetoclax represent a potential curative therapy for patients with hypodiploid leukemia and/or leukemias dependent on BCL-2 for survival**

Reference:
Pariury et al., Haematologica Early view Jan 26, 2023
https://doi.org/10.3324/haematol.2022.281443

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