A First-Generation Pediatric Cancer Dependency Map

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Supplementary Note: Methods

Ewing sarcoma and neuroblastoma cells used for validation with MCL1 inhibitor

All cell lines were cultured at 37°C in a humidified atmosphere containing 5% CO2. KELLY, SKNAS, and SKNBE2 lines were grown in Dulbecco's Modified Eagle's Media (DMEM) (Life Technologies) supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich). EWS502 was grown in DMEM supplemented with 15% FBS; TC106 was grown in RPMI1640 (Sigma-Aldrich) with 10% FBSx; SKNMC was grown in Eagle's Minimum Essential Medium (EMEM) (ATCC) supplemented with 10% FBS. All cells were grown in the presence of 10 units/mL of penicillin, 10 µg/mL streptomycin and 30 µg/mL of L-Glutamine, PSQ (Thermo Fisher Scientific). Cell lines were a kind gift from Todd Golub (Broad Institute, Cambridge MA). Cells were regularly tested for Mycoplasma by PCR (Sigma Aldrich). S63845, an MCL1 inhibitor, was obtained from Chemgood, LLC. Z-VAD-FMK, a pan-caspase inhibitor, was obtained from R&D Systems.

MCL1 CRISPR-Cas9 mediated disruption

Lentivirus was produced by transfecting HEK-293T cells with the lentiCRISPRv2 sgRNA vector (Addgene #52961), pCMV-VSV-G (Addgene #8454) and psPAX2 (Addgene #12260) as well as X-tremeGENE™ HP DNA Transfection Reagent (Roche) according to the manufacturer's instructions. CRISPR sgRNAs sequences for *MCL1* were obtained for lentiviral transduction, and Ewing sarcoma and neuroblastoma cells were incubated with 2 mL of virus and 8 mg/mL of polybrene (Sigma-Aldrich). Cells were selected in puromycin (Sigma-Aldrich) 48 hours post-transduction. Two to four days post-selection, cells were harvested for protein and viability assays. CRISPR guide sequences against *MCL1* were from the Achilles Project Avana library at the Broad Institute (http://www.depmap.org). LacZ control guide was designed not to cut anywhere in the human genome. sgLacZ sequence: AACGGCGGATTGACCGTAAT. Data were plotted using GraphPad Prism version 8.

MCL1 inhibitor and knockout viability studies

Cells were seeded onto 384-well tissue culture treated plates at a density of 25,000 cells/ml. The D300e digital dispenser (HP) was used to robotically plate drugs onto 384-well plates containing cells in two-fold dilutions, with four replicates per dose. For experiments involving the MCL-1 inhibitor S63845, a maximum concentration of 10 µM and a minimum concentration of 20 nM were used, normalized with DMSO controls. After treatment, plated cells were analyzed for cell viability at four days using the CellTiter-Glo® luminescence assay (Promega) per the manufacturer's instructions. For experiments involving Z-VAD-FMK, a maximum dose of 50 µM and a minimum dose of 6.25 µM were used, with cells treated in 2-fold dilutions, with four replicates per dose, normalized with DMSO controls. Cells were incubated for one hour after application of Z-VAD-FMK, and then S63845 was added as above in a matrix to plates, with four replicates per combination of Z-VAD-FMK and S63845. After combination treatment, plated cells were analyzed for cell viability at three days using the CellTiter-Glo® luminescence assay (Promega) per the manufacturer's instructions.

For *MCL1* knockout viability studies, eight replicates per CRISPR guide (sgRNA #1 CCTCACGCCAGACTCCCGGA and sgRNA #2 GTTTGGCCTCAAAAGAAACG) were plated as above on to 384 well plates. Plates were analyzed at days two, four, six, and eight using the Cell-TiterGlo® luminescence assay (Promega) per the manufacturer's instructions.

For all assays, luminescence was read on a Fluostar Omega Reader (BMG Labtech). IC50 values for S63845 were calculated from ATP luminescence measurements using log-transformed, normalized data in GraphPad Prism 8.0 (GraphPad Software, Inc.).

Data were plotted using GraphPad Prism version 8.

Western immunoblotting

Western immunoblotting was performed for appropriate protein biomarkers of drug response (MCL1, PARP, Caspase 3, and Cleaved Caspase 3). Cells were lysed in Cell Signaling Lysis Buffer (Cell Signaling Technology) supplemented with Complete, EDTA free Protease Inhibitor Cocktail (Roche Diagnostics) and Phos-STOP Phosphatase Inhibitor (Roche Diagnostics). Protein concentrations were determined by the Bradford protein assay. Protein samples were separated by SDS-PAGE along with the Precision Plus Protein™ Kaleidoscope™ Prestained Protein Standard (Bio-Rad) and transferred to PVDF membranes. Membranes were incubated with primary antibodies directed against MCL1 (Cell Signaling Technology Cat. No. 94296S), PARP (Cell Signaling Technology Cat. No. 9542S), Caspase 3 (Cell Signaling Cat. No. 9662S), Cleaved Caspase 3 (Cell Signaling Technology Cat. No. 9664S) and Tubulin (Sigma Cat. No. T6199). All antibodies were used at a dilution of 1:1000. Horseradish peroxidase (HRP) conjugated secondary antibodies were used at a dilution of 1:4000. Blots were visualized by enhanced chemiluminescence (ThermoFisher Scientific).