BH3 profiling as pharmacodynamic biomarker for the activity of BH3 mimetics

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Figure S1

- A) The genetic modifications and anti-apoptotic dependency of used cell lines.
- B) Selectivity of used BH3 peptides and BH3 mimetics for BH3 profiling assays in this study.
- C) Baseline mitochondrial priming of O_BCL-2 and O_MCL-1 cell lines, as determined by BH3 profiling (numbers following peptide names are used concentrations (µM). DMSO negative control; ALM (alamethicin), positive control).
- D) Five cell lines in Figure B were permeabilized with digitonin and incubated with BCL201 or S63845 for 45 min (mitochondrial exposure) and then examined for cytochrome *c* release.
- E) Workflow of BH3 profiling of treated human PBMC (peripheral blood mononuclear cells) cells. Zombie Yellow (ZY) was used as the live/dead staining.
- F) Gating strategy of human PBMC cells used for in vitro studies.
- G) Diagram showing how delta priming is calculated.

Data were presented as mean ± SEM of triplicate experiments.

Figure S1 В **Cell Lines Genetic Modifications** Phenotypic Features Α BAD HRK MS-1 FS-1 ABT-199 A-1331852 S63845 O_BCL-2 BCL-2 overexpression Dependency on BCL-2 BCL-2 O_MCL-1 Dependency on MCL-1 MCL-1 overexpression BCL-XL O_BCL-XL Dependency on BCL-XL BCL-XL overexpression MCL-1 O_BFL-1 BFL-1 overexpression Dependency on BFL-1 BFL-1 O_DKO BAX/BAK double knockout Deficiecy in mitochondrail apoptosis С D O_BCL-2 O_MCL-1 -- O_BCL2
-- O_MCL1
-- O_BCLXL
-- O_BFL1
-- O_DKO 100 ● 0_BCL2 ● 0_MCL1 ■ 0_BCLXL ● 0_BFL1 ● 0_DK0 Cytochrome c release % Cytochrome c release % 80 80 60 60 60 40 40 40 20 10 0 20 The order he he is a feet he he he OF THE CONCENTRATION OF THE PROPERTY OF THE PR 10⁻¹ 10⁰ S638451 (μM) 10⁻² 10⁻¹ BCL201 (μM) 10-2 10-3 10⁰ E F G DMSO 3 Live/dead staining Treatment with BH3 mimetics SSC Exposure to BH3 peptides PBMC isolation CD3 ALM Control Treated Delta Priming =Primingtreated - Primingbaselin CD14 High-throughput flow analysis Cytochrome c & surface protien staining Analysis