LETTER TO THE EDITOR

Acute myeloid leukemia-driven IL-3-dependent upregulation of BCL2 in non-malignant hematopoietic stem and progenitor cells increases venetoclax-induced cytopenias

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Supplementary Fig 1 – Flow cytometry gating, BCL2 expression and cell viability.

A) Gating strategy for B cells (CD4+CD8-CD19+), T cells (CD4+CD8-CD19+), monocytes (CD11b+Ly6 G+) and neutrophils (CD11b+Ly6 G+). B) Gating strategy for non-malignant HSC (Lin−Sca1+CD117+CD150+CD48−), multipotent progenitor (MPP) (Lin−Sca1+CD117+CD150−CD48+) and LSK (Lin−Sca1+CD117+). C) BCL2 protein expression is elevated in LSKs co-cultured with both MN1 and MEIS1/HOXA9 AML subtypes. LSKs were isolated from BM of young C57BL/6 mice and 5x10⁶ cells were co-cultured in transwells with either MN1 or MEIS1/HOXA9 cells for 48h. BCL2 protein level was quantified by mean fluorescence intensity (MFI) in LSKs co-cultured with AML cells (n = 6) compared to LSK-only controls (n = 5) using flow cytometry. **p < 0.01 using Mann-Whitney U Test. D) Blocking IL-3 does not make AML cells resistant to venetoclax. 5x10⁴ MN1 and MEIS1/HOXA9 cells were cultured with 10 µM venetoclax, 5 µg/mL anti-IL-3 or both for 24h. Percentage cell viability was quantified using the CellTiter-Glo® luminescent assay in MN1 and MEIS1/HOXA9 cells remaining untreated (n = 6), treated with venetoclax (n = 6), treated with anti-IL-3 (n = 6) and treated with venetoclax and anti-IL-3 (n = 6). E) Total BCL2 and phosphorylated BCL2 protein levels are elevated in LSKs treated with IL-3. 2x10⁵ LSKs were cultured in DMEM supplemented with 10% FBS and 1% Pen-Strep and treated with 100 ng/mL IL-3 for 24h. Total BCL-2 protein level and phosphorylated BCL2 within the BCL2-positive population was quantified by mean fluorescence intensity (MFI) in LSKs treated with IL-3 (n = 3) compared to LSK-only controls (n = 3) using flow cytometry. Data are non-significant using Mann-Whitney U Test. F) BCL2 protein levels were quantified by MFI in the lineage positive cell population of MN1 engrafted mice treated with venetoclax (n = 9) compared to control MN1 engrafted mice (n = 8). Data are non-significant using Mann-Whitney U Test. All data in C-F are represented as median + interquartile range.