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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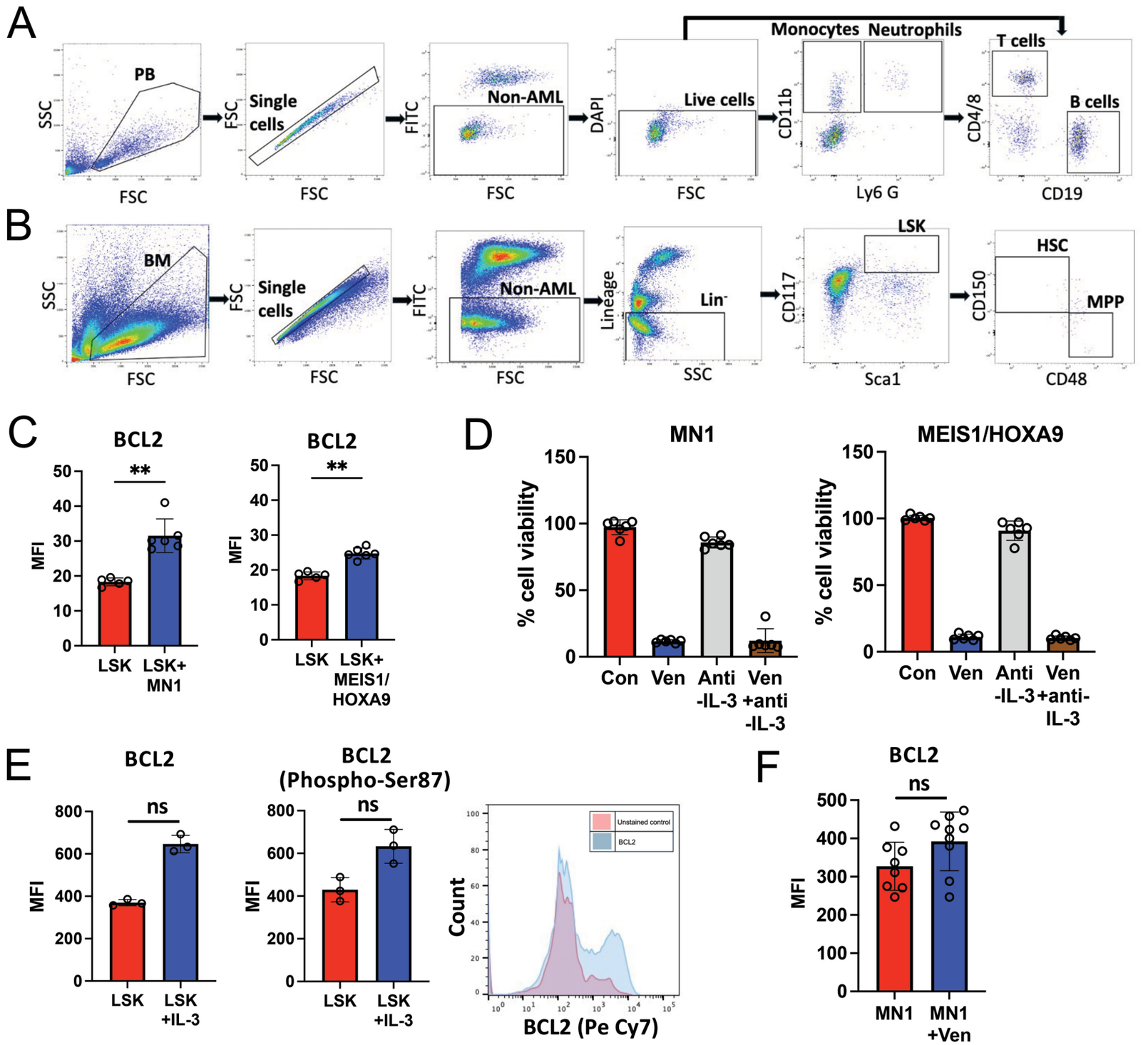
https://doi.org/10.3324/haematol.2023.283944

Received: July 19, 2023.
Accepted: December 28, 2023.
Early view: January 4, 2024.

Supplementary figure 1



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 $5x10^4$ 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. $5x10^4$ 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. $2x10^5$ 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.