Effective combination of FLT3 and BCL2 inhibitors in a preclinical model of lineage-ambiguous leukemia

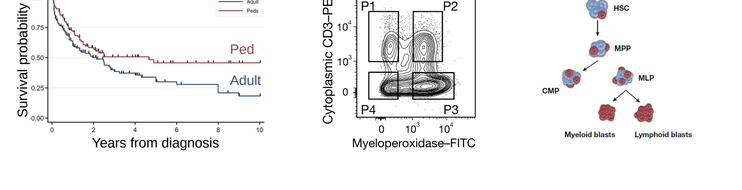
Lindsey E Montefiori[1†], Ilaria Iacobucci[1†], Amit Budhraja[2], Jamila Moore[3], Rebekah Baskin-Doerfler[4], Rebekah DeVries[3], Emily A Backhaus[1], Melissa Johnson[3], Walter Akers[3], Divyabharathi Chepyala[5], Cyrus M Mehr[1], Aman Seth[1], Paul E Mead[1], Joseph T Opferman[2] and Charles G Mullighan[1*]

Abstract

Acute leukemias of ambiguous lineage (ALAL) pose significant diagnostic and therapeutic challenges due to lack of clarity surrounding their cellular origins and driving genomic alterations, as well as poor long-term response to conventional chemotherapy. We recently identified a new subtype of ALAL defined by aberrant activation of the T cell transcription factor gene, *BCL11B*[1]. This subtype includes 30-40% of T/myeloid MPAL and ETP-ALL as well as 8% of AML. Activating *FLT3* mutations were identified in >80% of cases and all cases exhibited high *FLT3* expression, suggesting a therapeutic window. Moreover, *BCL11B*-activated (*BCL11B*-a) cases express high levels of *BCL2* compared to other leukemia lineages and a patient-derived xenograft (PDX) of a *BCL11B*-a case was specifically dependent on BCL2 compared to other BCL2 members and was primed for apoptosis. FLT3 and BCL2 inhibitors are currently included in treatment regimens in AML and ETP-ALL, however whether single agent FLT3 or BCL2 inhibitors are effective in *BCL11B*-a ALAL, or whether the combination exerts a synergist effect, is unknown. Here we tested single agent gilteritinib (a FLT3 inhibitor), single agent venetoclax (a BCL2 inhibitor), and the combination in a preclinical model of *BCL11B*-a ALAL. Single agent venetoclax was not effective while single agent gilteritinib reduced leukemic growth but did not clear disease. In contrast, the combination treatment was highly effective at clearing leukemic burden from the peripheral blood, bone marrow and spleen, even in highly engrafted animals, suggesting that FLT3 signaling is required to compensate for BCL2 inhibition in these cases.

Background

- ALAL accounts for 3-5% of acute leukemia cases and outcomes remain poor **(A)**[2].
- Most ALAL are diagnosed as MPAL, which is characterized by an immunophenotype including both myeloid and lymphoid markers **(B)**. A hematopoietic stem or early progenitor cell (HSPC) is the most likely cell of origin and may explain the multi-lineage differentiation capacity characteristic of this entity[3] **(C)**.



- We identified a new subtype of ALAL defined by aberrant activation of the T lineage transcription factor gene, *BCL11B*, in an HSPC[1]. This subtype includes 30-40% of T/myeloid MPAL and ETP-ALL and ~8% of AML **(D)**.
- *FLT3* activating mutations occur in >80% of *BCL11B*-activated (*BCL11B*-a) cases **(E)** and all cases show high *FLT3* expression and relatively high expression of the anti-apoptotic gene *BCL2* compared to other leukemias **(F)**.
- **AIM: To test the efficacy of FLT3 and BCL2 inhibitors in a pre-clinical model of *BCL11B*-a leukemia.**

Results

Figure 1: All immunophenotypic subpopulations of a *BCL11B*-a PDX are highly primed and dependent on BCL2

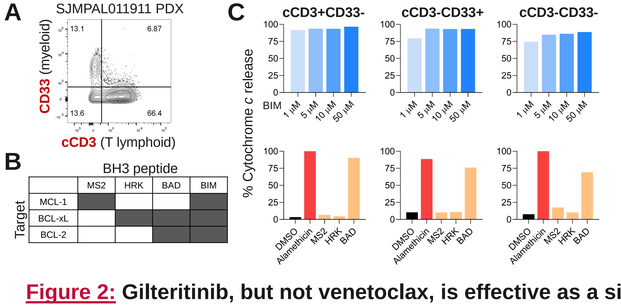


Figure 1. (A) Flow cytometry of *BCL11B*-a PDX SJMPAL011911 used in this study. **(B)** Table summarizing BH3 peptides (columns) and their targets (rows). Shaded cells show which peptides are active against which targets. **(C)** Dynamic BH3 profiling on treatment-naïve PDX cells (top, blue). Bottom (orange), intracellular staining BH3 profiling with peptides listed in **(B)**. Alamethicin is a positive control and DMSO is a negative control. All PDX populations (shown in **(A)**) depend on BCL2 (BAD stimulates Cytochrome *c* release, but not HRK or MS2).

Figure 2: Gilteritinib, but not venetoclax, is effective as a single agent while the combination significantly improves disease clearance in a *BCL11B*-a PDX

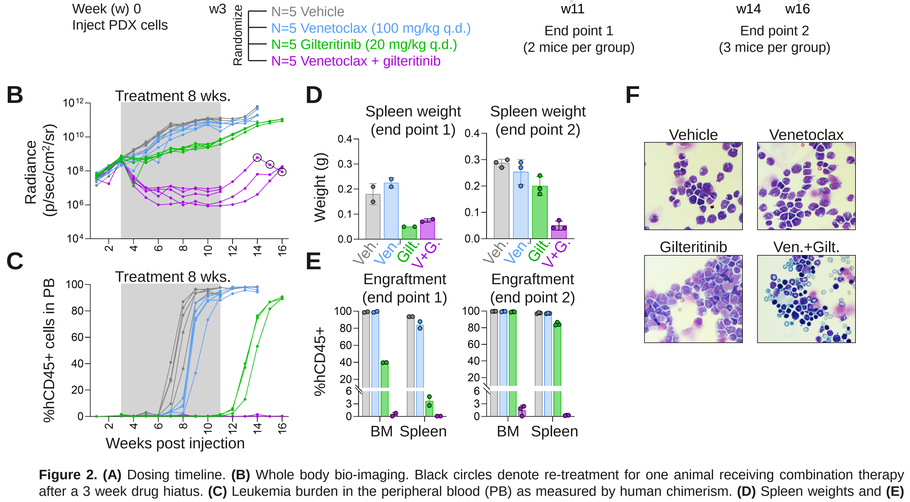


Figure 2. (A) Dosing timeline. **(B)** Whole body bio-imaging. Black circles denote re-treatment for one animal receiving combination therapy after a 3 week drug hiatus. **(C)** Leukemia burden in the peripheral blood (PB) as measured by human chimerism. **(D)** Spleen weights and **(E)** chimerism of the bone marrow (BM) and spleen at the two endpoints. **(F)** Cytospin images of the BM of a representative animal at end point 2.

Figure 3: Combination therapy is effective in PDXs with high leukemic burden

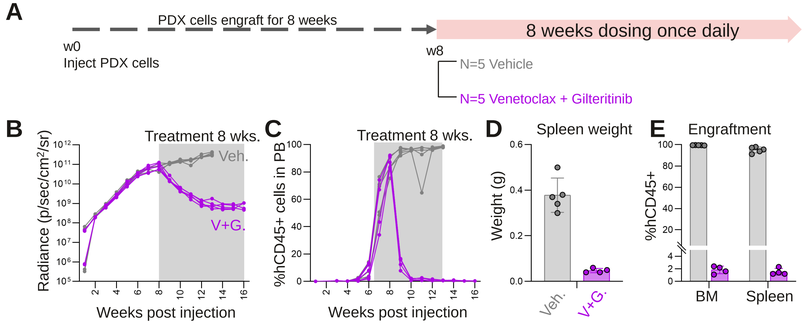


Figure 3. (A) Schematic of the dosing timeline. **(B)** Whole body bio-imaging for each mouse in each arm. The black circles denote re-treatment for one animal receiving the combination therapy after a 3 week drug hiatus. **(C)** Leukemia burden in the peripher blood as measured by human chimerism. **(D)** Spleen weights and **(E)** chimerism of the bone marrow and spleen at the two endpoints. **(F)** Cytospin images of the bone marrow of a representative animal at end point 2.

Conclusions

Conclusions

- A preclinical PDX model of *BCL11B*-a ALAL is dependent on BCL2 but not MCL-1 or BCL-xL, and is primed for apoptosis
- Single agent venetoclax shows a brief but ultimately minimal impact on leukemic growth in a *BCL11B*-a PDX in vivo
- Single agent gilteritinib decreases the rate of leukemic growth in vivo but does not clear disease
- The combination of venetoclax and gilteritinib is highly effective at eliminating leukemic cells and blocking continued growth
- Combination therapy is effective even in highly engrafted animals but does not completely clear disease

References

[1]Montefiori LE, Bendig S, Gu Z, Chen X, Polonen P, Ma X, et al. Enhancer hijacking drives oncogenic *BCL11B* expression in lineage ambiguous stem cell leukemia. Cancer Discov 2021;(11):1–22.
[2]Maruffi M, Sposto R, Oberley MJ, Kysh L, Orgel E. Therapy for children and adults with mixed phenotype acute leukemia: a systematic review and meta- analysis. Leukemia 2018;32:1515–28
[3]Alexander TB, Gu Z, Iacobucci I, Dickerson K, Choi JK, Xu B, et al. The genetic basis and cell of origin of mixed phenotype acute leukaemia. Nature 2018;562: 373–9.

Funding

This study was supported by the National Institutes of Health NCI R35 CA197695 (to C.G.M.), NCI 5F32CA254140 (L.E.M), NIH R01 CA201069 (to J.T.O), and by the Hematological Malignancy Program at St Jude Children's Research Hospital

[1]Department of Pathology, St. Jude Children's Research Hospital, Memphis TN USA; [2]Department of Cell and Molecular Biology, St. Jude Children's Research Hospital, Memphis TN USA; [3]Center for In Vivo Imaging and Therapeutics, St. Jude Children's Research Hospital, Memphis TN USA; [4]Flow Cytometry and Cell Sorting Shared Resource, St. Jude Children's Research Hospital, Memphis TN USA; [5]Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, Memphis TN USA; †equal contribution; * correspondence