Preclinical assessment of dual FLT3 and BCL-2 inhibition in lineage ambiguous leukemia

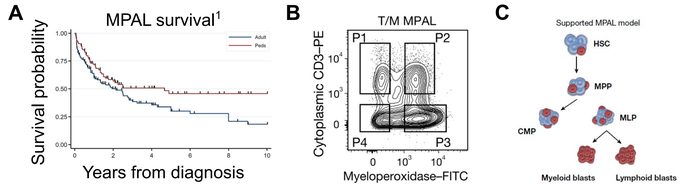
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Abstract

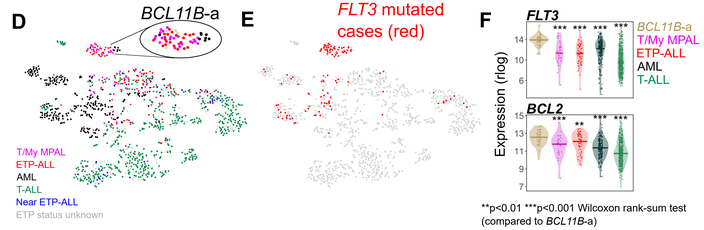
Acute leukemias of ambiguous lineage (ALAL) pose significant diagnostic and therapeutic challenges due to lack of clarity surrounding their cellular origins and driving genomic alterations, as well as poor long-term response to conventional chemotherapy. We recently identified a new subtype of ALAL defined 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 BCL-2 compared to other BCL-2 members and was primed for apoptosis. FLT3 and BCL-2 inhibitors are currently included in treatment regimens in AML and ETP-ALL, however whether single agent FLT3 or BCL-2 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 BCL-2 inhibitor), and the combination in two preclinical models 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 BCL-2 inhibitors in pre-clinical models of *BCL11B*-a leukemia.

Results

Figure 1: All immunophenotypic subpopulations of a T/myeloid MPAL *BCL11B*-a PDX are highly apoptotically primed and dependent on BCL-2

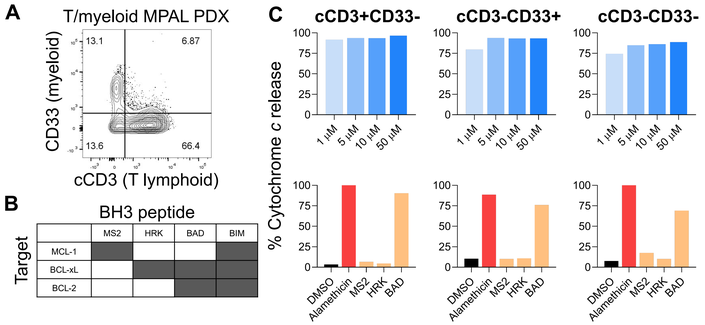


Figure 1. (A) Flow cytometry of *BCL11B*-a PDX SJMPAL011911 used in this study. **(B)** Table summarizing BH3 peptides (columns) and their targets (rows). Shaded cells show which peptides are active against which targets. **(C)** Dynamic BH3 profiling on treatment-naïve PDX cells (top, blue). Bottom (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

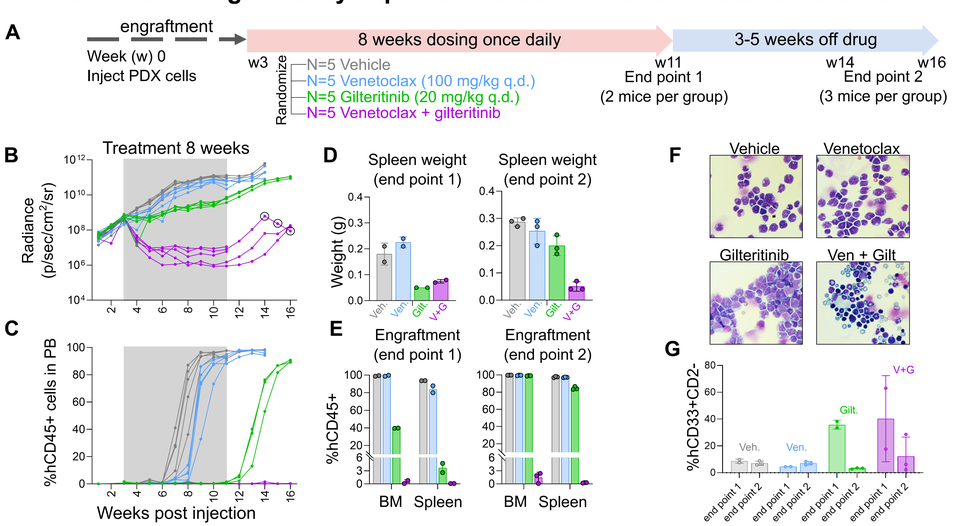


Figure 2. (A) Dosing timeline. **(B)** Whole body bio-imaging. Black circles denote re-treatment for one animal receiving combination therapy after a 3 week drug hiatus. **(C)** Leukemia burden in the peripheral blood (PB) as measured by human chimerism. **(D)** Spleen weights and **(E)** chimerism of the bone marrow (BM) and spleen at the two endpoints. **(F)** Cytospin images of the BM of a representative animal at end point 2. **(G)** Flow cytometry analysis of CD33+ in the remaining blast population at each time point.

Figure 3: Variable responses: Single agent venetoclax and gilteritinib are effective in an acute undifferentiated leukemia (AUL) *BCL11B*-a PDX

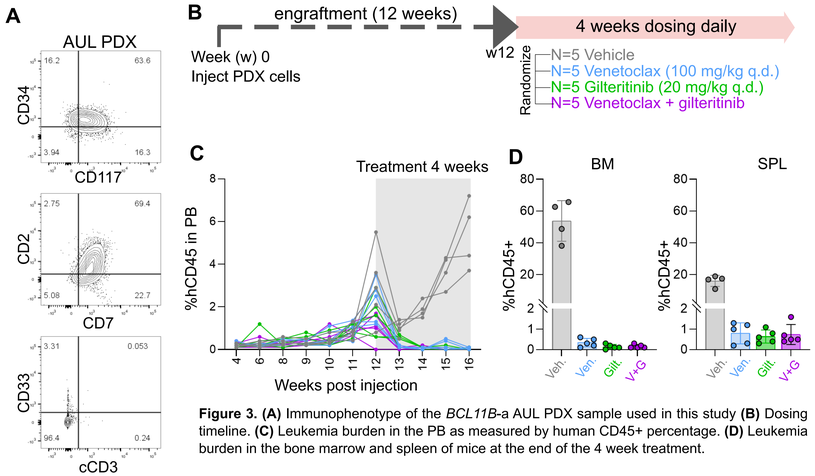


Figure 3. (A) Immunophenotype of the *BCL11B*-a AUL PDX sample used in this study **(B)** Dosing timeline. **(C)** Leukemia burden in the PB as measured by human CD45+ percentage. **(D)** Leukemia burden in the bone marrow and spleen of mice at the end of the 4 week treatment.

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 T/My MPAL *BCL11B*-a PDX
- 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
- A second *BCL11B*-a PDX model (AUL diagnosis) is sensitive to all three treatment arms, highlighting potential variability in response within a genetic subtype

References

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