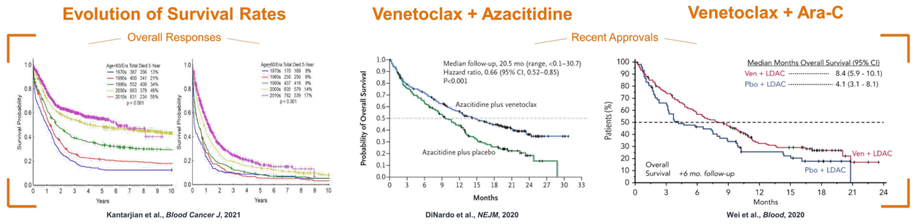
Superior anti-leukemic effects of combined acid ceramidase and Bcl-2 inhibition in acute myeloid leukemia

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INTRODUCTION

– Overview of acute myeloid leukemia –

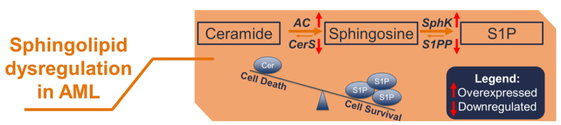
- ► Acute myeloid leukemia (AML) is an aggressive, heterogenous malignancy of the bone marrow.
- ► The median age at diagnosis for AML is 68.
- ► Intensive induction chemotherapy has been the main treatment option for the last five decades.
- ► The 5-year survival of patients <60 years vs patients >60 years is 40-50% and 10-15%, respectively.
- ► Venetoclax (ABT-199), a Bcl-2 inhibitor, was approved for AML in combination with Ara-C or azacitidine.
- ► Responses to ABT-199 regimens are incomplete and relapse rates remain high.

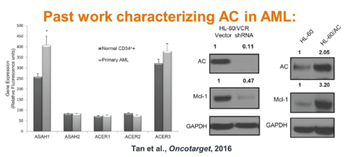


[*HIGH RELAPSE RATES NECESSITATES THE DEVELOPMENT OF NOVEL TREATMENTS TO AUGMENT CURRENT THERAPIES*]

– Harnessing the power of sphingolipids –

- ► Leukemic blast survival is influenced by cellular levels of ceramide, a bona fide pro-death molecule.
- ► Acid ceramidase (AC) catabolizes ceramides, is upregulated in AML, and correlates with poor outcome.
- ► AC overexpression promotes chemoresistance and upregulates Mcl-1, an ABT-199 resistance factor.
- ► AC inhibitor and ceramide analog, SACLAC, induces apoptosis in AML cell lines and patient samples.





[*AIM: TO ASSESS THE ANTI-LEUKEMIC EFFECTS OF COMBINED AC AND BCL-2 INHIBITION*]

METHODS

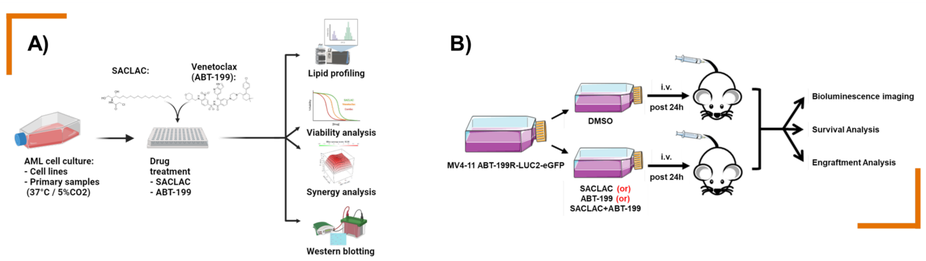


Figure 1. In vitro: human AML cell lines and patient samples were treated with vehicle (DMSO), SACLAC, ABT-199, or combination. Lipid profiling, cell viability (MTS), apoptosis, synergy analyses, and western blotting were performed after 48 hours (**A**). In vivo: AML cell lines were pretreated with vehicle, SACLAC, ABT-199, or combination. Twenty-four hours after treatment in flasks, 2.5 million viable cells were injected into 10-12 week old immunodeficient mice. Bioluminescence imaging, engraftment, and survival were monitored over time (**B**). Stats: Two-way ANOVA with Tukey's post test (Fig 2A-F, 3C, and 3E), uncorrected Fishers LSD (Fig 4A), Games-Howell test (Fig 4C), or Dunnet's test (Figure 5C); one-way ANOVA with Tukey's post test (Fig 3A-B); Welch's ANOVA with Dunnett's post test (Fig 4B); unpaired t-test (Fig 5D); log-rank test (Fig 4E and 5E). *, p< 0.05; **, p< 0.01; ***, p<0.0005, ****, p<0.0001

RESULTS

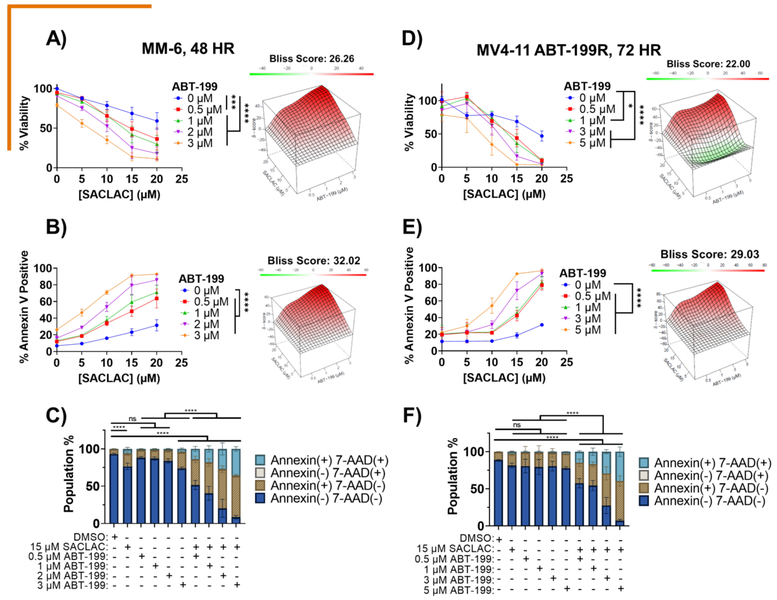


Figure 2. SACLAC and ABT-199 treatment synergistically induced cell death in cell lines with de novo (**A**,**B**,**C**; MM-6; 48 HR) and acquired (**D**,**E**,**F**; MV4-11 ABT-199R; 72 HR) ABT-199 resistance. Synergy analysis was performed with https://synergyfinder.fimm.fi/.

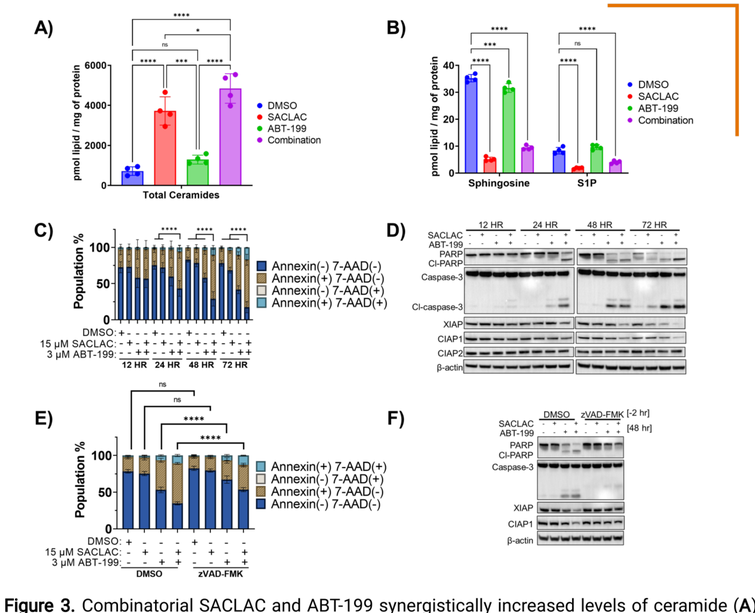


Figure 3. Combinatorial SACLAC and ABT-199 synergistically increased levels of ceramide (**A**) while decreasing sphingosine and S1P (**B**). Time course combination treatment increased cell death and levels of cleaved caspase-3 and PARP with concurrent decreases in XIAP and CIAP1 (**C**,**D**). zVAD-FMK treatment rescued cell death and XIAP and CIAP1 levels (**E**,**F**). Experiments were performed in MM-6.

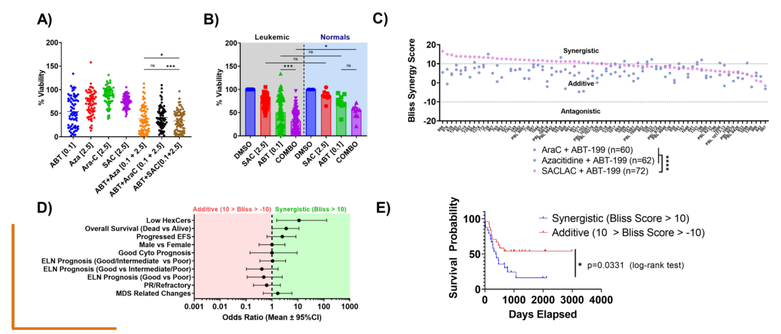


Figure 4. Viability of primary AML patient samples treated with ABT-199 alone or azacitidine, Ara-C, and SACLAC alone or in combination with ABT-199 (**A**). SACLAC and ABT-199 in normals (**B**). Synergy analysis of AML regimens and the SACLAC and ABT-199 combination (**C**). Low hexosylceramides and poorer patient survival after chemotherapy correlated with better response to SACLAC and ABT-199 in retrospective analysis (**D**,**E**).

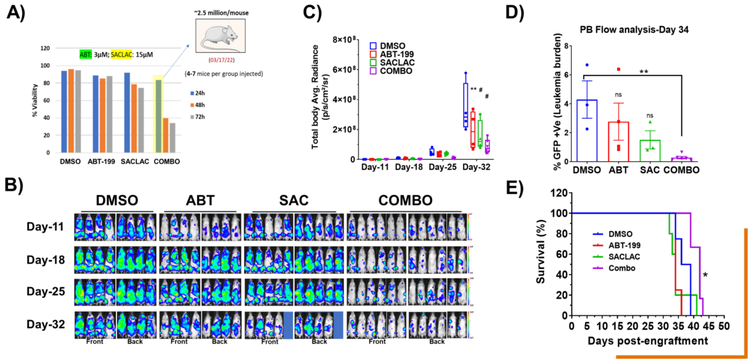


Figure 5. Extented time course monitoring of AC and Bcl-2 inhibition. Following 24 hour pre-treatment of LUC2-eGFP expressing MV4-11 ABT-199R cells with DMSO, SACLAC, ABT-199, or combination, 2.5 million viable cells were injected into 10-12 week old NRG-S male mice (**A**). Bioluminescence imaging (**B**,**C**), flow cytometry engraftment (**D**), and survival analyses (**E**) following injection.

CONCLUSIONS

Collectively, our studies in AML indicate that...

- ► AC and Bcl-2 inhibition results in synergistic killing in several AML cell lines.
- ► SACLAC+ABT-199 is comparable to AML standard treatments at reducing cell viability in primary AML patient samples.

Looking forward, we will...

- ► Assess the effect of AC, XIAP, and CIAP1 knockdown on ABT-199 sensitivity.
- ► Characterize the effects of AC inhibition on mitochondrial respiration and morphology.
- ► Further assess AC and Bcl-2 inhibition in vivo.

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