ALX148 Enhances the Depth and Durability of Response to Multiple AML Therapies

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Disclosures

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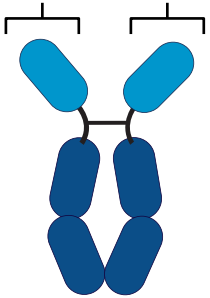
Background

High Affinity CD47 Binding Domains of SIRPα



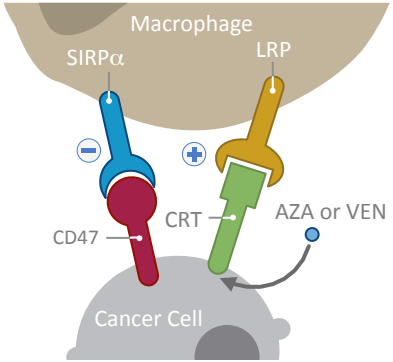
Optimized, picomolar binding affinity

Fc domain mutated to eliminate Fcγ receptor binding and minimize associated toxicity

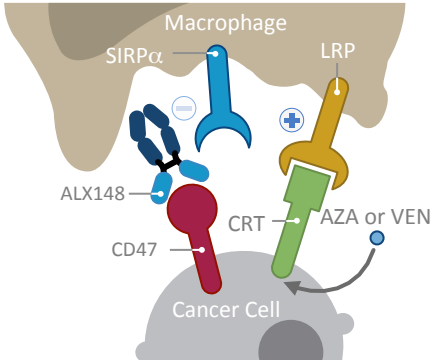
- Fc domain enables antibody-like PK
- Molecular weight half the size of typical antibody

- CD47, a myeloid checkpoint and marker of self, signals the macrophage to ignore the cell on which CD47 is expressed by binding its receptor, SIRPα.[1] Tumors upregulate CD47 to evade the immune response.
- ALX148 is a high affinity CD47 blocker fusion protein with an inactive human immunoglobulin Fc region designed to enhance the activity of anti-cancer targeted antibodies and checkpoint inhibitors with minimal hematologic toxicity.[2]

CD47/SIRPα



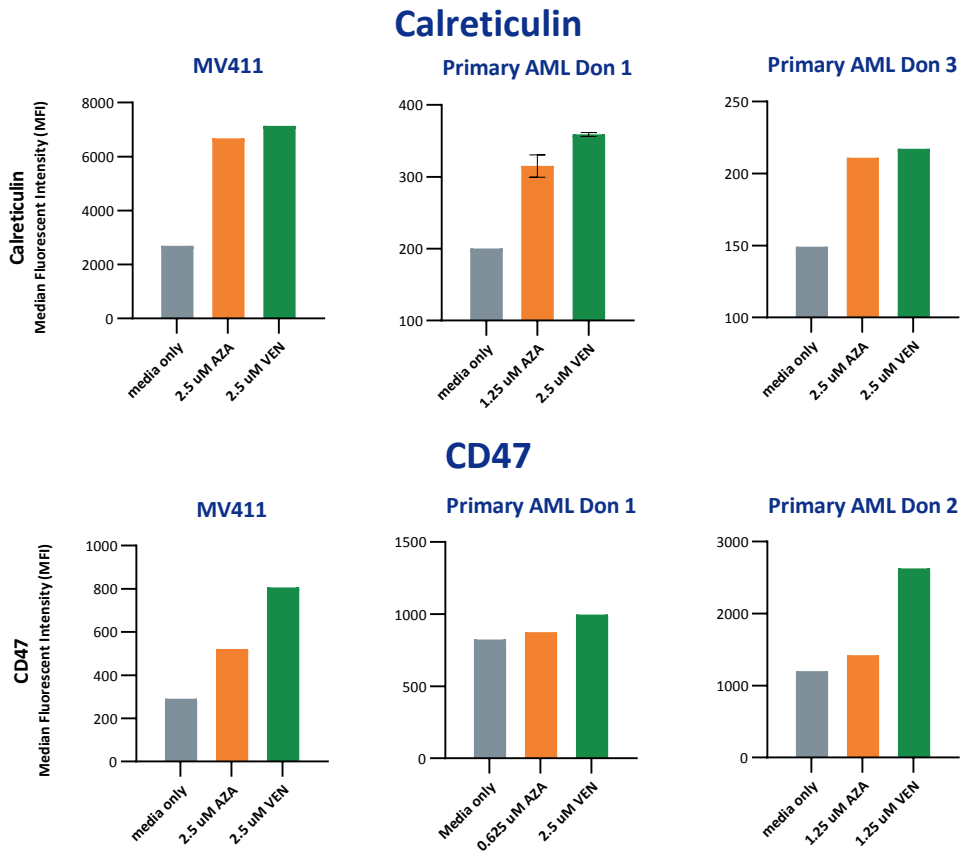
ALX148



- Calreticulin is a pro-phagocytic multifunctional protein that is translocated to the cell surface under conditions of cellular stress and apoptosis, marking the cell for phagocytic clearance.[3]
- Azacitidine (AZA) is a hypomethylating chemotherapeutic agent used in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) treatments that induces upregulation of both CD47 and calreticulin.
- Venetoclax (VEN) is a specific B-cell lymphoma-2 (BCL2) inhibitor that can restore apoptosis in malignant cells and has been recently granted approval in combination with AZA for treatment of newly diagnosed AML.[4]

CRT – Calreticulin; **LRP** – Low density lipoprotein receptor–related protein; **AZA** – Azacitidine; **VEN** – Venetoclax.

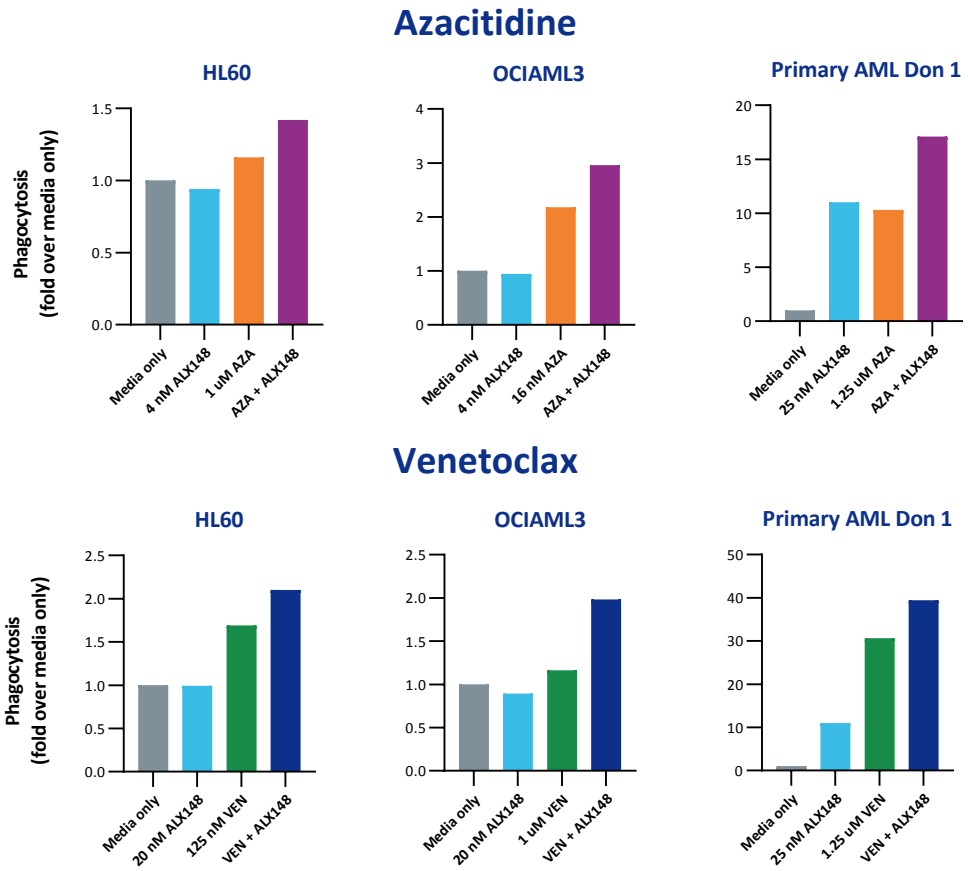
Azacitidine or Venetoclax Treatments Increase Surface Levels of Calreticulin and CD47 in AML Cell Lines and Primary AML Blasts



- AML cells were incubated for 24-48 hours with AZA or VEN, and calreticulin and CD47 expression were measured by flow cytometry.
- AZA and VEN increase expression of the pro-phagocytic signal calreticulin in both AML cell lines and primary AML blasts.
- AZA and VEN also increase expression of the CD47 anti-phagocytic signal.

Range of AZA and VEN concentration tested, representative Concentration with maximal effect shown

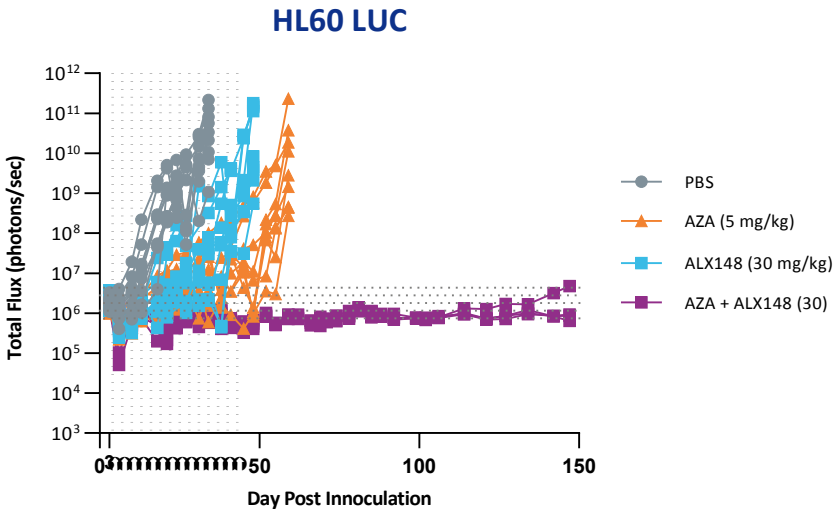
ALX148 Enhances Phagocytosis of AML Cell Lines and Primary AML Blasts in Combination with Azacitidine or Venetoclax

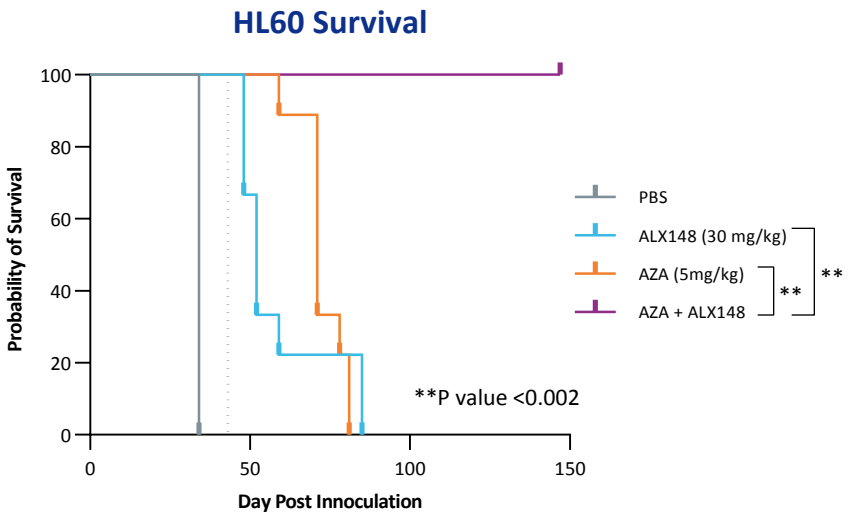


Range of AZA and VEN concentration tested, representative results shown

- AML cells were incubated with AZA or VEN for 24-48 hours and then co-cultured for 2 hours with human monocyte derived macrophages in the presence or absence of ALX148.
- Phagocytosis of AML cells was determined by flow cytometry as the percentage of macrophages that have engulfed AML (CFSE+) cells compared to total number of macrophages.
- ALX148 in combination with AZA or VEN enhanced phagocytotic elimination of AML cells by human macrophages compared to either treatment alone.

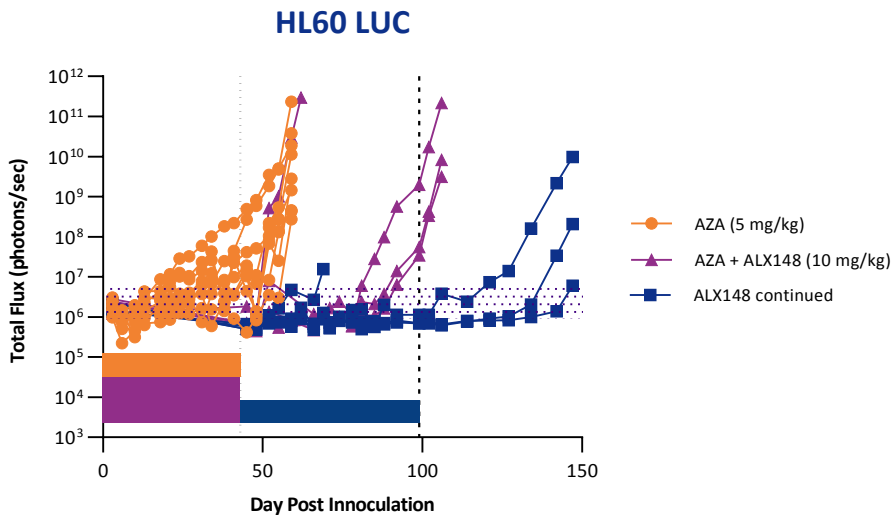
Combination of ALX148 with Azacitidine Demonstrated Significant and Durable Anti-Leukemic Activity and Prolonged Survival in Aggressive AML Tumor Model

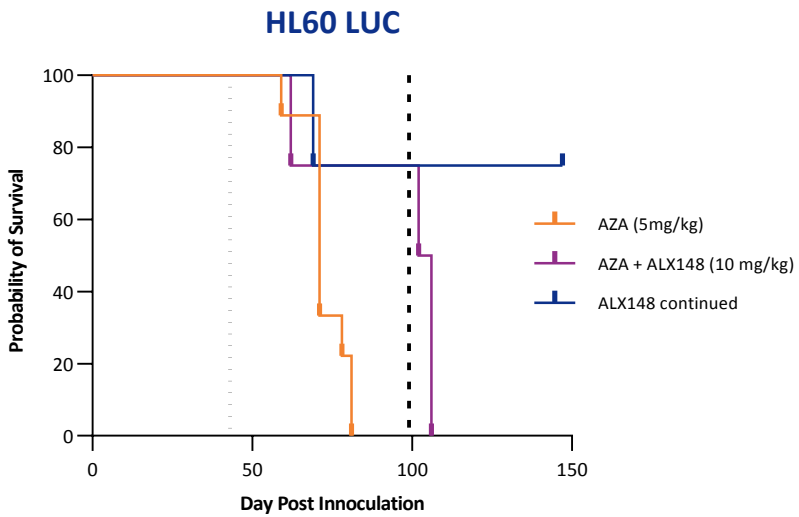




- Utilizing an aggressive AML tumor model, HL60 cells were transplanted by intravenous injection in NOD SCID mice with engraftment and tumor burden measured by bioluminescent imaging until end of study. AZA and ALX148 therapies were initiated on day 4 post engraftment and dosed intraperitoneally every 3 days for a total of 14 doses.
- AZA and ALX148 monotherapies produced moderate tumor growth inhibition with all mice succumbed to disease by day 85.
- Combination of AZA and ALX148 completely eliminated tumor growth with 100% animal survival up to study termination on day 147.

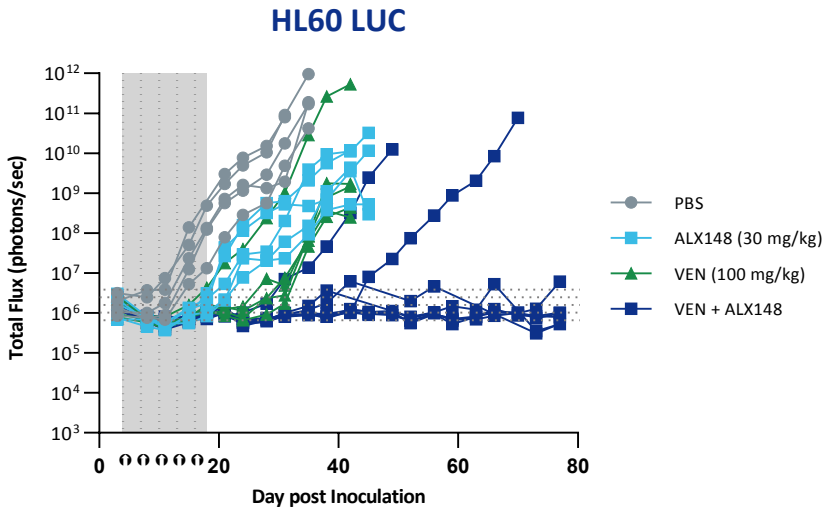
ALX148 Maintenance Therapy Controlled Disease and Extended Survival

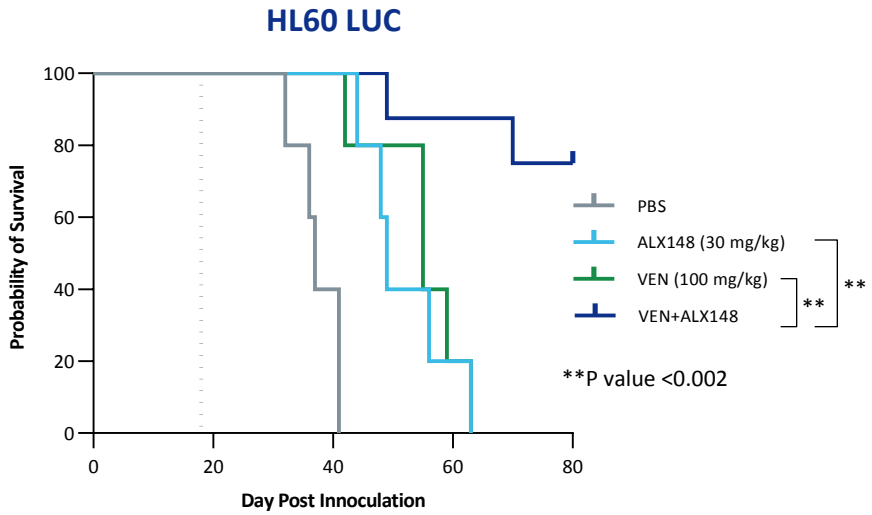




- AZA and ALX148 therapies were initiated day 4 post engraftment and dosed intraperitoneally every 3 days for a total of 14 doses.
- ALX148 maintenance monotherapy was continued in half the combination cohort for an additional 16 doses.
- After 14 total doses of AZA and ALX148 combination dosing, tumor inhibition was observed until day 105.
- ALX148 maintenance therapy extended tumor inhibition with 3 out of 4 mice surviving until study termination on day 147.

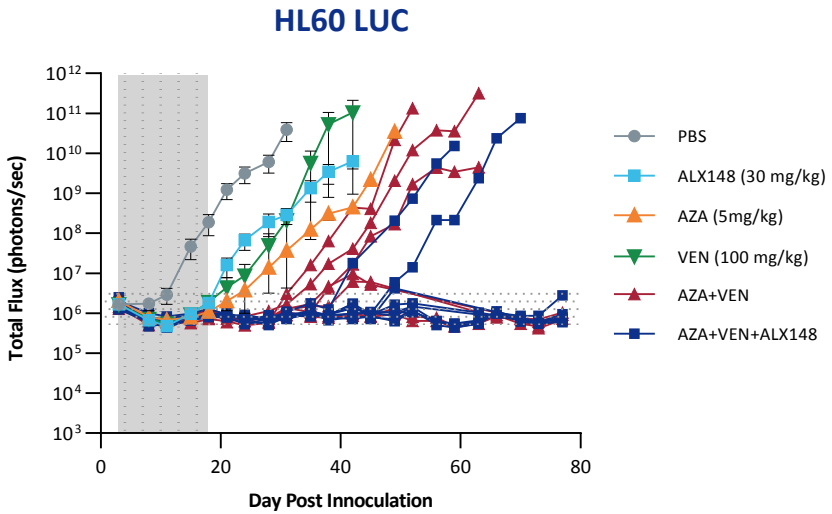
Combination of ALX148 with Venetoclax Abrogated Tumor Growth and Prolonged Survival in AML Tumor Model

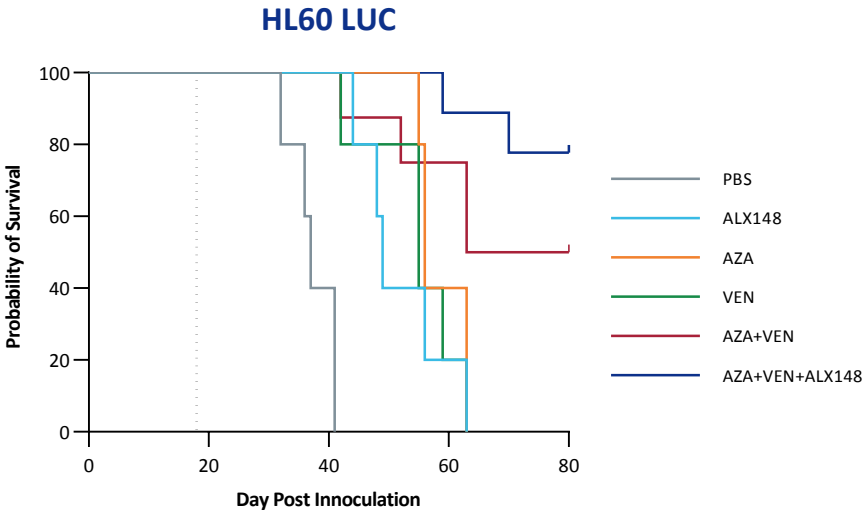




- VEN and ALX148 therapies were initiated day 4 post engraftment with ALX148 given intraperitoneally every 3 days for a total of 5 doses and for 14 consecutive days by oral gavage for VEN.
- VEN and ALX148 monotherapies produced moderate tumor growth inhibition but did not maintain durable responses with all mice succumbing to disease by day 40.
- In contrast, combination of VEN+ALX148 completely eliminated tumor growth in 6 out 8 mice within an 80 day evaluation period. Study is ongoing.

Combining ALX148 with both Venetoclax and Azacitidine Inhibited Tumor Growth and Prolonged Survival in AML Tumor Model





- AZA, VEN and ALX148 therapies were initiated day 4 post engraftment with AZA and ALX148 given intraperitoneally every 3 days for a total of 5 doses and for 14 consecutive days by oral gavage for VEN.
- AZA and VEN combination therapies yielded 4 out of 8 mice with progressive disease.
- In contrast, the combination of AZA+VEN+ALX148 completely eliminated tumor growth for 7 out of 9 mice within an 80 day evaluation period. Study is ongoing.

Conclusion

ALX148 in combination with VEN/AZA demonstrates superior tumor control and significant prolongation of survival compared to VEN, AZA, or VEN/AZA in aggressive murine models of AML

- AZA and VEN increase the cell surface expression of calreticulin and CD47 in AML cell lines and primary AML blasts *in vitro*.
- Co-treatment of ALX148 with AZA as well as VEN leads to increased clearance of AML cell lines and primary AML blasts by human macrophages *in vitro*.
- Combination treatment of ALX148 with AZA, VEN or AZA+VEN leads to tumor elimination and prolonged survival in AML xenograft models.
- ALX148 maintenance therapy significantly prolonged duration of tumor remission and overall survival after discontinuation of AZA treatment in AML xenograft models.
- These results support evaluation of ALX148 in combination with VEN/AZA in patients with AML.
- The ASPEN-02 clinical trial evaluates ALX148 in combination with AZA for patients with 1L MDS and is ongoing (NCT04417517), ASPEN-05 evaluating ALX148 in patients with AML is planned.

1. Weiskopf, K., *Eur J Cancer*. 2017 May;76:100-109; 2. Kauder, S.E. et al., *PLoS ONE*. 2018 August;13(8): e0201832; 3. Chao MP. et al., *Sci Transl Med*. 2010 Dec 22;2(63):63ra94;
4. https://www.fda.gov/drugs/fda-approves-venetoclax-combination-aml-adults