Combination Treatment with 5F9 and Azacitidine Enhances Phagocytic Elimination of Acute Myeloid Leukemia

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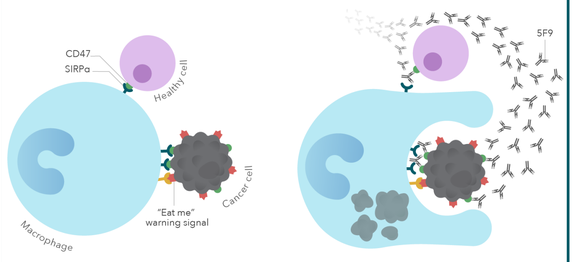
Introduction



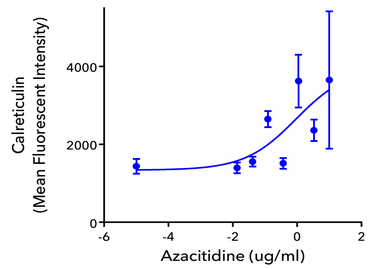
- CD47 is a "don't eat me" signal that is over-expressed on cancer cells and enables cancer cells to escape macrophage phagocytosis by interactions with its macrophage receptor, SIRPa (1)
- Calreticulin is a multifunctional protein involved in Ca^{2+} binding and storage found in the endoplasmic reticulum as well as a cell-surface pro-phagocytic marker that has been previously described in acute myeloid leukemia (AML)
- Azacitidine (AZA) is a hypomethylating and chemotherapeutic agent utilized in AML treatment and has been associated with increases expression of both CD47 and Calreticulin on AML blasts
- We therefore hypothesized that AML cells may be more efficaciously eliminated using a combination of AZA and 5F9
- This combined therapeutic strategy is currently being clinically investigated in AML patients (NCT03248479)

Methods

- Cells: HL60 (ATCC) human AML cells were previously transduced with lentivirus to express GFP and luciferase, human macrophages were derived from peripheral blood monocytes by incubation with human serum
- Mouse Strain: 6 – 8 week old female NSG (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ) (Jackson Labs)
- Reagents: anti-CD47 5F9 antibody (Forty Seven Inc), azacitidine/Vidaza (Sandoz), anti-Calreticulin antibody FMC75 (Enzo Life Sciences)
- Phagocytosis assay: Human HL60 cells were co-incubated with human macrophages in the presence of the indicated therapeutic molecules. Frequency of phagocytosis was determined by flow cytometry as the percentage of macrophages that have engulfed AML (GFP+) cells compared to total number of macrophages.
- Xenograft Model: HL60 cells (500,000 cells/per mouse) were engrafted by intravenous injection into 6 – 8-week-old NSG mice, HL60 engraftment was assessed by bioluminescence imaging (total flux (photons/sec)) and animals were randomized into 6 treatment cohorts with 8 animals per group and treatment initiated (see table). AML burden and survival were assayed by bioluminescence imaging.
- See results sections for additional method details

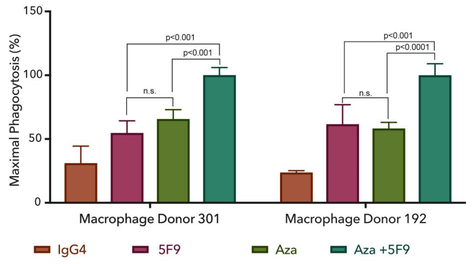
Results

Azacitidine Increases Expression of the "Eat Me" Signal Calreticulin on AML Cancer Cells



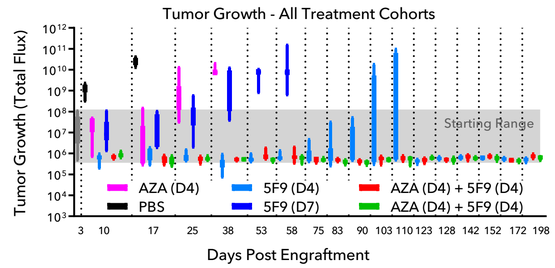
- AML cancer cells (HL60) were incubated for 4 hours with increasing concentrations of azacitidine as indicated and expression of calreticulin (CRT) was measured with an anti-CRT Ab by flow cytometry
- Azacitidine increases the expression of this pro-phagocytic "eat me" signal on the surface of AML cells

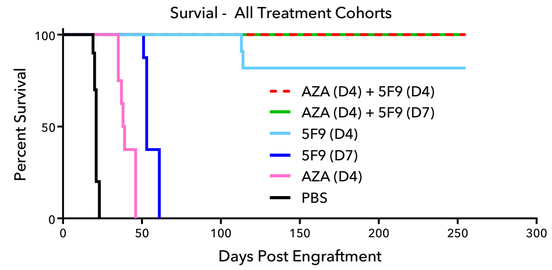
Combination of 5F9 with Azacitidine Enhances Phagocytosis of AML Cancer Cells

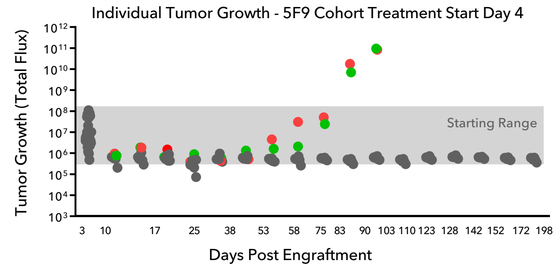


- AML cancer cells (HL60) were incubated for 24 hours with azacitidine (3μM). HL60 cells were then co-cultured for 2 hours with human monocyte derived macrophages in the presence or absence of the anti-CD47 Ab 5F9 (10ug/ml) or control IgG4 (10ug/ml)
- Phagocytosis of HL60 cells was determined by flow cytometry
- The combination of azacitidine with 5F9 significantly enhanced the phagocytic elimination of HL60 leukemia cells by human macrophages in *vitro* compared to single agent treatment with azacitidine or Hu5F9 alone
- These results were consistent across multiple assays with macrophages derived from multiple monocyte donors

Combination of 5F9 with Azacitidine Enhances Elimination of AML and Prolongs Survival







Treatment Cohorts - Dose and Schedule

Treatment Group	Dose Level	Treatment Schedule post engraftment
PBS	N/A	Day 4: 14 consecutive doses
Azacitidine	7.5 mg/kg	Day 4: 5 consecutive doses
5F9 (D4)	10 mg/kg	Day 4: 14 consecutive doses
5F9 (D7)	10 mg/kg	Day 7: 14 consecutive doses
5F9 (D4)+ Azacitidine	10 mg/kg 7.5 mg/kg	Day 4: 14 consecutive doses Day 4: 5 consecutive doses
5F9 (D7)+ Azacitidine	10 mg/kg 7.5 mg/kg	Day 7: 14 consecutive doses Day 4: 5 consecutive doses

- An aggressive AML xenograft mouse model was utilized to evaluate the *in vitro* phagocytosis results *in vivo*
- After transplantation of HL60 cells by intravenous injection into NSG mice, engraftment was confirmed by bioluminescence imaging and mice were randomized into cohorts. Treatment was performed as shown in the table above. HL60 growth and burden was assessed by bioluminescence imaging
- AZA and 5F9 monotherapies initiated on day 4 post-engraftment yielded two animals with progressive disease while the remainder of the cohort surviving without detectable evidence of AML cancer cells
- In contrast, monotherapy treatments initiated later on day 7 post-engraftment failed to produce durable responses with all animals perishing by days 46 and 61 post-engraftment, respectively
- Critically, both 5F9 and AZA co-treatment cohorts, regardless of timing to treatment initiation, demonstrated inhibition of AML growth as early as day 10 post HL60 engraftment (PE), and maintained elimination of growth and overall survival up to 255 days PE when the study was terminated

Conclusions

- AZA increases the cell surface expression of CRT, a pro-phagocytic marker
- 5F9 and AZA co-treatment leads to increased *in vitro* clearance of AML cancer cells by human macrophages
- Combinatorial treatment leads to increased *in vivo* clearance and significantly prolonged survival in AML xenograft models
- These results support the rationale for investigating a combinatorial treatment of 5F9 and AZA in AML patient
- A clinical trial with this combination in is currently ongoing (NCT03248479)

References

- (1) Jaiswal, S., M. P. Chao, Et Al. (2010). "Macrophages As Mediators Of Tumor Immunosurveillance." Trends Immunol **31**(6): 212-219

For Disclosures See: https://ash.confex.com/ash/2018/webprogram/Paper120170.html