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

Enhances Phagocytic Elimination of Acute Myeloid Leukemia

AML cancer cells (HL60) were incubated for 4 hours. Phagocytosis assay: Human HL60 cells were co-cultured with 3µM 5F9 and azacitidine for 24 hours and the phagocytic marker, calreticulin (CRT), was measured by flow cytometry. Azacitidine significantly enhanced the phagocytic elimination of HL60 leukemia cells by human macrophages in vitro compared to single agent treatment with azacitidine or Hu5F9 alone.

- 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 Ca2+ 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 efficiently eliminated using a combination of AZA and 5F9.
- This combined therapeutic strategy is currently being clinically investigated in AML patients (NCT03248479).

Introduction

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-Prkdcscid Il2rgtm1晋/Il2rgtm1(Tcra-Tg)1Jcsk (Jackson labs).)
- Reagents: anti-CD47 5F9 antibody (Forty Seven Inc), azacitidine/Vidaza (Sanodio), 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 (GP+R+) 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/s/crat)) and animals were randomized into 6 treatment cohorts with 8 animals per group and treatment initiated (see table). AML burden and survival were assessed by bioluminescence imaging.
- See results sections for additional method details.

Results

- An aggressive AML xenograft mouse model was utilized to evaluate the in vivo phagocytic 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 persisting 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

References


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