RBC-Specific CD47 Pruning Confers Protection and Underlies the Transient Anemia in 5F9 Anti-CD47 Treatment

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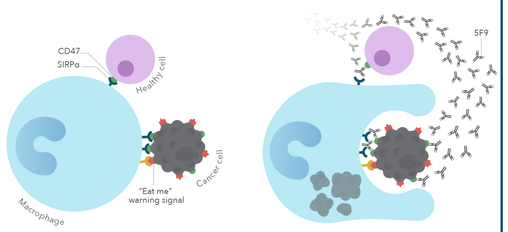
Introduction



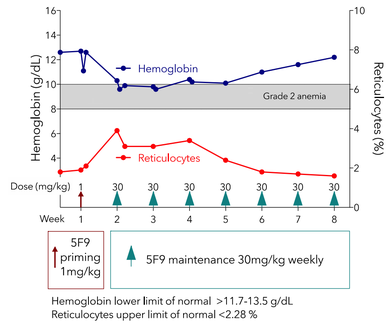
- CD47 is a "don't eat me" signal that is over-expressed on cancer cells and enables cancer cells to escape macrophage phagocytosis
- 5F9 binding to CD47 enables tumor cell phagocytosis and activates an anti-tumor T-cell response *in vitro* and *in vivo*
- 5F9 is being evaluated as an anti-cancer therapy in multiple clinical trials both as a mono- and combination therapy
- Oldenborg et al. (1) established that red blood cell (RBC) removal is dependent on the balance between accumulation of pro-phagocytic signals and loss of inhibitory anti-phagocytic signals
- We therefore hypothesized that an initial lower 5F9 "priming" dose would be sufficient to trigger clearance of aged RBCs and would yield an overall younger pool of RBCs, less vulnerable to subsequent higher 5F9 therapeutic "maintenance" doses
- 5F9 is therefore dosed on an initial lower "priming" dose followed by higher subsequent "maintenance" doses

Methods

- CD47 receptor occupancy analysis was performed on patient peripheral blood and bone marrow samples from NCT02216409 (solid tumor) and NCT02678338 (AML) trials
- Flow cytometry analysis for CD47 expression and 5F9 binding was conducted with anti-IgG4 and anti-CD45, and Sytox™ blue viability dye. Red blood cells (RBCs) were defined as CD45 negative and Sytox™ blue negative events and white blood cells (WBCs) as CD45 positive Sytox™ blue negative events
- Mouse strains for non-clinical studies: C57BL/6J (JAX), FcG1,3 KO (Fcer1g KO, B6.129P2-*Fcer1g*^{tm1Rav}N12, Taconic), FcG2 KO (Fcgr2b KO, B6.129S4-*Fcgr2b*^{tm1TtK}N12, Taconic), NSG (NOD.Cg-*Prkdc*^{scid}Il2rg^{tm1Wjl}/SzJ,JAX), Rag2gcKO (Rag2/Il2rg, B10;B6-*Rag2*^{tm1Fwa}Il2rg^{tm1Wjl}, Taconic)
- Antibodies: Anti-CD47 (MIAP410), Anti-CD47 (AF1866), Anti-CD47 (Hu5F9-G4), Anti-Gr-1 (RB6-8C5), Anti-IgG4 (G17-4), CD45 (2D1), Anti-IgG1 (RMG1-1)
- Other Reagents: Clodronate (Liposoma)
- See results sections for additional method details

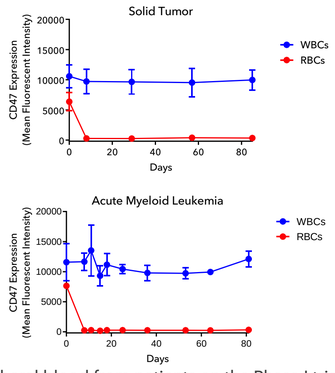
Results

Prime - Maintenance Dosing Regimen Limits Clearance of Aged RBCs to Transient Anemia



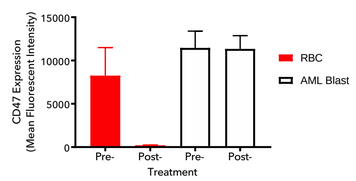
- Hemoglobin levels and reticulocyte counts of a patient dosed with 1mg/kg priming dose followed by 30mg/kg maintenance dose (NCT02216409)

CD47 Pruning is Specific to RBCs and Does not Occur on WBCs



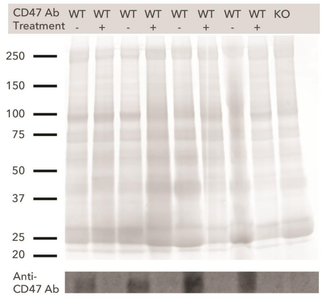
- Peripheral blood from patients on the Phase I trials for solid tumors (NCT02216409) and hematologic malignancies (NCT02678338) was assayed for CD47 expression and 5F9 binding by flow cytometry
- CD47 pruning (loss) was only observed on RBCs (CD45-) but not WBCs (CD45+) and occurred with the initial (priming) dose
- Pruning was also confirmed with a 5F9 non-competing anti-CD47 antibody (data not shown)

CD47 Pruning Does not Occur on Acute Myeloid Leukemia Blasts



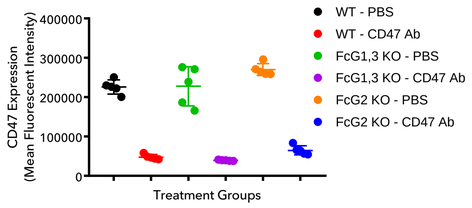
- Bone marrow from patients in the Phase I trial for hematologic malignancies (NCT02678338) was assayed for CD47 expression and 5F9 binding on AML blasts using flow cytometry
- AML blasts are defined by CD45 vs SSC profile and RBCs as CD45-

CD47 Protein Levels on RBCs are Decreased After Anti-CD47 Antibody Treatment



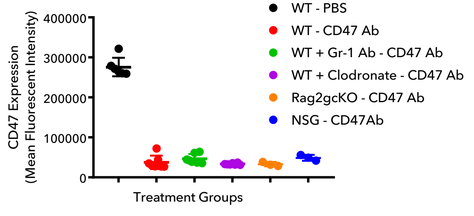
- RBCs isolated from peripheral blood of C57BL6 wild type mice (WT) pre-treated with anti-CD47 antibody (MIAP410) were evaluated for CD47 expression by western blot
- RBCs isolated from anti-CD47 antibody treated mice (+) (n=4) and a CD47 knock out (KO) mouse showed a significant decrease in measurable CD47 protein by anti-CD47 antibody (AF1866) staining compared to untreated mice (-) (n=4) that exhibited robust staining

Anti-CD47 Mediated RBC Pruning is Fc-Independent



- C57BL6 wild type (WT), γ chain knock out (KO) which prevents cell surface expression of Fc-γRI and III or Fc-γRII KO mice were treated with anti-CD47 antibody (MIAP410) and peripheral blood erythrocytes were evaluated for expression of CD47 by flow cytometry
- The degree of CD47 pruning was comparable across all three treatment groups suggesting CD47 antibody mediated pruning is Fc-independent

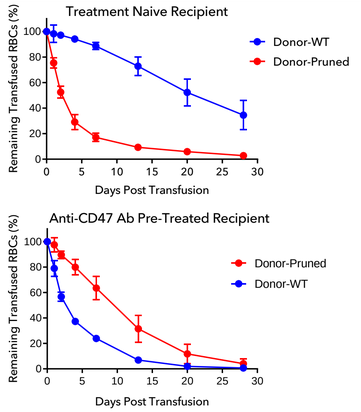
CD47 Pruning is Independent of Neutrophils, Macrophages, T-, B-, NK-, Cells and C5 Complement



- C57BL6 WT mice were pre-treated with anti-Gr-1 antibody (RB6-8C5) to ablate neutrophils and clodronate to ablate macrophages (including red pulp macrophages)
- C57BL6 WT mice -depleted of neutrophils and macrophages-, Rag2gcKO, and NSG mice (lacking T, B, and NK -cells) were treated with anti-CD47 antibody (MIAP410)
- Similar to the clinical setting, anti-mouse CD47 antibody treatments resulted in pruning of CD47 in all mice, thereby suggesting that neutrophils, macrophages, T, B, and NK -cells are not necessary for anti-CD47 antibody mediated CD47 pruning
- CD47 pruning in NSG mice with a deficiency for complement factor 5 (C5) also suggests lack of complement involvement

Results

CD47 Pruning is Protective for RBCs



- Fluorescently labeled RBCs from anti-CD47 antibody untreated (WT) and anti-CD47 antibody treated (Pruned) mice were transfused into treatment naïve and anti-CD47 antibody pre-treated recipients.
- Pruned RBCs were rapidly cleared in treatment naïve recipients while exhibiting a significantly improved survival in pre-treated recipients
- These data suggest CD47 pruning is protective for RBCs in the context of continued anti-CD47 antibody therapy

Conclusions

- 5F9 priming dose is sufficient to induce CD47 pruning on RBCs and is maintained over the course of treatment
- 5F9 mediated CD47 pruning is specific to RBCs and is not detected on WBCs or acute myeloid leukemia blasts
- Anti-CD47 antibody induced RBC-specific CD47 pruning is protective for RBCs over the course of treatment
- To our knowledge, anti-CD47 antibody mediated pruning represents a novel RBC antigen depletion phenomenon that is independent of known RBC regulatory compartments, including the spleen, liver (organ resection data in mice not shown), major cellular effector cells, complement, and is Fc-independent.
- Concerns for potential anti-CD47 antibody mediated effects, i.e. hemagglutination, decrease significantly given these new findings

References

- (1) Oldenborg, P. A. *et al.* Role of CD47 as a marker of self on red blood cells. *Science* 288, 2051-2054 (2000).

For Disclosures See: https://ash.confex.com/ash/2018/webprogram/Paper115674.html