**Figure 1:** CD47 regulates the interaction between tumor cells and macrophages. **BACKGROUND**
• CD47 is a transmembrane protein of unknown function.
• It has multiple binding partners (Figure 1) and negatively regulates phagocytosis

**SRF231 ANTIBODY GENERATION**
• Fully human anti-CD47 antibodies were generated using mice carrying human variable heavy and light transgenes.
• Several antibodies were screened for desired characteristics, including no hemagglutination or RBC phagocytosis.

**SRF231 BINDING CHARACTERISTICS**
• SRF231 binds with high affinity to human CD47 (Table 1). SRF231 is a potent blocker of the CD47-SRPSR5 interaction (Figure 2).

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**Table 1:** Blot ec analysis of the interaction between SRPSR and human CD47.

**SRF231 PROMOTES PHAGOCYTOSIS OF HEME CANCER CELL LINES AND PRIMARY AML CELLS IN VITRO AND HAS ANTI-TUMOR ACTIVITY IN VIVO**

**Figure 2:** SRF231 promotes phagocytosis of heme cancer cell lines and primary AML cells in vitro and has anti-tumor activity in vivo.

**Figure 3:** SRF231 promotes phagocytosis of hematologic tumor cell lines and primary AML cells in vitro. Human monocyte derived macrophages were cultured with the indicated CFSE-labeled tumor cell lines (A) or primary AML cells (B). Cells were incubated for 2 hr in the presence of SRF231. Co-cultures were stained for CD14 and analyzed by flow cytometry. Phagocytosis reported as % CD14+ macrophages that were CFSE+ double-exposed. Phagocytosis was the percentage of tumor cells that were CFSE+ at each antibody concentration. SRF231 was selected as the lead candidate antibody.

**Figure 4:** Enhanced phagocytosis is preferential for tumor cells over normal leukocytes and RBC. (A) Human macrophages were cultured 2 hr with CellTrace Violet labeled Jurkat and CFSE-labeled normal T cells (n = 11) and analyzed by flow cytometry. SRF231 was used at a concentration of 10 µg/ml. Phagocytosis was the percentage of macrophages that were CFSE+ double-exposed (% SRF231 +) (B) Antibodies were incubated with 0.5% human RBC overnight. Aggregation is % of conjugates determined by analysis of side-scatter height vs. side-scatter width. Positive control is in-house anti-CD47 clone that causes hemagglutination.

**Figure 5:** Enhanced phagocytosis is preferential for tumor cells over normal leukocytes and RBC. (A) Human macrophages were cultured 2 hr with CellTrace Violet labeled Jurkat and CFSE-labeled normal T cells (n = 11) and analyzed by flow cytometry. SRF231 was used at a concentration of 10 µg/ml. Phagocytosis was the percentage of macrophages that were CFSE+ double-exposed (% SRF231 +) (B) Antibodies were incubated with 0.5% human RBC overnight. Aggregation is % of conjugates determined by analysis of side-scatter height vs. side-scatter width. Positive control is in-house anti-CD47 clone that causes hemagglutination.

**MULTIPLE MYELOMA CELLS ARE SENSITIVE TO SRF231 IN VITRO AND IN VIVO**

**Figure 6:** Activity of SRF231 against multiple myeloma cells in vitro and in vivo. (A) Myeloma cell lines were cultured for 2 hr with CFSE labeled human AML cells in the presence of SRF231. Co-cultures were stained for CD47 and analyzed by flow cytometry. Phagocytosis reported as % CD47+ macrophages that were CFSE+ double-exposed. Phagocytosis was the percentage of tumor cells that were CFSE+ at each antibody concentration. SRF231 was selected as the lead candidate antibody.

**MACROPHAGE ACCUMULATION AND SHIFT TOWARD M1 PHENOTYPE IN SRF231-TREATED TUMOR XENOGRAFTS**

**Figure 7:** Analysis of macrophage accumulation by IHC staining of Raji xenograft tumors. SRF231 was injected i.p. (2 mg/kg) on d0. Mice treated with 100 µg SRF231 (red) or isotype (blue) were treated with isotype control or up to 3 doses of 200 µg SRF231 i.p. (A) Macrophages were counted in viable tumor areas. (B) Tumor burden of SCID mice was measured weekly. T Cell Input % was calculated as the mean tumor volumes.

**EFFECT OF MACROPHAGE DEPLETION ON SRF231 ANTI-TUMOR ACTIVITY**

**Figure 8:** Macrophages contribute to SRF231 mediated anti-tumor activity. CLD mice were inoculated with Raji cells s.c. When tumors reached 100-150 mm3, mice (n = 10/group) were treated with 100 µg clodronate liposomes (blue) or saline (red). One day following the second clodronate treatment, mice were treated with either 100 µg SRF231 i.p. (SRF231) or saline (Isotype). Data shown as mean tumor volumes ± SEM.

**CONCLUSIONS**
• SRF231 is a high affinity, fully human antibody against human CD47.
• SRF231 promotes robust tumor cell phagocytosis of hematological cell lines and primary tumor cells preferentially over T cells or RBC in vitro.
• SRF231 shows potent in vivo anti-tumor efficacy in preclinical models of AML, lymphoma and multiple myeloma as monotherapy or in combination settings.
• SRF231 is currently in IND-enabling studies and is expected to enter clinical trials in 2017.

**References**