Lack of CD47 Membrane Mobility Contributes to the Poor Erythrocyte Binding of SIRPαFc, a Novel CD47-Blocking Cancer Immunotherapeutic

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Introduction
- CD47 binds to SIRPα on the surface of macrophages and delivers a “do not eat” signal to suppress phagocytosis
- Tumor cells frequently overexpress CD47 and exploit this pathway to evade macrophage-mediated destruction
- Blocking CD47 using a soluble decoy receptor (SIRPαFc) has emerged as a promising strategy to neutralize the suppressive effects of CD47 and promote the eradication of tumor cells
- One concern with CD47-based therapies is the expression of CD47 on red blood cells (RBCs), which has the potential to act as a large antigen sink and cause hematological toxicities
- We previously reported that SIRPαFc binds very poorly to human RBCs (AACR 2014); here we present additional data with an expanded donor set and provide mechanistic insights in the unusual species selectivity of this phenomenon

SIRPαFc: A Novel Biologic that Blocks the CD47 “Do Not Eat” Signal
- CD47 mAbs
- CD47 Fc
- CD47 Fc does not agglutinate human RBCs
- SIRPαFc binds very poorly to human RBCs
- SIRPαFc binds monkey RBCs despite a lower affinity for monkey CD47
- Blocking CD47 with SIRPαFc results in the inhibition of tumor cell-mediated killing of tumor cells
- SIRPαFc may enable macrophage-mediated killing of tumor cells in vitro
- SIRPαFc has potential in vivo anti-leukemic activity in AML xenograft models
- SIRPαFc is in preclinical development as a therapy for AML and other malignancies

The Presence of Detergent-Insoluble CD47 in RBC Membranes Correlates with Poor SIRPαFc Binding
- Detergent-insoluble CD47
- SIRPαFc binding is reduced selectively in detergent-insoluble RBCs

Targeting CD47 Induces Anemia in Non-Human Primates
- Male cyno monkeys dosed with 5 mg/kg SIRPαFc (hIgG1 Fc) by slow IV injection
- Animals 2001B and 2003B received four doses of drug (days 1, 4, 8, 11)
- Animal 2002B received three doses of drug (days 1, 4, 8), dosing discontinued due to clinical symptoms of anemia

Conclusions
- Using an expanded donor pool (n=43) we have confirmed that human SIRPαFc binds very poorly to human RBCs and does not induce hemagglutination, despite ample CD47 expression and strong reactivity with anti-CD47 antibodies
- The poor binding of SIRPαFc is independent of gender, ABO or Rh blood groups
- Despite nearly 10-fold weaker affinity for cyno CD47, human SIRPαFc binds strongly to cyno RBCs
- The failure of SIRPαFc to bind RBCs correlates with the presence of detergent-insoluble CD47 in RBC membranes, consistent with a model in which CD47 mobility is required to form high affinity clusters with SIRPαFc
- The binding of SIRPαFc to cyno RBCs results in clinically significant anemia in monkeys; we speculate that similar RBC toxicity is likely to occur in humans treated with CD47-blocking antibodies but not with a poor RBC-binding SIRPαFc therapeutic

CD47 mAbs
- Total
- SIRPαFc
- Anti-CD47 Western Blot
- hemagglutination

Fluorescence Intensity
- Human RBCs
- SIRPαFc
- cyno RBCs
- Conc (nM)
- FI (Geo Mean)
- Controls
- BRIC126
- 2D3
- B6H12
- CC2C6
- 5F9
- mIgG1
- mIgG2b
- SIRPαFc
- HUman SIRPαFc
- cyno SIRPαFc
- Human RBCs
- cyno RBCs
- Mouse RBCs
- Hb (g/L)
- Reference Range
- Hypochromic Anemia
- Hemoglobin (g/L)
- Total
- SIRPαFc
- Anti-CD47 Western Blot
- Hb (g/L)
- Reference Range
- Hypochromic Anemia
- Hemoglobin (g/L)
- Total
- SIRPαFc
- Anti-CD47 Western Blot
- Hb (g/L)
- Reference Range
- Hypochromic Anemia
- Hemoglobin (g/L)
- Total
- SIRPαFc
- Anti-CD47 Western Blot
- Hb (g/L)
- Reference Range
- Hypochromic Anemia
- Hemoglobin (g/L)
- Total
- SIRPαFc
- Anti-CD47 Western Blot
- Hb (g/L)
- Reference Range
- Hypochromic Anemia
- Hemoglobin (g/L)
- Total
- SIRPαFc
- Anti-CD47 Western Blot
- Hb (g/L)
- Reference Range
- Hypochromic Anemia
- Hemoglobin (g/L)
- Total
- SIRPαFc
- Anti-CD47 Western Blot
- Hb (g/L)
- Reference Range
- Hypochromic Anemia
- Hemoglobin (g/L)
- Total