Antibodies to SIRPα enhance innate and adaptive immune responses to promote anti-tumor activity

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Background & Objective
Signal regulatory protein α (SIRPα) is an immune checkpoint receptor expressed primarily on myeloid cells and tumors. SIRPα suppresses innate immunity upon interaction with its ligand CD47. Targeting the CD47-SIRPα pathway represents a novel therapeutic approach to enhance anti-cancer immunity. Ongoing clinical trials to initiate this pathway through targeting CD47 have shown promising results in reducing tumor burden. Unlike CD47 which is expressed ubiquitously, SIRPα expression is more restricted. Therefore, targeting SIRPα may result in differential safety and efficacy profiles vs. that of CD47 targeted therapies.

Objectives of study:
• Characterize anti-SIRPα clone 21 for binding to human, mouse and cynomolgus monkey SIRPa.
• Reconstruct in vitro and in vivo models of human and cynomolgus monkey SIRPa binding and interactions.
• Identify optimal ligand output for humanized 21.
• Evaluate clones 21 for anti-tumor activity in both xenograft and syngeneic tumor models and elucidate the mechanisms driving efficacy.
• Evaluate 21 in cytokineemyogenic models for PK, pharmacodynamics (PD) and tolerability.

Methods

Surface Fluorescent Resonance (FRET) For FRET determination, SIRPα antibodies were subjected to a "two-step" binding assay and measured for intramolecular anti-SIRPα antibodies. Epitope tanning was carried out using a classical sandwich approach.

Humanization: Children variable region of the light chain was grafted onto human tandem light chain framework.

Cell binding on primary human, mouse and monkey monocytes. Permitted humanized 21 were labeled with Alexa 488, and differentially expressed on the surface of the antibodies stained at 37°C. Monocytes from human and cynomolgus monkey PBMC were identified CD14+/CD19- and mouse by expression of CD4, CD8, and CD11b.

Phagocytosis:
Mixed lymphocyte reaction. Freshly collected in CD47-coated wells were generated from purified monocytes treated with 4 μM CD47 and stimulated with LPS/IFN-γ. DCs and T cells at a 1:5 ratio were incubated along with MCs and antibodies.

Phagocytic uptake: PKMCs were incubated with 10μg/mL of antibody. Cell viability was assayed with a control of cell-specific antibody two days post treatment.

Tumor models and immunophenotyping: RAJ and MC cells were implanted in NOD-SCID and NOD-SCID, respectively. Experiments were performed as previously described.

Reactivity, Permeability and Pharmacodynamics study in cynomolgus monkeys. Antibodies were administered intravenously on day 1 and 8 at 10 or 30 mg/kg. Serum concentrations were determined using SIRPα capture enzyme-linked immunosorbent assay. Receptor occupancy was measured using flow cytometry (data not available).

Immunoassay.

Results

H21 binds human, monkey and mouse SIRPa

• H21 binds SIRPα across species and with similar affinity as parent clone 21.
• Similar binding profile of 21 on primary human, cynomolgus and mouse monocytes.

On v/vL human monocytes, H21 binds with the lowest EC50 as compared to known anti-SIRPα antibodies.

H21 binds v1 and v2 SIRPα alleles and induces phagocytosis

• H21 induces phagocytosis by homozygous v1 and v2 and heterozygous v1/v2 macrophages while a allele specific anti-SIRPα antibody (H7) does not induce phagocytosis by v1/v1 and v1/v2 macrophages.

21 enhances anti-tumor activity in combination with rituximab in the Raji xenograft model

• Combination of 21 with rituximab induced tumor regression. Treatment led to 6/10 and 2/10 tumor free-mice in combination with control and high drug only groups, respectively.

Concluding remarks

• A panel of anti-SIRPα antibodies with diverse binding profiles, SIRP reactivity and broad epitope coverage was identified and will be useful tools for interrogating SIRP biology and the CD47-SIRPα checkpoint pathway.

• Anti-SIRPα clone 21 was selected for humanization and displays high affinity binding to both human SIRPα alleles v1 and v2, cynomolgus monkey and mouse SIRPα.

• H21 induces phagocytosis by homozygous v1 and v2 and heterozygous v1/v2 macrophages and potentiates the efficacy of anti-tumor and anti-CD163 antibodies in xenograft and syngeneic models, respectively.

• Clone 21 in combination with anti-IL-6 induces DC activations, increases M1 tumor associated macrophages (TAMs) and T cell effector function thus bridging innate and adaptive immunity in MC38 tumor bearing mice.

• FcR/PD of clone 21 is cytokineemyogenic showed typical antibody PK and complete SIRPα occupancy. H21 was well tolerated with no adverse signs observed in clinical evaluations, histopathology and serum chemistry.

21 enhances anti-tumor activity in combination with anti-PD-1 in MC38 syngeneic tumor model

• Clone 21 potentiates anti-CD163 tumor inhibition in MC38 syngeneic model.

• Tumors were harvested two days post last dose.

21 alone and in combination with anti-PD-1 induced systemic dendritic cell activation and increased IFNγ expression in spleen T cells stimulated as well with PMA/Allophycocyanin.

• Increased inducible nitric oxide synthase (iNOS) expression, (NOS2) and T cell function in group treated with 21 in combination with anti-PD1.

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