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Antibodies to SIRPα enhance innate and adaptive immune responses to 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 neurons[1]. 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 inhibit this pathway through targeting CD47 have shown promising results in reducing tumor burden[2]. Unlike CD47 which is expressed ubiquitously, SIRPα expression is more restricted. Therefore, targeting SIRPα may result in differential safety and efficacy profiles versus that of CD47-targeted therapies.

Objectives of our study:

- Characterize anti-SIRPα clone 21 for binding to human, mouse and cynomolgus monkey SIRPα.
- Phagocytosis of target cells by prevalent alleles of human SIRPα (v1, v2) macrophages.
- Identify optimal IgG isotype for humanized 21 (h21).
- Evaluate clone 21 for anti-tumor activity in both xenograft and syngeneic tumor models and elucidate the mechanism driving efficacy.
- Evaluate h21 in cynomolgus monkeys for pharmacokinetics (PK), pharmacodynamics (PD) and tolerability

Methods

- ***Surface Plasmon Resonance (SPR)***. For K_D determination, SIRP analytes were injected in a "one-shot" kinetic mode and flowed over immobilized anti-SIRPα antibodies. Epitope binning was carried out using a classical sandwich approach[3].
- ***Humanization.*** Chicken variable regions of the light chain were grafted onto human lambda light chain IGVL3 frameworks.
- ***Cell binding on primary human, monkey and mouse monocytes***. Parent and humanized 21 were labeled with Alexa Fluor 647 and 1:4 titration of the antibodies starting at 500nM were incubated with human, cynomolgus PBMCs and mouse splenocytes. Monocytes in human and cynomolgus PBMCs were identified by $CD14^+CD16^-$ and in mouse splenocytes by $LY6C^+MHCII^-$.
- ***Phagocytosis***. *In vitro* monocyte-derived macrophages were polarized using M-CSF or human serum as previously described[4].
- ***Mixed Lymphocytes Reaction.*** Mature DCs cultured in CD47-Fc coated wells were generated from purified monocytes treated with IL-4 and GM-CSF and stimulated with LPS/IFNγ . DCs and T cells at a 1:5 ratio were incubated along with 10μg/mL of antibody.
- ***PBMC depletion.*** PBMCs were incubated with 10μg/mL of antibody. Cell viability was assessed with a cocktail of cell-specific antibodies two days post treatment.
- ***Tumor models and immunophenotyping.*** Raji and MC38 cells were implanted in NOD SCID and C57BL/6 mice. Harvesting and processing of tumors and spleen from MC38 tumor bearing mice were performed as previously described[4].
- ***Tolerability, Pharmacokinetics and Pharmacodynamics study in cynomolgus monkeys***. Antibodies were administered intravenously on days 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 a flow cytometry assay with labeled H21.

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.
- 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 monocytes.
- H21 induces phagocytosis by SIRPα homozygous and heterozygous v1 and v2 macrophages and potentiates the efficacy of anti-tumor and anti-PD-1 antibodies in xenograft and syngeneic models, respectively.
- Clone 21 in combination with anti-PD-1 induces DC activation, increases M1 tumor associated macrophages (TAMs) and T-cell effector function thus bridging innate and adaptive immunity in MC38 tumor-bearing mice.
- PK/PD profile of h21 in cynomolgus monkey showed typical antibody PK and complete SIRPα occupancy. H21 was well tolerated with no adverse signs observed in clinical evaluations, hematology and serum chemistry

References:

1. Barclay AN, Van den Berg TK (2014) *Annu Rev Immunol* 32:25–50.
2. Lakhani N, et al. (2018) *SITC*, Abstract P335.
3. Sim J, et al. (2019) *Keystone Symposia*, Abstract Poster 3022
4. Kauder SE, et al. (2018) *PLoS One* 13(8):e0201832.

Results

Immunization in chicken produced diverse high affinity anti-SIRPα antibodies that can potentiate macrophage phagocytosis

- A diverse panel of antibodies bind to human SIRPα v1 and v2 with high affinity ranging from low nanomolar to picomolar (K_D).
- A select number of clones (21, 119, 135, 136) bind similarly to human SIRPα alleles and cynomolgus SIRPα; in addition, clones 21 and 136 cross-react with mouse SIRPα alleles, enabling preclinical mouse studies.

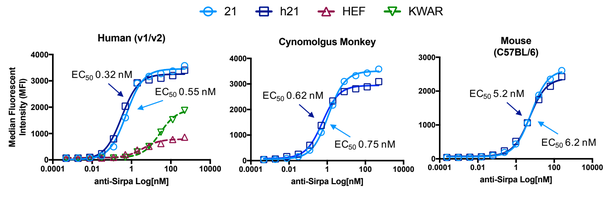
Bin	Antibody	Source	V1	V2	Cyno	NOD	BL6	BALB/c	SIRPγ	SIRPβ1	CD47 Blocking
1	119	H	1.83E-10	6.82E-11	1.12E-10	NB	NB	NB	2.67E-10	3.42E-10	Block
1	135	H	1.51E-10	2.90E-11	9.69E-11	NB	NB	NB	5.39E-10	1.10E-10	Block
1	21	S	<1.00E-12	<1.00E-12	3.61E-12	5.47E-10	4.60E-10	1.05E-09	<1.00E-12	<1.00E-12	Block
2	115	H	4.26E-10	1.86E-09	2.41E-09	NB	NB	NB	B	B	Kick-off
3	136	H	4.58E-10	1.63E-09	2.15E-09	5.54E-10	1.27E-08	3.50E-10	2.39E-08	4.35E-09	Non-block
4	3	W	1.62E-10	7.67E-11	2.29E-09	1.63E-09	3.65E-09	1.16E-09	8.36E-08	1.63E-09	Non-Block
4	173	H	9.37E-10	9.28E-09	4.46E-08	NB	NB	NB	NB	B	Non-Block
4	209	S	1.71E-10	5.01E-09	3.90E-08	NB	NB	NB	8.99E-09	B	Non-Block
4	213	S	6.05E-09	1.69E-09	4.49E-08	NB	NB	NB	2.02E-09	1.71E-08	Non-Block
5	123	H	6.05E-10	NB	1.62E-09	NB	NB	NB	7.47E-10	7.07E-08	Non-Block
5	149	H	8.73E-10	2.38E-10	7.64E-09	NB	NB	NB	1.89E-09	2.06E-10	Non-Block
5	161	H	1.03E-09	1.27E-10	6.35E-09	NB	NB	NB	2.84E-09	2.63E-09	Non-Block
5	162	H	4.50E-10	1.57E-08	1.26E-08	NB	NB	NB	3.97E-09	3.00E-09	Non-Block
5	194	H	4.97E-10	NB	9.11E-10	NB	NB	NB	9.47E-10	5.36E-08	Non-Block
5	218	S	1.23E-10	2.76E-10	5.99E-11	NB	NB	NB	6.36E-11	B	Non-Block
6	45	W	6.83E-11	1.34E-10	NB	NB	NB	NB	2.71E-08	1.06E-08	Non-Block

- All antibodies are screened as full IgG1 except 173, 209, 213, 123, 149, 161, 162, 194, 218 are screened as scFv-Fc
- NB = no binding; B = binding confirmed and exact K_D value was not determined
- W=wt, H=OmniChicken, S=SynVH chickens

H21 binds human, monkey and mouse SIRPα

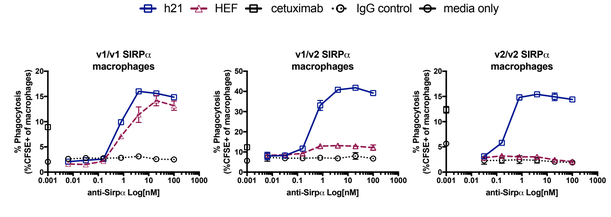
- H21 binds SIRPα across species and with similar affinity as parent clone 21.
- Similar binding profile of h21 and 21 on primary human, cynomolgus and mouse monocytes.
- On v1/v2 human monocytes, h21 binds with the lowest EC_{50} as compared to known anti-SIRPα antibodies.

K_D (M)	V1	V2	cynomolgus	mouse
21	<1.00E-12	<1.00E-12	3.61E-12	4.60E-10
h21	2.06E-12	4.60E-12	3.89E-12	9.25E-09
HEF	1.94E-09	NB	NB	NB
KWAR	5.23E-10	9.57E-09	5.25E-09	NB



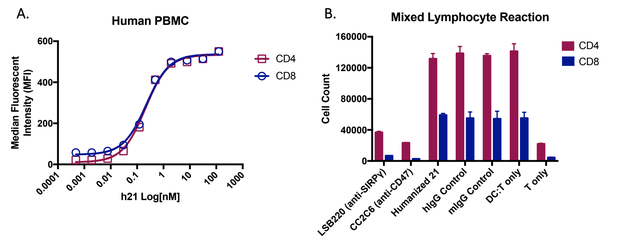
H21 binds v1 and v2 SIRPα alleles and induces phagocytosis

- H21 induces phagocytosis by homozygous v1 and v2 and heterozygous v1/v2 macrophages while allele specific anti-SIRPα antibody (HEF) fails to induce phagocytosis by v1/v2 and v2/v2 macrophages.



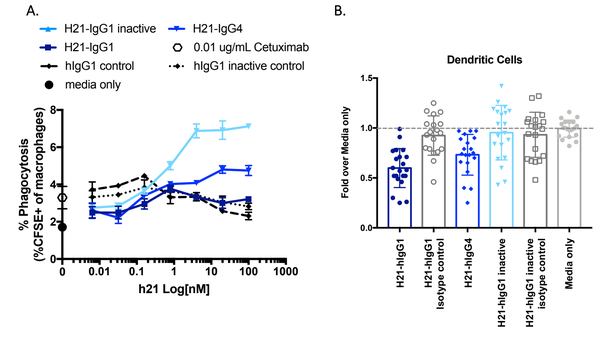
H21 binds SIRPγ but does not impact T-cell proliferation in mixed lymphocyte reaction

- H21 binds similarly to SIRPγ on primary human CD4 and CD8 T-cells (A).
- $CD4^+$ and $CD8^+$ T-cell proliferation in a mixed lymphocyte reaction with allogeneic monocyte derived dendritic cells are inhibited by specific anti- SIRPγ and anti-CD47 antibodies but not by h21 (B).



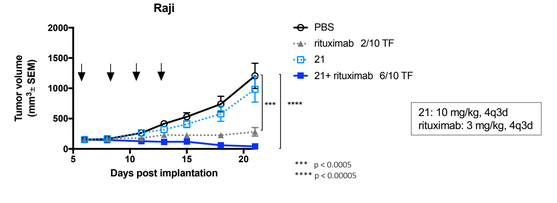
Reduced effector function is optimal for potentiating phagocytosis and minimizing Fc-mediated dendritic cell depletion

- H21 with reduced effector function potentiates cetuximab induced phagocytosis of DLD-1 cells, while IgG1 and IgG4 diminish the effect (A).
- H21 with effector function depletes dendritic cells ($HLADR^+Lin^-$) in PBMCs while h21 with inactive Fc function has no effect (B).
- H21 does not affect cell counts for B-cells, T-cells, NK cells and monocytes.



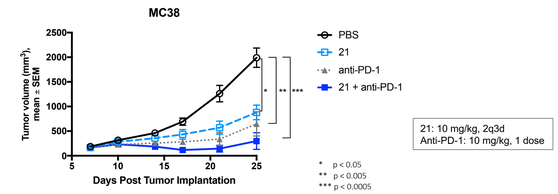
H21 enhances anti-tumor activity in combination with rituximab in the Raji xenograft model

- Combination of h21 with rituximab induced tumor regression. Treatment led to 6/10 and 2/10 tumor free mice in combination and rituximab only groups, respectively.

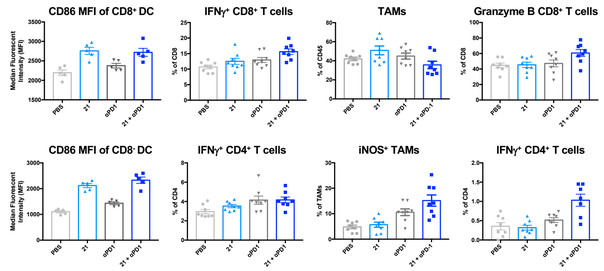


21 enhances anti-tumor activity in combination with anti-PD-1 in MC38 syngeneic tumor model

- Clone 21 potentiates anti-PD-1 tumor inhibition in MC38 syngeneic model



- Tissues were harvested two days post last dose.
- 21 alone and in combination with anti-PD-1 induced splenic dendritic cell activation and increased IFNγ expression in splenic T-cells stimulated *ex vivo* with PMA/ionomycin.
- Increased inducible nitric oxide synthase (iNOS) expressing TAMs (M1 phenotype) and T-cell function in group treated with 21 in combination with anti-PD-1.



H21 was well tolerated in cynomolgus monkeys with favorable PK and extended receptor occupancy

- H21 was administered on days 1 and 8 via intravenous injections at 10 and 30 mg/kg
- No test article related adverse signs were observed in clinical evaluations, hematology and serum chemistry.
- PK parameters show extended half-life expected of antibody and full target occupancy was observed on monocytes.
- No test article related changes were observed in B-cells, NK cells, T cells and monocytes for treated animals.