Anti-SIRPα Antibodies Stimulate Macrophage Phagocytosis to Cancer Cells in Both CD47-dependent and CD47-independent Manners

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BACKGROUND

Signal-regulatory protein alpha (SIRPα), is an inhibitory receptor expressed on myeloid cells and dendritic cells. Ligation of CD47 to SIRPα delivers a "don't eat me" signal to suppress phagocytosis. Tumor cells frequently overexpress CD47 to evade macrophage-mediated destruction. Currently, agents targeting CD47 have proceeded to clinical trials and demonstrated promising anti-tumor activity. However, these agents have been associated with hemolytic anemia and thrombocytopenia. In addition, universal expression of CD47 causes antigen sink, which leads to reduced efficacy of anti-CD47 antibody. We therefore consider targeting CD47 receptor, SIRPα, to achieve an improved efficacy with a better safety profile. We have developed 2 classes of anti-SIRPα antibodies: CD47-SIRPα interaction "blocker" and "non-blocker". Both groups of antibodies functionally stimulate phagocytosis of multiple cancer cell types by macrophages.

METHODS

Using SIRPα extracellular domain (ECD), SIRPα overexpression stable cell line and plasmid encoding SIRPα as immunogens, mice were immunized and anti-SIRPα antibodies were generated by hybridoma technology. Pan-allele/SIRP family homologue binding properties, and species cross-reactivity profile were evaluated by ELISA and FACS. *In vitro* function activity was determined by phagocytosis assay. In vivo safety profile was assessed in hCD47/hSIRPα double knock-in mice. Lead clone was humanized via CDR grafting and back mutation screening. Stress tests were carried out to evaluate the developability of candidate antibody.

RESULTS

Figure 1. Immunization in mouse produced diverse anti-SIRPα antibodies that can potentiate macrophage phagocytosis

Antibody	Cross Reactivity			Specificity				Blocking		Phagocytosis (MDM/Target cell)			Affinity (KD, M)		Binding Domain	Epitope Group
	Human	Cyno	Mouse	α V1	α V2	β	γ	hSIRPα /CD47	hSIRPγ /CD47	Jurkat	Raji	DLD1	α V1	α V2	IgV	
ES004-B1	+	+	-	+	+	+	-	+	-	+++	++	++	1.14E-08	2.06e-08	+	I-a
ES004-B2	+	+	-	+	+	+	-	+	-	+++	++	++	1.61E-08	2.83e-08	+	I-a
ES004-B3	+	+	-	+	+	+	-	+	-	+++	++	++	8.92E-09	1.47e-08	+	I-a
ES004-B4	+	+	weak	+	+	+	weak	+	-	+++	+++	++	1.11E-09	2.62e-09	+	I-b
Competitor 1	+	+	-	+	+	+	+	+	+	-	-	-	N/A	N/A	N/A	I-a
Competitor 2	+	+	-	+	+	+	+	+	+	weak	-	+	2.66E-09	1.47E-08	N/A	I-a
Competitor 3	+	-	-	+	-	+	weak	+	weak	-	-	-	N/A	N/A	N/A	I-c
ES004-N1	+	+	-	+	+	-	-	-	-	+	+	+	1.66E-08	2.85e-08	-	II
ES004-N2	+	+	-	+	+	+	-	-	-	++	+++	++	4.40E-09	2.52e-08	-	III
ES004-N3	+	+	-	+	+	+	weak	-	-	++	+++	++	5.90E-09	2.56e-08	-	IV
ES004-N4	+	+	-	+	+	+	weak	-	-	+++	+++	++	3.07E-09	4.72e-09	-	IV
ES004-B5	+	-	-	+	-	+	-	weak	-	+++	++	+	1.49E-09	N/A	+	V

RESULTS

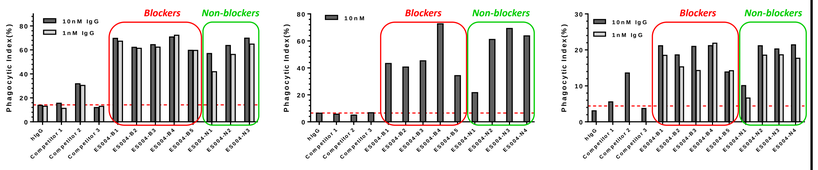
Figure 2. ES004-B4 and ES004-N2 bind to unique epitopes

Coating (0.1ug)	Competitors (20ug), % Inhibition											
	ES004-B1	ES004-B3	ES004-B2	Competitor 2	Competitor 3	Competitor 1	ES004-B4	ES004-N1	ES004-N2	ES004-N3	ES004-N4	ES004-B5
ES004-B1	90	96	95	83	36	92	96	4	5	-19	5	19
ES004-B3	94	94	93	69	14	83	91	3	6	1	4	7
ES004-B2	94	92	90	71	7	87	93	-16	-10	-5	-13	-10
Competitor 2	85	82	49	95	27	54	4	1	1	6	5	5
Competitor 3	89	91	81	94	68	70	51	1	2	4	4	7
Competitor 1	97	97	94	93	25	93	98	0	4	8	7	10
ES004-B4	92	89	86	-6	2	50	93	-1	6	-1	-3	-1
ES004-N1	-3	-1	-8	45	-10	14	8	91	3	2	7	8
ES004-N2	33	34	14	-14	-14	-19	10	-17	93	-5	7	-1
ES004-N3	2	4	6	1	1	2	0	1	-1	81	79	26
ES004-N4	2	8	3	0	-14	-6	-4	-16	2	79	83	17
ES004-B5	0	4	1	-4	-3	-5	3	7	-1	10	14	91

Bin I: blocker; Bin II: non-blocker; Bin III: non-blocker; Bin IV: non-blocker; Bin V: blocker

✓ ES004 lead antibodies bind to different epitopes compared to competitor antibodies

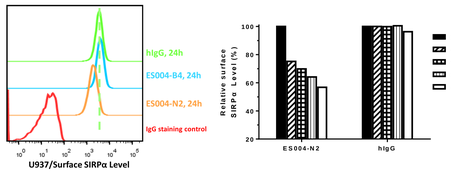
Figure 3. ES004-B4 and ES004-N2 induce potent phagocytosis



✓ ES004 lead antibodies induced strong macrophage phagocytosis against multiple tumor cell lines

✓ Competitor anti-SIPRα antibodies did not induce (or induce very weak) phagocytosis

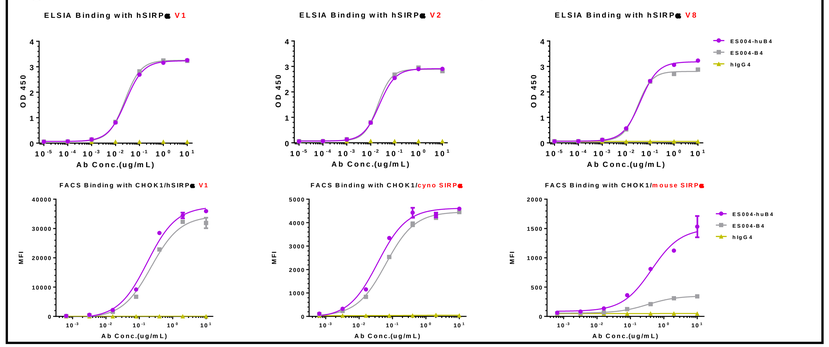
Figure 4. ES004-N2 but not ES004-B4 down-regulates cell surface SIRPα level



✓ Incubation of ES004-N2 but not ES004-B4 led to down-regulation of U937 surface SIRPα level

✓ ES004-N2 mediated down-regulation of cell surface SIRPα level is time dependent

Figure 5. Humanized ES004-huB4 binds human, monkey and mouse SIRPα



RESULTS

Antibody	Affinity (KD, M)		
	hSIRPα V1	hSIRPα V2	C57BL/6
ES004-huB4	8.60E-10	1.43E-09	2.59E-08
ES004-B4	1.89E-09	1.39E-09	5.27E-06

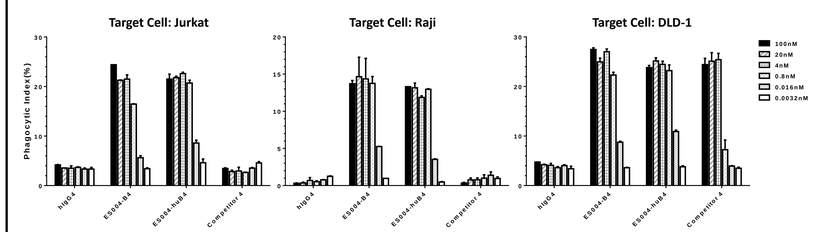
ES004-huB4 binds

✓ human v1, v2 and v8 SIRPα alleles

✓ Cyno SIRPα

✓ Mouse SIRPα (C57BL/6)

Figure 6. ES004-huB4 induces potent phagocytosis

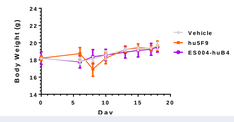


✓ ES004-huB4 induced strong macrophage phagocytosis against multiple tumor cell lines

✓ Competitor anti-SIPRα antibody 4 did not induce phagocytosis against Jurkat and Raji cells

Figure 7. ES004-huB4 shows good safety profile in hSIRPα/hCD47 Double KI Mice

Day	0	6	7	8	9	10	11	12	13	14	15	16	17	18
Administration		√		√		√								
Routine Blood Test	√		√				√							√
Weight	√	√		√		√			√		√		√	√



Group	Treatment	Dosage	Adverse Effects (N=3)		
			Erythropenia	Thrombocytopenia	Weight loss (>10%)
1	Vehicle	N/A	0	0	0
2	hu5F9 analogue	10mg/kg, Q2d x3	3(9%, 19%, 36%) after the 3rd administration, recovered when observation ended	2(23%, 52%) after the 1st administration, recovered when observation ended	2 after the 1st administration, recovered when observation ended
3	ES004-huB4	10mg/kg, Q2d x3	1(24%) after the 3rd administration, recovered when observation ended	1(34%) after the 1st administration, recovered when observation ended	0

✓ ES004-huB4 demonstrated lower anemia and thrombocytopenia risks compared to anti-CD47 antibody hu5F9 analogue

CONSCLUSIONS

In summary, we have developed 2 anti-SIRPα antibodies with "Best-in-Class" potential: 1) CD47/SIRPα interaction "Blocker" ES004-B4, 2) CD47/SIRPα interaction "Non-Blocker" ES004-N2. Both antibodies greatly enhance macrophage-mediated tumor cell destruction, likely through different mechanisms of action. The "Non-Blocker" ES004-N2 may induce macrophage phagocytosis partially via down-regulation of cell surface SIRPα level. We are currently advancing the development of ES004-B4 and ES004-N2 into clinical candidates.