Generation of a Mutant SIRPα Fusion Protein with Highly-improved Affinity and Favorable Safety Profile

Abstract # 6357

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Introduction

CD47 has been validated to be expressed on various tumor types including acute myeloid leukemia, myelodysplastic syndrome and other hematologic or solid tumors. As primary 'don't eat me' signal, high expression of CD47 on tumor cells interacts with SIRPα negatively regulating the phagocytosis level.

However, poor safety profile as well as insufficient combinatory effect remain to be the issues limiting the clinical outcomes of CD47-targeting molecules. Herein, we used rational design strategies to generate SCR9168 with potent affinity improvement, and maintained favorable safety profiles in different animal species, including mice and cynomolgus monkeys.

Methods and Materials

Mutagenesis was introduced to the residues critical for CD47/SIRPα binding interface. Affinity was measured by surface plasmon resonance (SPR) analysis. *In vitro* activities were determined by biochemical- or cell-based binding, blocking and antibody-dependent cellular phagocytosis (ADCP) assays. The efficacy *in vivo* was assessed with the OE19 xenograft model. Pharmacokinetics (PK) and safety profiles were monitored in both mice and cynomolgus monkeys.

Results

The affinity of SCR9168 achieves 178 pM for human CD47 which is nearly 50-fold increase in comparison with wild-type SIRPα. Enhanced affinity has also been confirmed in binding to OE19 or DLD1 cell lines, as well as in blocking human SIRPα binding to Raji cells.

SCR9168 remains strong binding potency to monkey, mouse or rat CD47 which allows the PK and safety assessment using these animal species. The combined effects of SCR9168 and multiple therapeutic antibodies targeting tumor-associated antigens (TAAs) have been evaluated with ADCP assays using human monocyte-derived macrophages. SCR9168 markedly increases the ratio of phagocytic cells combining with antibodies, such as cetuximab. However, due to the inert function of Fc fragment, no phagocytosis of red blood cells or platelets was caused after the incubation *in vitro*.

In addition, SCR9168 treatment in combination with trastuzumab leads to significantly improved suppression of tumor growth in a dose-dependent manner. Moreover, administration of two doses at day 1 and day 11 in cynomolgus monkeys results in no toxicity events related to SCR9168 based on the data from hematological analysis.

Highly Improved Affinity to Human CD47

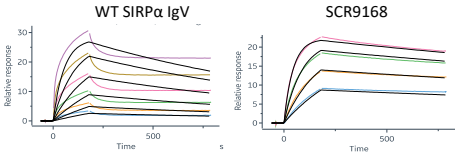

Figure 1. Curve-fitting for SPR results. SCR9168 was generated from engineered of human SIRPα IgV domain fused with inert human IgG Fc fragment. SPR was applied to define the affinity of this mutant SIRPα protein. 178 pM indicated a significant increase in human CD47 binding affinity.

Table 1. Highly Improved Affinity to Human CD47.

	ka (1/Ms)	kd (1/s)	KD (M)
WT SIRPα V1	2.22E+05	1.63E-03	7.34E-09
ALX148	1.52E+06	1.39E-04	9.16E-11
SCR9168	1.47E+06	2.62E-04	1.78E-10

Potent Binding or Blocking Activity Determined at Biochemical or Cell Level

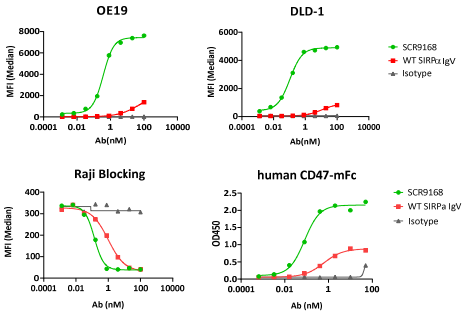

Figure 2. (Upper) Flow cytometry analysis of SCR9168 binding for the human tumor cell lines with endogenous CD47 expression. (Lower left) Flow cytometry analysis of SCR9168 blocking the interaction between wild-type SIRPα and Raji cells. (Lower right) Biochemical analysis of SCR9168 binding to the recombinant CD47 protein.

Cross-reactivity to Cyno or Mouse CD47

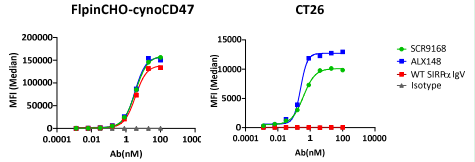

Figure 3. Flow cytometry analysis of SCR9168 binding for the recombinant cyno CD47 cell line or mouse tumor cell line.

Promote ADCP Level without Inducing the Phagocytosis of either RBC or Platelet

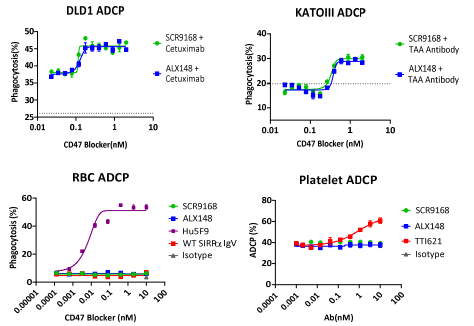

Figure 4. The percentage of macrophages engulfed with cells was shown in the figures. (Upper) Fixed dose of TAA antibodies and mutant SIRPα were applied. (Lower) No obvious phagocytosis of red blood cells or platelets was observed after SCR9168 treatment.

Strong Tumor Inhibition Effect Combining with Trastuzumab in a Dose-dependent Manner

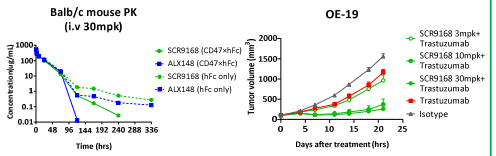

Figure 5. (Left) PK study was carried out in Balb/c mice. (Right) OE19 tumor cells were implanted to NOD-SCID mice. Mutant SIRPα in combination with trastuzumab showed tumor inhibition effect in a dose-dependent manner.

PK Profile in Cynomolgus Monkeys with No Obvious Safety Issue

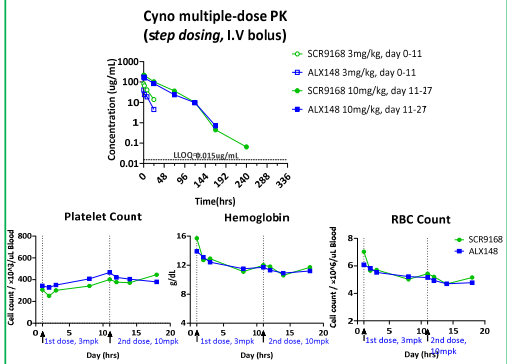

Figure 6. As mutant SIRPα proteins retain the cross-reactivity to cyno CD47, PK and potential toxicity studies were carried out in cynomolgus monkeys. The monkeys were treated with 3 mpk at Day 1 and 10 mpk at Day 11, respectively. The nonlinear serum concentration is correlated with CD47 antigen sink. The serum exposure of ALX148 was undetectable at Day 8 with 10 mpk, while the exposure of SCR9168 remained detectable at Day 10. No toxicity or adverse events related to drugs were found in observation, body weight as well as hematological analysis of red blood cell, platelet and hemoglobin levels.

Table 2. Serum Exposure of Multi-dose Treatments in Cynomolgus Monkeys .

Dosage	Cpd	T1/2 (h)	Tmax (h)	Cmax (ug/mL)	AUClast (h*ug/mL)	Cl_obs (mL/hr/kg)
3 mpk	ALX148	8.79	0.25	40.64	380.86	6.85
	SCR9168	9.83	0.25	80.39	900.40	2.74
10 mpk	ALX148	22.91	0.25	172.12	6638.58	1.50
	SCR9168	20.17	0.25	215.12	8527.32	1.17

Conclusions

SCR9168 demonstrates the best-in-class potential among SIRPα mutein molecules. It elicits improved and dose-dependent efficacy in phagocytosis or tumor suppression combining with therapeutic antibodies, such as trastuzumab or cetuximab. Favorable safety profile with no phagocytosis of RBC or platelets *in vitro* as well as no hematological toxicity observation in cynomolgus monkeys allows broader dose range exploration in early clinical phase.

SCR9168 is currently in development stage and IND enabling will be expected in the end of 2023.