BSI-082. a Fully Human Anti-SIRPα Antibody with Both High Specificity and Broad Coverage of SIRPα Variant Populations

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Abstract

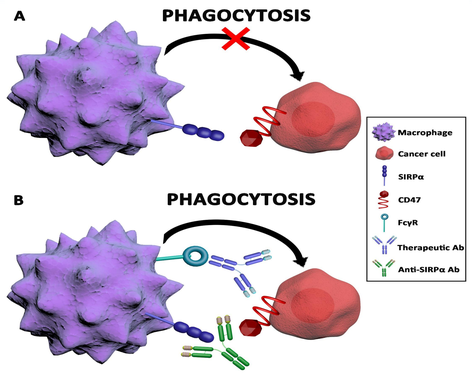
Background: SIRPα is an inhibitory immunoreceptor, expressed on macrophages that interacts with CD47 expressed on tumor cells to release a "don't eat me" signal, resulting in immune evasion of tumor cells. Antibodies targeting CD47 or SIRPα are expected to block the CD47/SIRPα signaling pathway, thereby stimulating macrophage-based phagocytosis of tumor cells. Anti-CD47 antibodies usually have side effects related to anemia and thrombocytopenia since CD47 is expressed on normal cells, including red blood cells and platelets. Targeting SIRPα on the macrophage is an alternative approach to block CD47/SIRPα signaling without the side effects observed in anti-CD47 antibody treatment. In addition to SIRPα there are two other members of the SIRP family – SIRPβ and SIRPγ. These isoforms share high sequence homology with SIRPα but have very different functionality. Moreover, SIRPα is polymorphic in humans, among which SIRPαV1, SIRPαV2 and SIRPαV8 are the main SIRPα variants in diverse ethnic groups. Together, the key challenge of anti-SIRPα antibody discovery is to identify lead candidates that specifically bind to SIRPα and block the SIRPα/CD47 interaction while also recognizing the majority of SIRPα variants.

Experimental procedures: Humanized mice were used for immunization and the proprietary H^3 (High-throughput, High-content and High-efficiency) antibody screening platform was used to identify a lead candidate - BSI-082. Affinity, specificity and CD47/SIRPα blocking activity were evaluated by SPR, ELISA and FACS. Functional activity of antibodies was investigated using monocyte-derived macrophages and Raji cells in the presence of Rituximab with and without anti-SIRPα antibody. Antibody internalization was evaluated by using the Biosion SynTracer™ antibody discovery platform. Anti-tumor activity of BSI-082 was conducted in combination with rituximab in immunodeficient transgenic mice expressing human SIRPα (B-NDG/hSIRPα) that grafted with B-luciferase-GFP Raji cells.

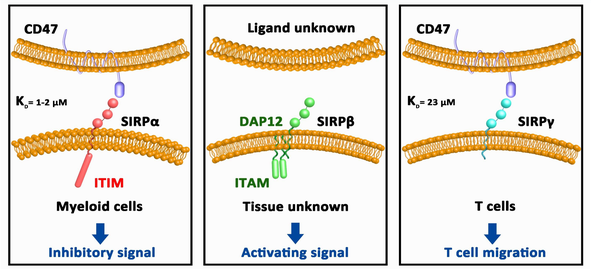
Results: BSI-082 is a fully human IgG1κ mAb that binds to SIRPα and blocks the SIRPα/CD47-mediated signaling pathway. BSI-082 is a highly selective SIRPα binder with weak binding to SIRPβ and no binding to SIRPγ. In addition, BSI-082 binds three main SIRPα variants (V1, V2 and V8) with high affinity. Through antibody engineering, BSI-082 harbors an engineered Fc domain that prevents FcγR binding and enhances FcRn binding, thus significantly reducing ADCC while also extending half-life. In the presence of rituximab, BSI-082 promotes macrophage-mediated phagocytosis. BSI-082 exhibits effective internalization, which would deplete SIRPα on macrophages and enhance immune response. BSI-082 significantly inhibits tumor growth *in vivo* when used in combination with rituximab.

Conclusion: BSI-082 exhibits best-in-class biophysical properties, SIRPα specificity, broad SIRPa variant binding and superior functional characteristics, supporting the initiation of development activities including manufacturing and IND-enabling studies.

Targeting SIRPα/CD47 Axis in Immunotherapy

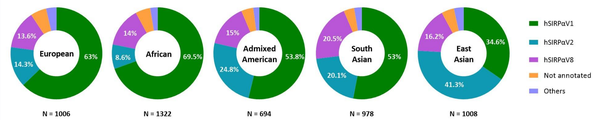


The Human SIRP Family



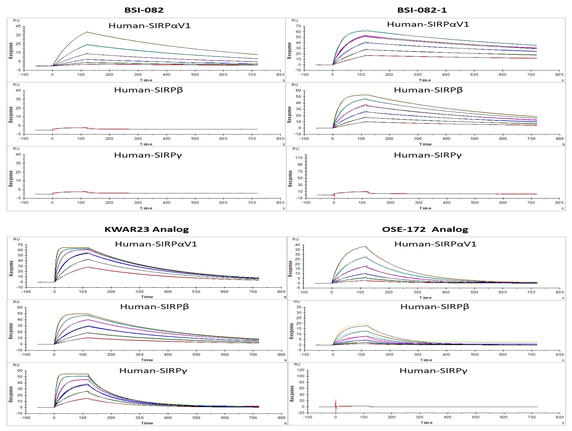
Proteins	Identity	Proteins	Identity	Proteins	Identity
SIRPα-ECD & SIRPβ-ECD	84.8%	SIRPα-ECD & SIRPγ-ECD	80.1%	SIRPβ-ECD & SIRPγ-ECD	78.3%

The Main SIRPα Variants in Human

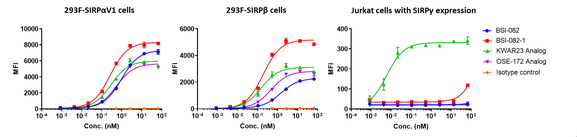


Proteins	Identity	Proteins	Identity	Proteins	Identity
SIRPαV1 & SIRPαV2	89.6%	SIRPαV1 & SIRPαV8	93.1%	SIRPαV2 & SIRPαV8	95.7%

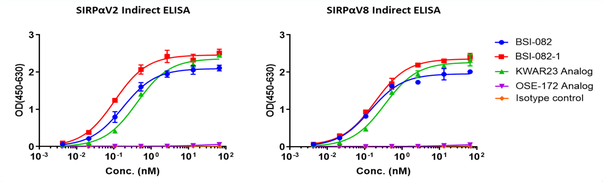
Evaluation of Antibodies Binding to SIRP Family Members



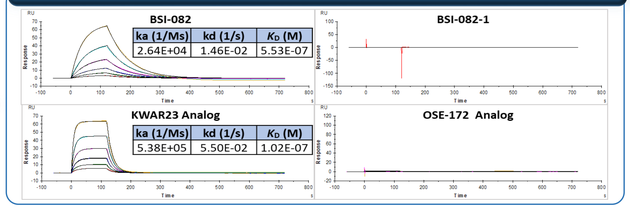
Antibody	Human SIRPαV1			Human SIRPβ			Human SIRPγ		
	ka (1/Ms)	kd (1/s)	K_D (M)	ka (1/Ms)	kd (1/s)	K_D (M)	ka (1/Ms)	kd (1/s)	K_D (M)
BSI-082	1.29E+05	1.88E-03	1.46E-08	No binding			No binding		
BSI-082-1	1.28E+06	1.05E-03	8.24E-10	7.13E+05	2.00E-03	2.81E-09	No binding		
KWAR23 Analog	1.26E+06	3.72E-03	2.94E-09	9.35E+05	2.98E-03	3.18E-09	6.42E+06	1.56E-02	2.43E-09
OSE-172 Analog	2.90E+05	7.75E-03	2.67E-08	3.04E+05	9.28E-03	3.05E-08	No binding		



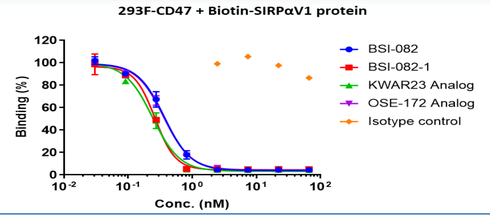
Evaluation of Antibodies Binding to the Main SIRPα Variants



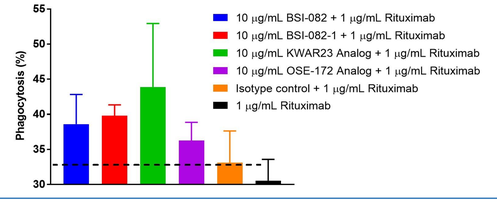
Evaluation of Antibodies Binding to Monkey SIRPα



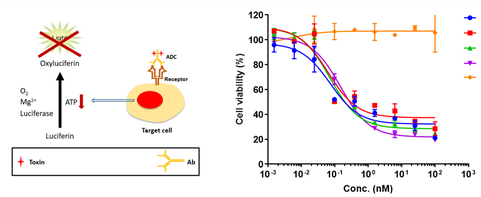
Antibodies Block SIRPα and CD47 Interaction



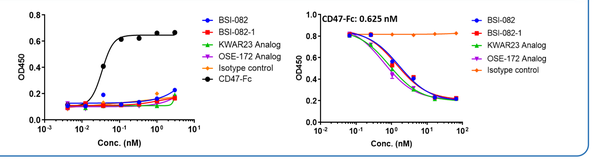
Antibodies Enhance Macrophage-Mediated Phagocytosis of Raji Cells



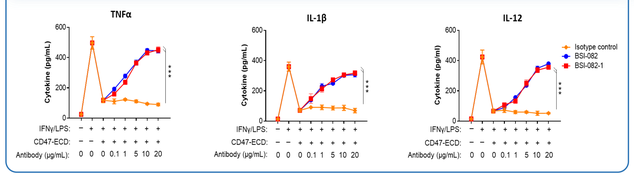
Evaluation of Endocytosis of Antibodies



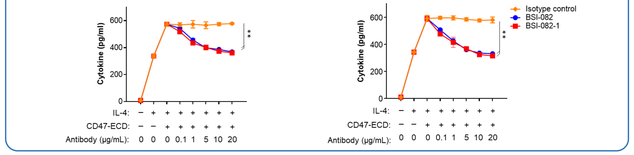
Antibodies Antagonize CD47-Mediated Phosphorylation of SIRPα in THP1 Cells



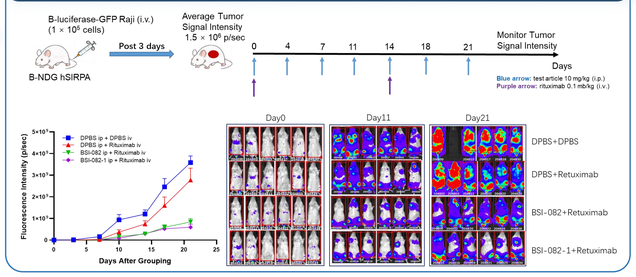
BSI-082 and BSI-082-1 Increase Cytokines Production under M1 Activation in the Presence of CD47



BSI-082 and BSI-082-1 Decrease Cytokine Production under M2 Condition in the Presence of CD47



BSI-082 and BSI-082-1 Enhance Anti-Tumor Activity of Rituximab *in vivo*



Summary

Drug		BSI-082	BSI-082-1	KWAR23 Analog	OSE-172 Analog
Affinity	SIRPαV1 Binding (K_D)	1.46E-08 M	8.24E-10 M	2.94E-09 M	2.67E-08 M
Selectivity	SIRPβ Reactivity	No/Low	Yes	Yes	Yes
	SIRPγ Reactivity	No	No	Yes	No
Variant Binding	SIRPαV2	Yes	Yes	Yes	No
	SIRPαV8	Yes	Yes	Yes	No
Cross-reactivity	Monkey SIRPα	No	Yes	Yes	No
Functional Activity	SIRPα/CD47 Blocking	Yes	Yes	Yes	Yes
	Antagonistic Activity	Yes	Yes	Yes	Yes
	Endocytosis	Yes	Yes	Yes	Yes
	Anti-tumor Activity	Yes	Yes	/	/

Note: Red – favorable characteristics