BSI-510, a Novel Bispecific Molecule Combining an Anti-Siglec-15 Antibody with Cytokine GM-CSF for Enhanced Antitumor Efficacy

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Abstract

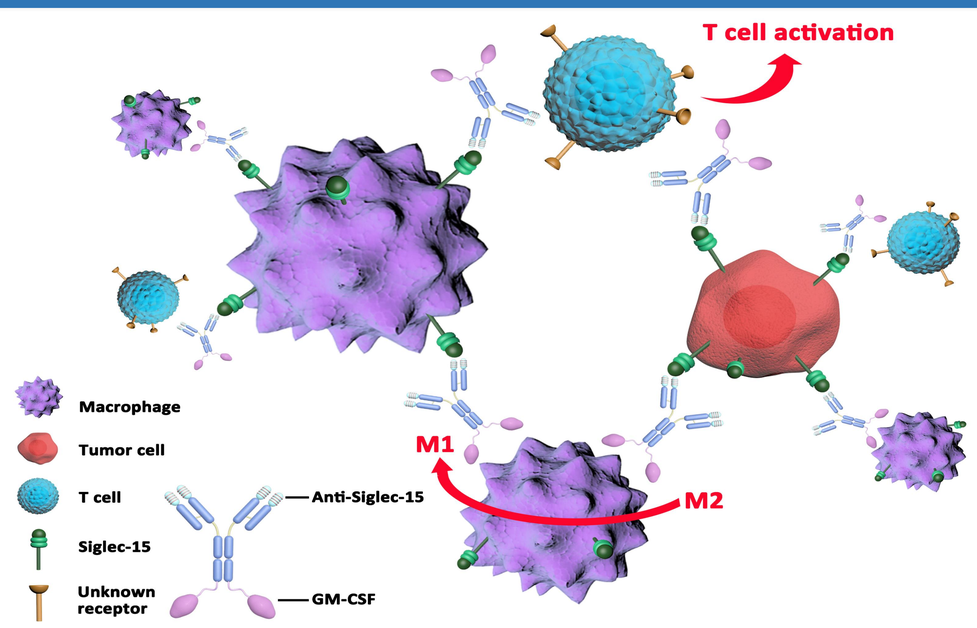
Background: Siglec-15 has increased expression levels not only in solid tumors but also in tumor associated macrophages. It is an immune suppressive molecule that inhibits T cell activity in the tumor microenvironment (TME). Siglec-15 blockade can increase T cell proliferation and activity. In the TME there is an accumulation of tumor suppressed M2 macrophages; thus, therapies that can convert M2 to M1 macrophages can enhance tumor killing. GM-CSF is a key cytokine that induces differentiation of pro-tumor M2 macrophages into anti-tumor M1 macrophages. Here we report the development of a novel bispecific fusion molecule, composed of a newly discovered anti-Siglec-15 antibody fused with GM-CSF. BSI-510 is expected to target Siglec-15 on M2 macrophages and to reprogram M2 to M1 through GM-CSF stimulation, thus boosting antitumor efficacy through activating both T cells and macrophages.

Methods: An anti-Siglec-15 mAb was identified from humanized mice immunized with recombinant Siglec-15-ECD-Fc and screened by our proprietary H^3 (High-throughput, High-content and High-efficiency) platform. Human GM-CSF was fused to the C-terminus of the anti-Siglec-15 antibody via a flexible linker. The affinities of the fusion molecule to Siglec-15 and GM-CSFR were determined by SPR. The binding of the molecule to Siglec-15 was further confirmed by ELISA and FACS. *Ex vivo* T cells proliferation assay was used to evaluate the function of anti-Siglec-15 antibody portion of the molecule, and TF-1 proliferation assay and macrophage polarization assay were used to evaluate the function of GM-CSF part. MC38-Siglec15 and MC38-mSiglec15 syngeneic murine models were used to evaluate the tumor inhibition activity of a surrogate bispecific fusion molecule (anti-Siglec15 x mouse GM-CSF).

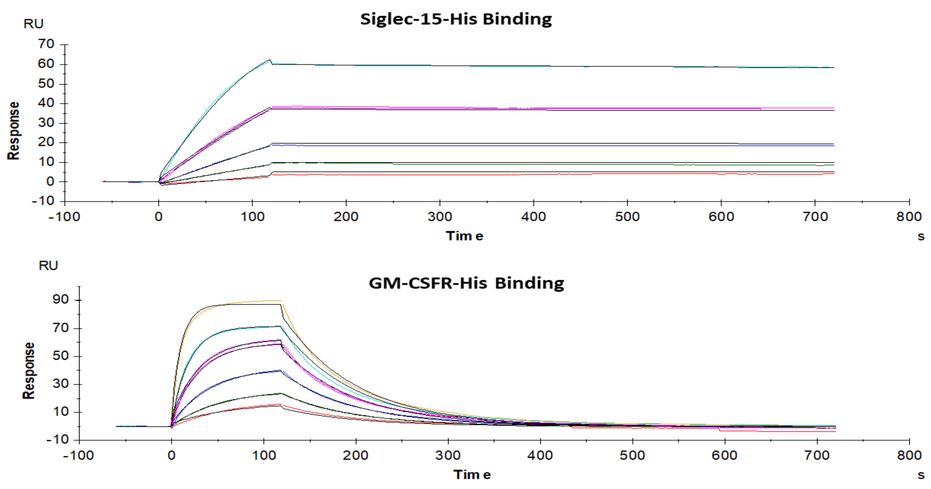
Results: BSI-510 demonstrated comparable potency to the parental anti-Siglec-15 antibody regarding Siglec-15 binding and T cell proliferation. It also exhibited comparable potency to recombinant protein GM-CSF in GM-CSFR binding and TF-1 proliferation. In addition, treatment with BSI-510 induced a significant shift from M2 to M1 macrophages, whereas anti-Siglec-15 alone did not show any repolarization activity. A BSI-510 surrogate (anti-Siglec-15 x mouse GM-CSF) showed superior antitumor efficacy in syngeneic murine models with a shift of M2 macrophages to M1. Expression of Siglec-15 in M2 macrophages was confirmed in human tumor samples.

Conclusions: BSI-510 is a first-in-class anti-Siglec-15 x GM-CSF bispecific fusion molecule combining simultaneous reversal of T cell inhibition and M2/M1 macrophage repolarization. BSI-510 demonstrates favorable biophysical and functional characteristics, supporting the initiation of development activities including manufacturing and IND-enabling studies.

MOA of Anti-Siglec-15 x GM-CSF Bispecific Molecule

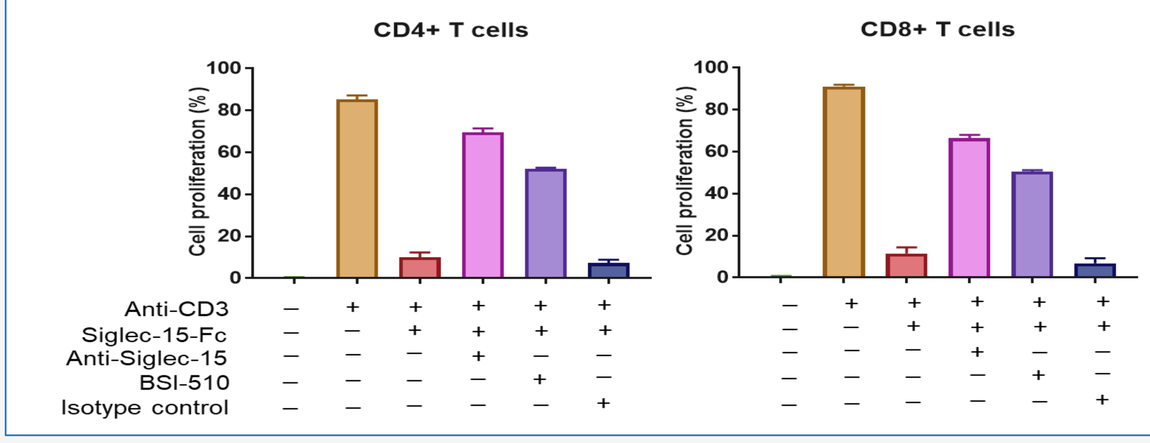


BSI-510 Exhibits High Binding Affinity to both Siglec-15 and GM-CSFR

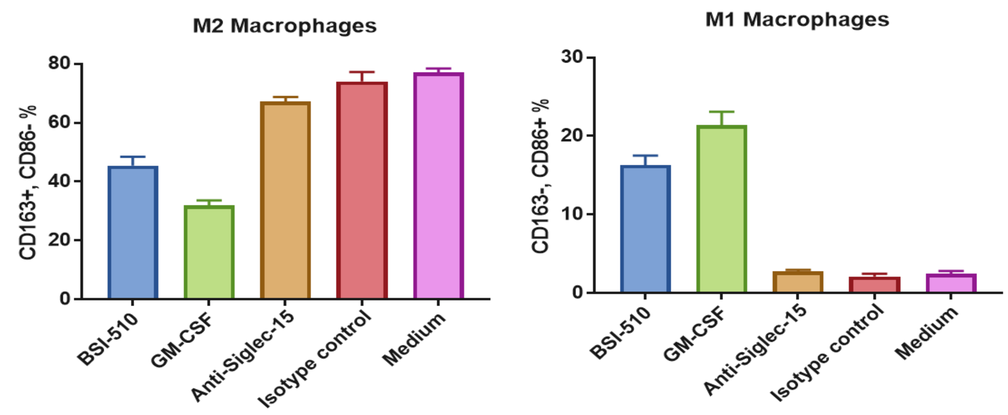


Antibody	Antigen: Siglec-15-His			Antigen: GM-CSFR-His		
	ka (1/Ms)	kd (1/s)	K_D (M)	ka (1/Ms)	kd (1/s)	K_D (M)
BSI-510	4.15E+06	6.09E-05	**1.47E-11**	1.02E+06	1.24E-02	**1.23E-08**

BSI-510 Reverses Siglec-15-Mediated T Cell Suppression

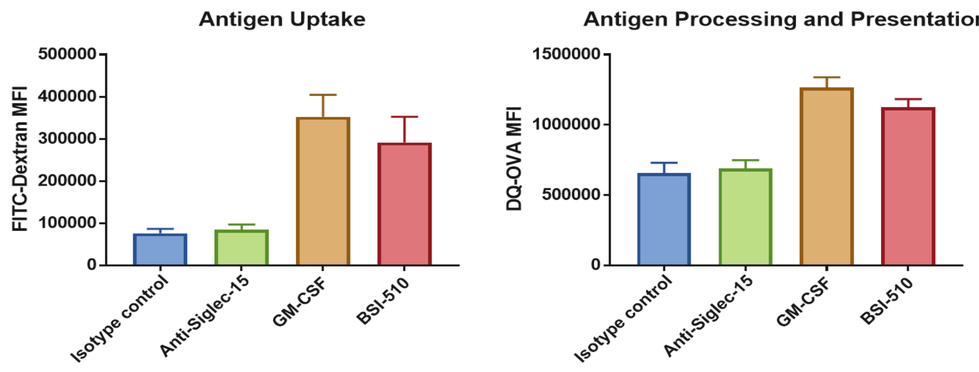


BSI-510 Reprograms M2 to M1 Macrophages



CD14+ cells were isolated from human PBMCs, induced with M-CSF for 6 days, and then incubated with the testing articles for 3 days. CD163 and CD86 expression was determined FACS.

BSI-510 Promotes Antigen Uptake, Processing and Presentation



Human PBMCs-derived macrophages from a healthy donor were cultured with M-CSF for 6 days, and then incubated with testing articles for 3 days. The cells were mixed with FITC-Dextran or DQ-OVA and evaluated by FACS.

Anti-Siglec-15 x mouse GM-CSF Shows Antitumor Efficacy