SBT6290, a Systemically Administered Nectin4-Directed TLR8 ImmunoTAC Therapeutic, is a Potent Human Myeloid Cell Agonist For the Treatment of Nectin4-Expressing Tumors

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Introduction

SBT6290 is a product candidate comprised of a selective TLR8 agonist conjugated to a Nectin4-specific monoclonal antibody, designed for systemic delivery and tumor-localized activation of myeloid cells. Nectin4 is a cell surface adhesion molecule that is overexpressed in multiple solid tumor types including bladder, triple negative breast, head and neck, and non-small cell lung cancers, with limited expression in normal tissues. Many solid tumors, including those expressing Nectin4, are resistant to immunotherapy due to immune-suppressive mechanisms, loss of HLA, low neoantigen availability, and/or minimal T cell infiltrates. These tumors, however, are often replete with myeloid cells. Activation of these cells has emerged as a promising approach in overcoming resistance mechanisms to current cancer immunotherapies. TLR8 is highly expressed in myeloid cell types prevalent in human tumors, including conventional DCs and macrophages. Agonism of TLR8 in human myeloid cells activates a broad spectrum of anti-tumor immune mechanisms, including proinflammatory cytokine production, repolarization of suppressive myeloid cells, and the priming of CTL responses.

Here we present preclinical data supporting the continued development of SBT6290 for Nectin4-expressing tumors.

- SBT6290 activates multiple-tumor immune mechanisms in *in vitro* studies.
- SBT6290 activity is dependent upon Nectin4 expression on tumor cells and the engagement of Fc gamma receptors on the surface of myeloid cells.
- A mouse surrogate of SBT6290 (SBT6290-S) confers single agent anti-tumor activity in a Nectin4-expressing mouse tumor model.

Figure 1: SBT6290 is Comprised of a TLR8 Agonist Conjugated to a Nectin4-Specific Monoclonal Antibody Designed for Tumor-Localized Activity

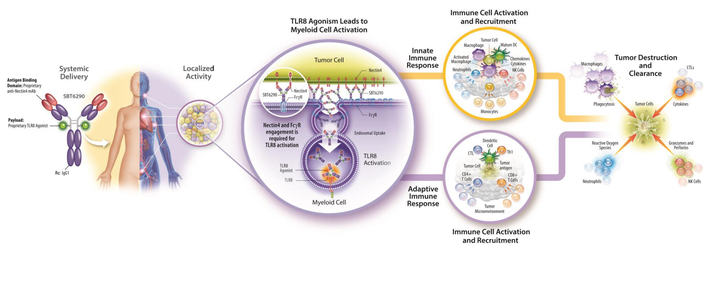


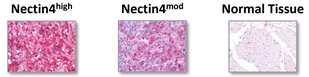
Table 1: Human Myeloid Cell-Restricted Expression Profile Supports Development of a TLR8-Selective Payload

	Cell Type	TLR4	TLR7	TLR8	TLR9	STING	RIG-I
Myeloid Cells	Dendritic Cells	+++	+/-	++++	-	++	++
	Macrophages	++++	+	+++	-	++	++
Non-Myeloid	Fibroblasts	++	++	-	-	+++	+++
	Endothelial Cells	+++	++	-	-	+	++
Tumor	Nectin4+ Tumor Cells	-	-	-	-	++	++

Table 1: Expression levels were determined using publicly available RNA-Seq datasets

Figure 2: Multiple Solid Tumors Overexpress Nectin4 and Display Substantial Myeloid Infiltrate

A) Nectin4 Expression On Tumors and Normal Tissue



B) Frequency of Nectin4 Overexpression in Select Solid Tumor Indications

Disease Indication	Nectin4 Overexpression (% patients)
Bladder	60-83
Breast	53-78
HNSCC	18-59
NSCLC	27-55

C) Solid Tumor Indications That Overexpress Nectin4 are Infiltrated by Myeloid Cells

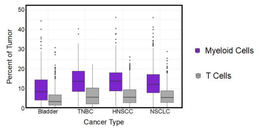


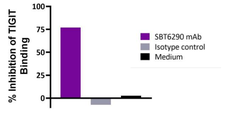
Figure 2: **A)** Representative images of Nectin4 expression in tumor and normal tissue as detected by IHC; bladder carcinoma and normal bladder tissue are shown. **B)** as described in Challita-Eid et al. Cancer Res. 2016 May 15;76(10):3003-13. **C)** Percent of tumor comprised of myeloid cells and T cells for bladder, triple negative breast cancer (TNBC), head and neck squamous cell carcinoma (HNSCC) and non-small cell lung cancer (NSCLC) as Thorsson, *V., et al.* (2018), The Immune Landscape of Cancer. Immunity 48(4), 812 - 830.e14.

Figure 3: SBT6290 Displays Selective Binding to Nectin4 and Blocking of the TIGIT:Nectin4 Interaction

A) The SBT6290 Binding Domain is Selective for Nectin4

Nectin Family Members	Binding Affinity
Nectin4	<1 pM
Nectin1	No binding detected; affinity is >1μM
Nectin2	
Nectin3	

B) SBT6290 Binding Domain Blocks TIGIT Binding to Nectin4-Expressing Tumor Cells



C) SBT6290 Binds with High Affinity to Tumor Cells Expressing Nectin4

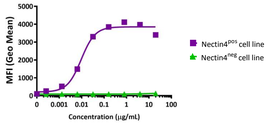
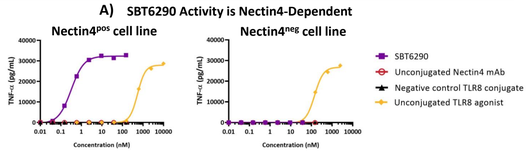


Figure 3: **A)** Binding affinities were analyzed by the Octet system. **B)** The binding of TIGIT-Fc to Nectin4-expressing tumor cells was blocked by the binding domain of SBT6290. Nectin4 was recently described to be a ligand for TIGIT (Reches et al., J Immunother Cancer. 2020; 8(1): e000266). **C)** Binding was detected by flow cytometry.

Figure 4: SBT6290 Activates Human Myeloid Cells in a Nectin4 and Fc-Dependent Manner

A) SBT6290 Activity is Nectin4-Dependent



B) SBT6290 Activity Requires Fc Engagement

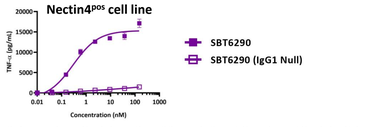
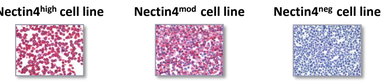


Figure 4: **A)** PBMCs were co-cultured with Nectin4-expressing or Nectin4 negative cell lines as indicated. Unconjugated Nectin4 mAb is the unconjugated monoclonal antibody utilized in SBT6290. Negative control TLR8 conjugate is a conjugate incorporating an irrelevant monoclonal antibody conjugated to the TLR8 agonist utilized in SBT6290. Unconjugated TLR8 agonist is the unconjugated TLR8 agonist used in SBT6290. **B)** A Nectin4-expressing tumor cell line was co-cultured with PBMCs in the presence of indicated conjugates. SBT6290 (IgG1 Null) contains the mutations L234A, L235A, G237A and K322A in the IgG1 Fc domain, inhibiting binding to Fc gamma receptors.

Figure 5: Nectin4 Expression Levels Observed in Tumors Support SBT6290 Activity

A) Cell Lines with Nectin4 Overexpression That Correlates with That Found in Human Tumors



B) SBT6290 Has Similar Activity in Settings of Moderate and High Nectin4 Expression

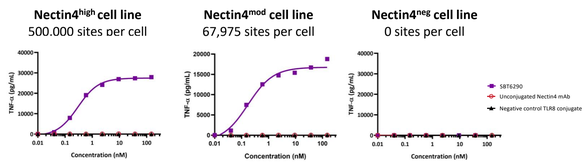


Figure 5: **A)** Nectin4 expression on cell lines corresponds to the expression detected on tumor samples shown in Figure 2A. **B)** Cell lines used in Figure 5A were co-cultured with PBMCs in the presence of the indicated conjugates or control antibody and TNF-α was measured.

Figure 6: SBT6290 Induces a Broad Spectrum of Anti-Tumor Immune Mechanisms in a Nectin4-Dependent Manner

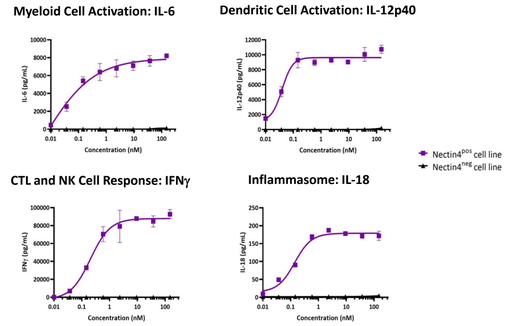


Figure 6: PBMCs were co-cultured with the indicated cells in the presence of SBT6290.

Figure 7: Nectin4-Directed TLR8 Agonism is Equipotent on Human Macrophage Subtypes

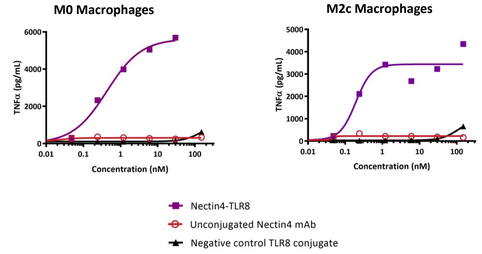
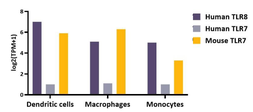


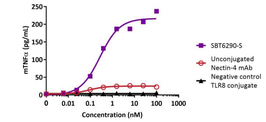
Figure 7: Macrophages differentiated from human peripheral blood monocytes were co-cultured with Nectin4^{pos} cells in the presence of indicated-conjugates or control antibody. No TNFα production was observed when macrophages were co-cultured with Nectin4^{neg} cells (data not shown).

Figure 8: SBT6290 Mouse Surrogate (SBT6290-S) Matches the Functional Profile of SBT6290 on Myeloid Cells

A) TLR7 Expression in Mouse Myeloid Cells is Comparable to That of TLR8 in Human Myeloid Cells



B) SBT6290-S Induces Activation of Mouse Myeloid Cells

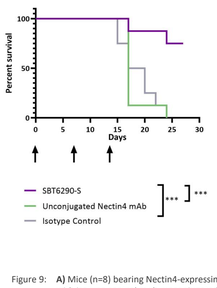


ImmunoTAC™ Conjugate	EC_{50} (nM)
SBT6290	0.3 (human)
SBT6290-S	0.4 (mouse)

Figure 8: **A)** RNA expression data was obtained from public databases. **B)** Mouse bone marrow-derived macrophages were co-cultured with Nectin4^{pos} cells or Nectin4^{neg} cells in the presence of the indicated conjugates or control antibody. 24 hours later, supernatants were evaluated for mouse TNFα production by ELISA.

Figure 9: SBT6290-S Demonstrates Single Agent Activity in the Nectin4-Expressing EMT6 Model

A) Single Agent Anti-tumor Activity



B) Systemic Administration of SBT6290-S Induces Cytokines and Chemokines in the Tumor

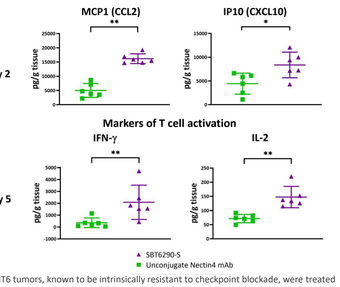


Figure 9: **A)** Mice (n=8) bearing Nectin4-expressing EMT6 tumors, known to be intrinsically resistant to checkpoint blockade, were treated with isotype control mAb, unconjugated Nectin4 mAb, or SBT6290-S, all at 10 mg/kg. Arrows indicate doses administered. *** p<0.001. **B)** Mice were treated as in (A) with one dose of the indicated conjugate and antibody. Tumors were harvested at Days 2 and 5 and levels of the indicated cytokines in the tumors were assessed. Statistical significance was determined by Mann-Whitney test. **p<0.01, *p<0.05

Conclusions

- SBT6290, a Nectin4-directed monoclonal antibody conjugated to a TLR8-specific agonist, activates myeloid cells in a Nectin4-dependent manner, enabling tumor-localized activity via systemic delivery.
- SBT6290 induces multiple anti-tumor immune mechanisms including proinflammatory cytokine production, inflammasome activation, and T and NK cell cytolytic activity.
- Treatment with SBT6290 surrogate in mice results in increased overall survival in a tumor model known to be intrinsically resistant to checkpoint blockade.
- We believe these data support the continued development of SBT6290 for Nectin4-expressing tumors.

SITC 2020 Abstract ID: 712