A Systemically Administered, Conditionally Active TLR8 Agonist for the Treatment of HER2-Expressing Tumors

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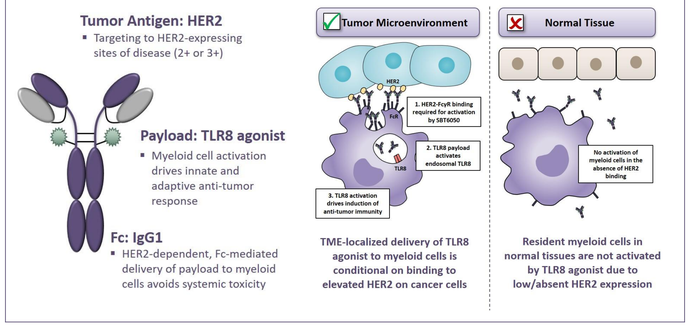
Introduction

Clinical development of systemically administered innate immune cell agonists has been hindered by acute toxicities due to peripheral activation of the targeted cell types. Intratumoral administration, the route of delivery typically used for innate immune/myeloid cell agonists, is limited by tumor accessibility and a dependence on abscopal responses.

Agonism of TLR8 (toll-like receptor 8) has been shown to drive anti-tumor immune responses. Here, we describe a TLR8 agonist conjugate, SBT6050 designed for systemic administration, that utilizes cell surface expression of HER2 to localize activation of TLR8 for the treatment of HER2-expressing tumors.

- SBT6050 activates human monocytes and macrophages only in the presence of HER2-expressing tumor cells with moderate or high expression levels.
- Systemic delivery of a SBT6050 surrogate in mice shows durable, single agent efficacy in a checkpoint refractory tumor model without the induction of peripheral cytokine production or associated CRS-like toxicity.
- SBT6050 is currently in preclinical development for patients with moderate or high HER2-expressing tumors and is projected to enter the clinic in 2020.

SBT6050 is Designed for Systemic Administration with TME-Localized Activity

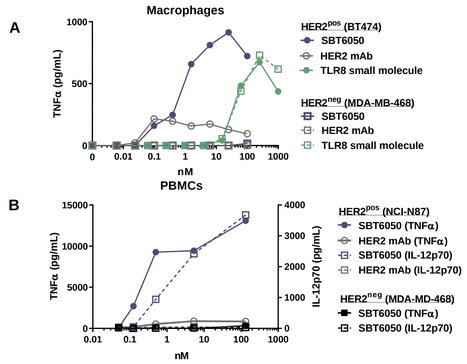


Human TLR8 Expression Profile Supports Development of a TLR8-Selective Payload

		TLR4	TLR7	TLR8	TLR9	STING	RIG-I
Myeloid Cells	Dendritic Cells	+++	+	++++	-	++	++
	Macrophages	++++	++	+++	-	++	++
	MDSC	+++	+++	+++	-	++	++
Non-Myeloid	Fibroblasts	++	++	-	-	+++	+++
	Endothelial Cells	+++	++	-	-	+	++
Tumor	HER2+ Tumor Cell	-	-	-	-	++	++

Expression levels were determined using publicly available RNA-Seq datasets

Figure 1: SBT6050 Induces a HER2-Dependent Pro-Inflammatory, Th1-Polarizing Myeloid Cell Response



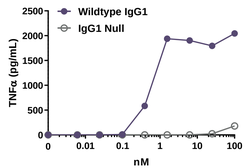
In vitro differentiated macrophages (A) or PBMCs (B) were co-cultured with HER2^{pos} or HER2^{neg} tumor cell lines in the presence of SBT6050, unconjugated anti-HER2 mAb or TLR8 small molecule agonist, as indicated. In contrast to SBT6050, TLR8 small molecule activity was not dependent on HER2 (A). SBT6050 displays enhanced potency compared to the TLR8 small molecule alone (A). TNFα and IL-12 are shown as representative indicators of pro-inflammatory and Th1-polarizing myeloid cell responses, respectively (B).

Figure 2: Delivery of TLR8 Agonist to Myeloid Cells Requires Moderate to High HER2 Expression and Fc:FcγR Interactions

A

Tumor Cell Line	HER2 Expression by IHC	HER2 Molecules/Cell (x10^6)	Activation
SKBR3	3+	1.1	+++
BT474	3+	0.65	+++
NCI-N87	3+	0.47	+++
MDA-MB-453	2+	0.08	+++
ZR-75-1	2+	0.04	++
MDA-MB-175-VII	1+	0.02	-
MDA-MB-468	0	<0.01	-

B

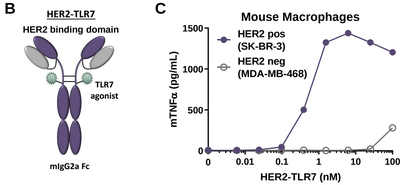


Activation determined by PBMC co-culture assay (A). Purified monocytes co-cultured with BT-474 tumor cells together with TLR8 conjugates bearing either WT IgG1 or IgG1Null, a mutant IgG1 Fc rendered incapable of binding to FcγRs by targeted mutations in Fc: L234A, L235A, G237A and K322A (B).

Figure 3: HER2-TLR7 is a Mouse Surrogate for SBT6050

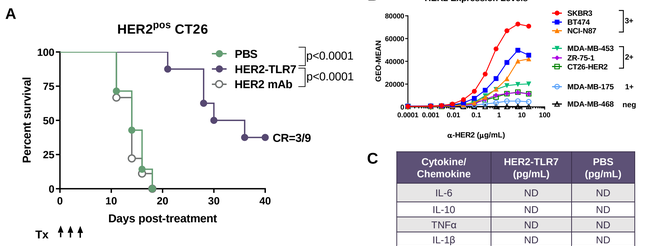
A

Cell Type	Mouse TLR7	Human TLR8
Dendritic Cells	+++	+++
Macrophages	+++	+++
Monocytes	+++	+++
NK Cells	-	-
T Cells	-	-
Tumor Cells	-	-



Mice do not express a functional homolog of human TLR8, but mouse TLR7 phenotypically matches the myeloid expression of human TLR8 (A). HER2-TLR7 surrogate (B) mediates HER2-dependent macrophage activation similar to that of SBT6050 (C).

Figure 4: HER2-TLR7 Monotherapy Results in Tumor Clearance Without Significant Systemic Cytokine Release

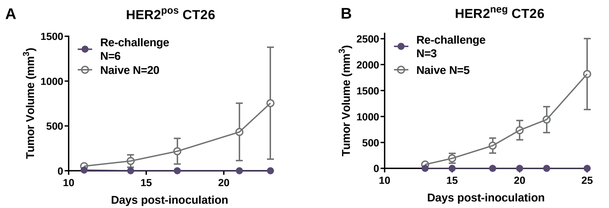


C

Cytokine/ Chemokine	HER2-TLR7 (pg/mL)	PBS (pg/mL)
IL-6	ND	ND
IL-10	ND	ND
TNFα	ND	ND
IL-1β	ND	ND
MCP-1	60	ND

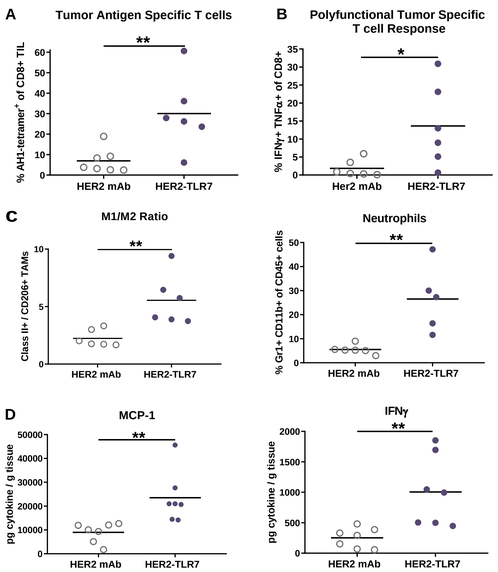
Mice bearing subcutaneous HER2^{pos} CT26 tumors were treated intravenously with HER2-TLR7 at 5 mg/kg, unconjugated HER2 mAb at 5 mg/kg, or PBS; CR=Complete Response (A). Relative HER2 expression of cell lines determined by flow cytometry (B). Cytokine/chemokine expression as assessed in blood drawn 24 hours after dosing; ND=Not Detected (C).

Figure 5: HER2-TLR7 Induces Durable Anti-Tumor Immunity That Protects Against Tumor Re-challenge



Mice cleared of HER2^{pos} CT26 tumor with HER2-TLR7 treatment were re-challenged with HER2^{pos} (A) or HER2^{neg} (B) CT26 cells. Re-challenge was performed 60 days after initial tumor clearance. Naïve mice were included as a control for tumor cell growth.

Figure 6: HER2-TLR7 Treatment Activates Intratumoral Innate and Adaptive Immune Responses



HER2^{pos} CT26 tumor-bearing mice were treated intravenously with 5 mg/kg HER2-TLR7 or unconjugated HER2 mAb. On days 2 (A) and 7 (B, C) post-treatment, intratumoral immune cell populations were analyzed by flow cytometry for anti-tumor T cells (A, B) and innate immune cells (C). Intratumoral cytokines were assayed on day 2 post-treatment (D). Statistical significance was determined by unpaired T-test. *p<0.05, **p<0.01, p<0.001.

Conclusions

- SBT6050 activates human myeloid cells only in the presence of HER2-expressing cells, enabling systemic administration with tumor-localized activity.
- Systemic administration of a SBT6050 mouse surrogate results in durable anti-tumor efficacy in the absence of peripheral cytokine production, consistent with tumor-localized activity.
- In mouse HER2-expressing tumors, a surrogate molecule of SBT6050 drives activation of both innate and adaptive immune response characterized by activation of tumor-associated myeloid cells, infiltration of neutrophils, persistent increases in local cytokine and chemokine production, and the generation of a robust, neo-Ag specific anti-tumor CTL response.
- SBT6050 is currently in preclinical development for patients with moderate or high HER2-expressing tumors and is projected to enter the clinic in 2020.

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