

# Novel Class of STING Agonists for Immuno-oncology

Sreerupa Challa<sup>1</sup>, Shenghua Zhou<sup>1</sup>, Anjaneyulu Sheri<sup>1</sup>, Samantha Delaney<sup>1</sup>, Rayomand Gimi<sup>1</sup>, Seetharamaiyer Padmanabhan<sup>1</sup>, Geeta Meher<sup>1</sup>, Diane Schmidt<sup>1</sup>, Dillon Cleary<sup>1</sup>, Santosh Khedkar<sup>2</sup>, Nezam Afdhal<sup>1</sup>, and Radhakrishnan Iyer<sup>1</sup>

<sup>1</sup>Spring Bank Pharmaceuticals, Inc., 113 Cedar Street, Milford, MA 01757, USA. <sup>2</sup>ChemBio Discovery Solutions, Lexington, MA 02421, USA.

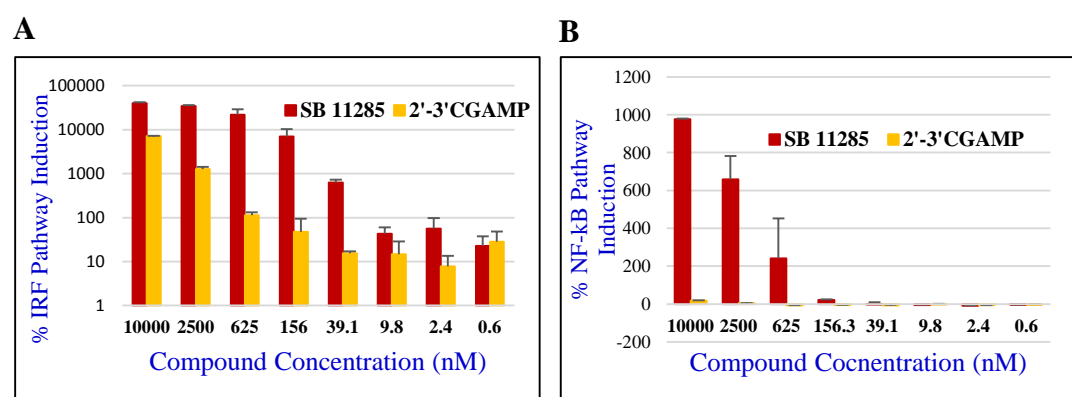
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## Abstract

Activation of Stimulator of Interferon Genes (STING) pathway in tumor cells and/or antigen presenting cells (APCs) within the tumor microenvironment (TME) can induce type I Interferon production leading to apoptosis of tumor cells, as well as, induction of adaptive immune response. Herein, we describe the discovery of highly potent and selective first-in-class STING agonist for application in immuno-oncology. The lead STING agonist SB 11285 has potent immune-modulating, as well as, anti-tumor activities and is being advanced for additional preclinical studies for application in immuno-oncology.

## Results

### SB 11285 is a potential STING agonist

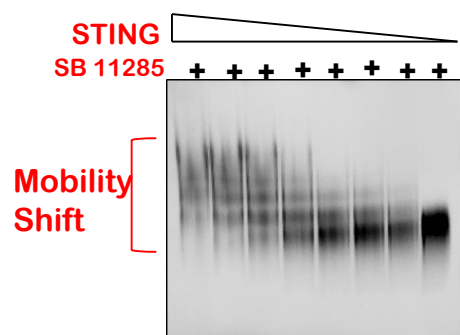


Name	IRF EC <sub>50</sub>	NF-kB EC <sub>50</sub>
SB 11285	7 ±4 nM	121.7 ±53.4 nM
2'-3'-cGAMP	1287 ±169 nM	> 50 μM

**SB 11285 induces IRF and NF-κB signaling:** THP-1 dual cells carrying both IRF and NF-κB reporters were treated with various concentrations of SB 11285 or 2'-3'-cGAMP. (A) IRF activity was assessed using QUANTI-luc and (B) NF-κB activity was determined by measuring SEAP levels. % induction was calculated from fold change in luminescence/absorbance compared to DMSO-treated sample. EC<sub>50</sub> values are generated by curve fit in Xlfit.

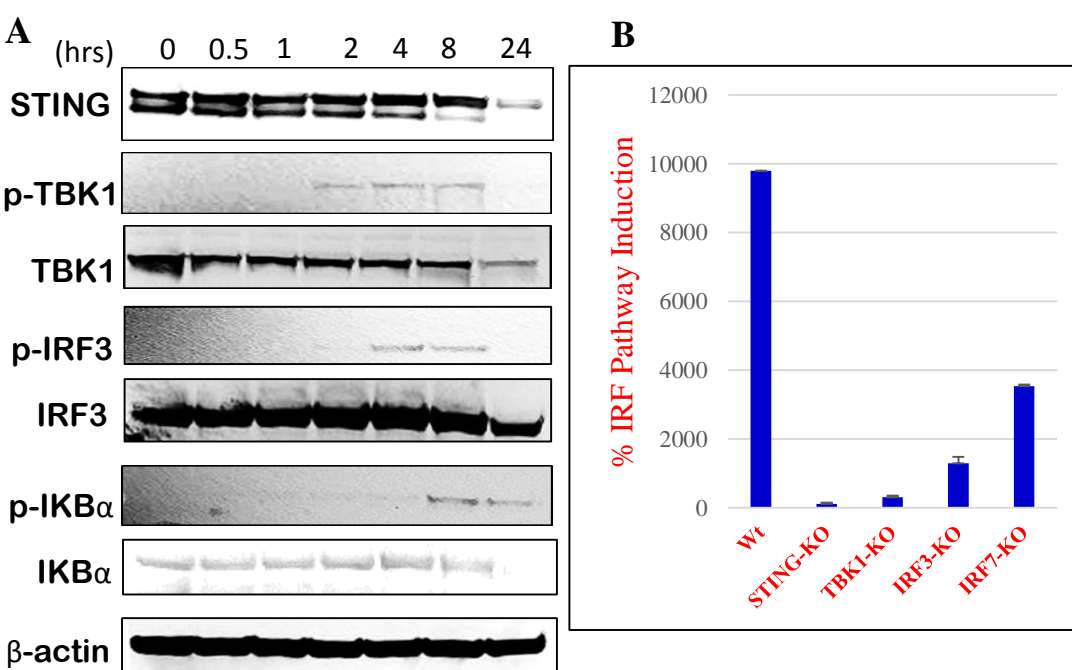
**SB 11285 is 1000-fold and 200-fold more active in inducing IRF and NF-κB respectively compared to 2'-3'-cGAMP.**

### SB 11285 binds to STING



**Gel Shift Assay:** A close structural analog of SB 11285 carrying a fluorescent substituent was synthesized for Gel Shift Assay. 250μM of SB 11285 with 20μM to 0μM of STING was incubated and subjected to electrophoresis on native gel for monitoring shift in the band size.

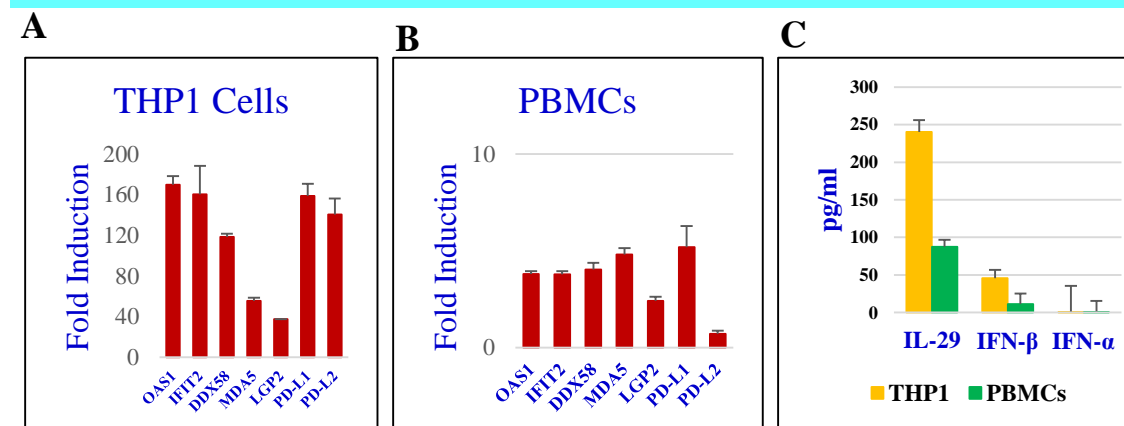
### SB 11285 activates known STING Signaling pathway



**Evaluation of SB 11285 induced STING signaling pathway:** (A) Expression of STING and phosphorylation status of IRF3, TBK-1 and IκB-α in THP-1 Cells was evaluated in THP-1 cells after treating with 5μM SB 11285 (B) The role STING, TBK1 & IRF3/7 in SB 11285-induced STING signaling was evaluated in Raw Macrophages. Wt, STING KO, TBK1 KO, IRF3 KO and IRF7 KO RAW-Lucia ISG Cells were either treated with 50μM of SB 11285 or DMSO. After 20h incubation, IRF activity was assessed using QUANTI-luc and % induction was calculated from fold change in luminescence/absorbance compared to DMSO treated sample.

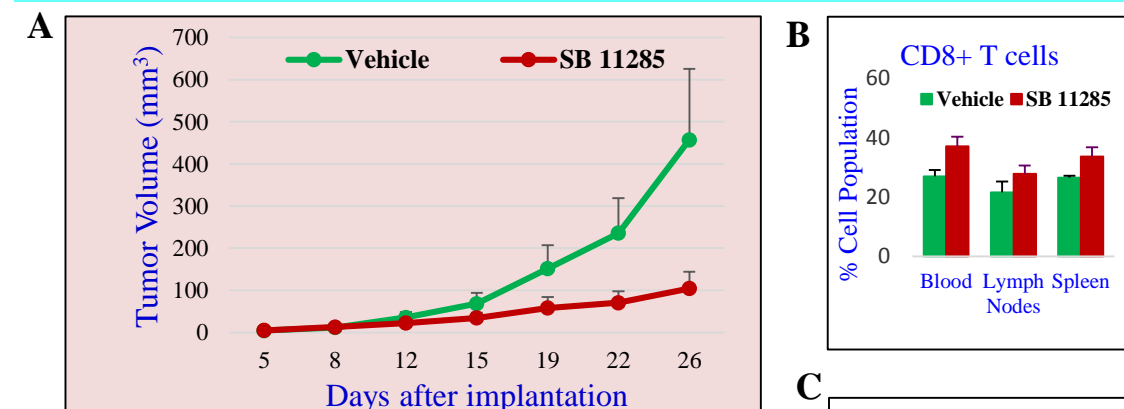
## Results

### SB 11285 induces IFN secretion and ISG expression



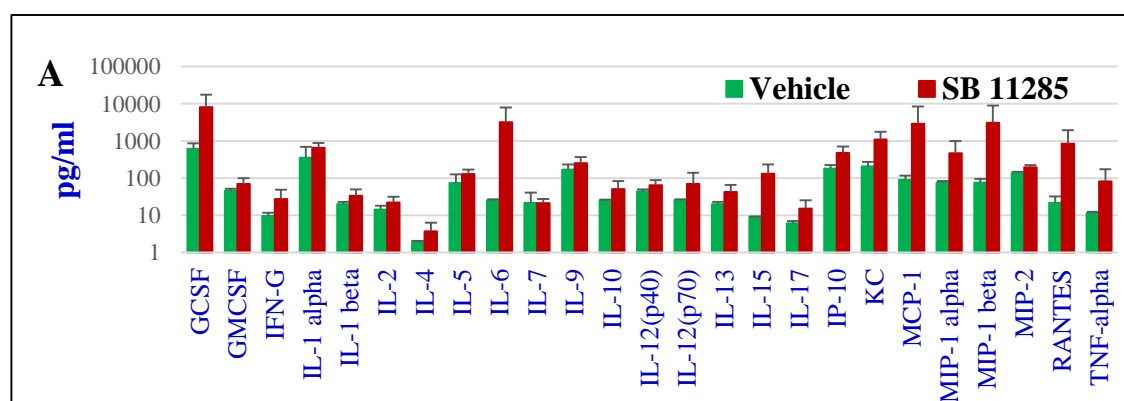
**Evaluation of IFN secretion and gene expression after SB 11285 treatment:** THP-1 cells (A) and PBMCs (B) were treated with 5μM of SB 11285 for 20 hrs and gene expression was evaluated by Taqman Assays. Fold Induction was calculated by ΔΔCt method. (C) THP-1 and PBMCs cells were treated with 10μM of SB 11285 for 20 hrs and secretion of type I and type III Interferons was evaluated by Procartaplex multiplex assay.

### SB 11285 inhibits tumor growth in 4T1 breast cancer model after intraperitoneal (i.p) administration



**Evaluation of SB 11285 in 4T1 Breast Cancer Model:** Briefly, 4T1 cells were implanted subcutaneously in mammary Fat Pad on day 1. SB 11285 was administered (i.p) on days 5, 7, 9, 11, 13, 17 & 19 @ 10 mg/kg in saline. (A) Tumor volumes measured for the entire study. (B) % of CD8+ T cells and (C) % of CD4+ T cells in spleen, lymph nodes and blood were measured on day 19 by flow cytometry.

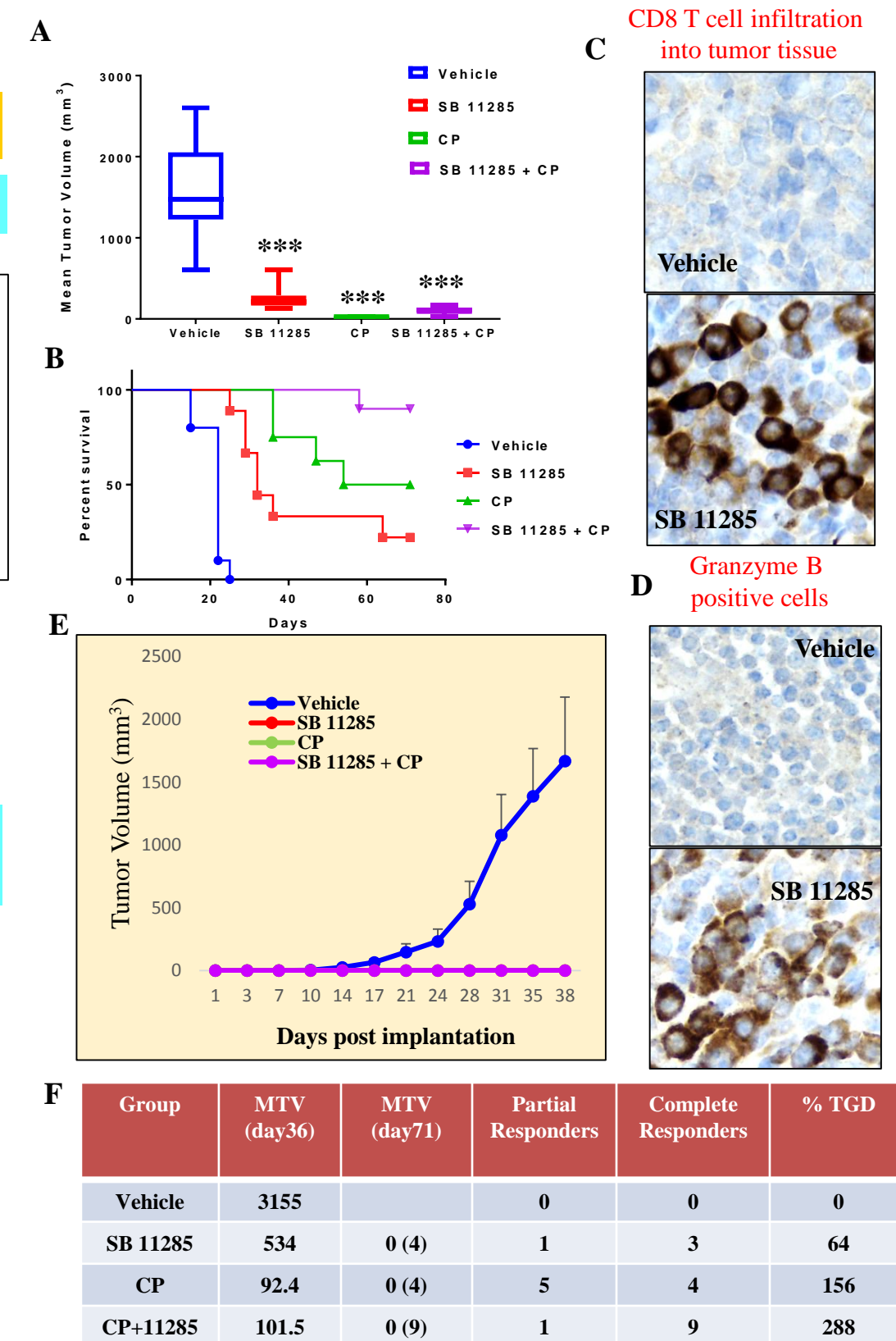
### IFN induction and cytokine expression profile in vivo after SB 11285 intraperitoneal (i.p) administration



A group of five, C57BL/6 mice were given intraperitoneal dosing of either saline or 10mg/kg SB 11285. Four hours after injection, serum was collected to investigate cytokine secretion. (A) MCYTOMAG-70K-PMX MILLIPLEX MAP Mouse Cytokine/Chemokine Magnetic Bead Panel and (B) Procartaplex Luminex IFNα-β Mouse Cytokine Magnetic Bead Panel were used.

## Results

### SB 11285 significantly inhibits tumor growth in A20 lymphoma model and induces immune memory



**Evaluation of SB 11285 in Syngeneic A20 Lymphoma Model:** Briefly, A20 cells were implanted subcutaneously in the right flank region of mice. Dosing was initiated when tumor size reached 100mm<sup>3</sup>. SB 11285 was administered intratumorally (i.t) for 5 times on days 3,4,6,8 & 10 @ 100 μg/50 μl in saline. Cyclophosphamide (CP) was administered intraperitoneally (i.p) for 2 times on days 1 & 2 @ 100 mg/kg. (A) Tumor volumes measured on day 22 after initiation of treatment. Statistical Significance for Kruskal Wallis Dunn's or Mann-Whitney U test: ns = non-significant, \* = 0.01 < P ≤ 0.05, \*\* = 0.001 < P ≤ 0.01, \*\*\* = P ≤ 0.001 when compared to Vehicle Group. (B) Kaplan Meier survival analysis for a period of 71 days. (C) Immunohistochemistry of tumor tissues with anti-CD8 Antibody and (D) anti-Granzyme B antibody was performed one day after end of dosing (day 11) to evaluate infiltration of CD8 T cells and NK cells into tumor tissue. The images shown are a representative pictures from a group of 4. (E) After the end of the study, the complete responders were re-challenged with A20 cells implanted subcutaneously on opposite flank. The animals were monitored and tumor volumes were measured for 38 days post-implantation. (F) Response summary of the TGI and TGD studies in A20-lymphoma model.

## Conclusions

We have discovered highly potent first-in-class STING agonists that show excellent selectivity in induction of IFN, NF-κB, ISGs, and cytokines. The lead STING agonist SB 11285 has potent anti-tumor activities, is synergistic with anti-cancer agents, induces anti-tumor immune memory and is being advanced for additional preclinical studies for application in immuno-oncology. [rchalla@springbankpharm.com](mailto:rchalla@springbankpharm.com) [kiyer@springbankpharm.com](mailto:kiyer@springbankpharm.com)