A Novel Class of STING Agonists that Self-Assemble into Nanostructures are Potent Anti-Cancer Immuno-Therapeutic Agents

Anjaneyulu Sheri[1], Seetharamaiyer Padmanabhan[1], Geeta Meher[1], Rayomand H. Gimi[1], Dillon Cleary[1], Santosh Khedkar[2], Sreerupa Challa[1], Shenghua Zhou[1], Vishal Nair[1], Leena Suppiah[1], Diane Schmidt[1], Nezam Afdhal[1] and Radhakrishnan P. Iyer[1]

[1]Spring Bank Pharmaceuticals, Inc. 35 Parkwood Dr, Suite 210, Hopkinton, MA 01748; [2]ChemBio Discovery, Inc., Lexington, MA 02421.

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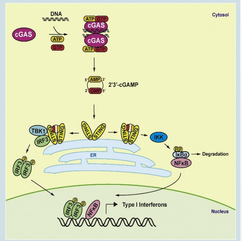
Introduction

Targeting innate immune signaling pathways that induce type I IFN production to re-program the tumor microenvironment and restore anti-tumor immunity represents a novel immunotherapeutic approach for cancer. In this regard, agonists such as cyclic dinucleotides (CDNs) that activate the Stimulator of Interferon Genes (STING) pathway have emerged as an attractive therapeutic strategy. Using structure-based drug design and focused library synthesis, we have discovered novel CDNs, that self-assemble into nanostructures. These CDNs show potent STING agonist activity in vitro, and demonstrate potent anti-tumor activity in orthotopic and subcutaneous syngeneic murine tumor models when administered by i.v., i.p., and i.t. routes. The CDNs also induce immune memory, show abscopal effect and are synergistic with other anti-cancer agents including checkpoint inhibitors.

cGAS-STING mediated cytosolic DNA sensing and activation type I IFN signalling



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Materials and Methods

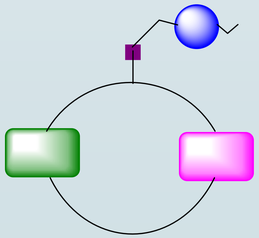
- Focused libraries of CDNs were synthesized using phosphoramidite chemistry
- **Binding affinity** with human STING CTD was determined by SPR
- **STING-dependent Induction of IRF and NF-kB** was assessed as % fold change in luminescence using cells, with reporter constructs
- **Self-assembly to nanostructures** was determined by SEM
- **In vivo efficacy** was assessed by measurement of mean tumor volumes following administration of compounds by i.v. or i.t. in the A20 lymphoma, CT26 carcinoma, B16 melanoma, and 4T1 breast cancer models
- Flow cytometry, multiplexing assays and immuno-histochemistry of blood, and tissues were carried out to assess MOA

Results

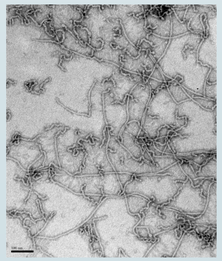
Lead compounds that induce IRF induction in reporter assays (EC_{50} 1 to 10 nM) were found to self-assemble into nanostructures. The lead analog, SB 11285, showed highly potent and durable anti-tumor response in multiple tumor models with M.E.D 10 μg (i.t.), and 1 mg/kg (i.v.).

SB 11285 Scanning Electron Microscopy Image

SB 11285

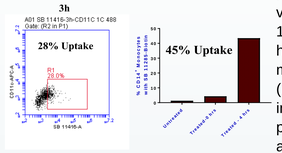


Self-assembled SB 11285 forms Nanostructures



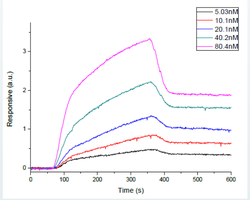
Uptake by Immune Cells

Significant uptake of SB 11285 by myeloid dendritic cells and monocytes



PBMCs were treated with Biotin-SB 11285 at various time points to evaluate uptake of SB 11285 into cells. The cells were then harvested for staining with various surface markers such as CD14 (monocytes), CD 11c+ (myeloid dendritic cells) before performing intracellular cytokine staining with streptavidin probe for Biotin-SB 11285. Cells were analyzed by flow cytometry. A representative plot is shown.

Binding Affinity by SPR

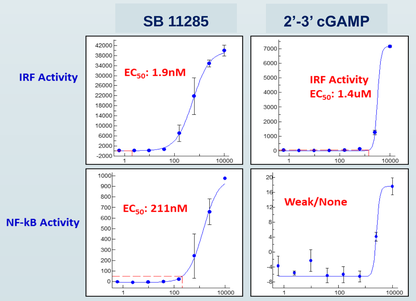


Evaluation of binding affinity of biotinylated SB 11285 to wt STING-CTD.

The equilibrium dissociation constant (Kd) Value was 1.22 X 10^{-7} M (122 nM)

Biotin-SB 11285 was immobilized onto streptavidin chips. Various concentrations of biotin-SB 11285 dissolved in water was manually coated onto PlexArray Nanocapture Sensor Chips and STING protein was injected into the flow cell. SPR measurements were performed at 4°C and signal changes after binding were recorded as the assay value. Real-time binding signals were recorded and kinetic analysis was performed using Plexera Analysis.

STING-dependent Induction of IRF and NF-kB

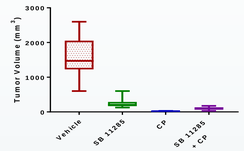


IRF and NF-kB induction of SB 11285 and natural STING Ligand 2'-3' cGAMP in THP-1 cells: THP-1 dual cells carrying promotors to measure both IRF and NF-kB activity were treated with various concentrations of SB 11285 (or) 2'-3' cGAMP. IRF activity was assessed using QUANTI-luc and NF-kB activity was determined by measuring secreted embryonic alkaline phosphatase (SEAP) levels. % induction was calculated from fold change in luminescence/absorbance compared to DMSO treated samples. EC_{50} values were generated by curve fit in Xlfit.

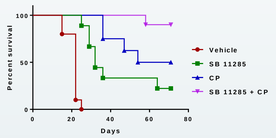
In Vivo Efficacy

Intratumoral (IT) administration of SB 11285 results in potent and durable anti-tumor activity and induces immune memory in the A20 lymphoma mouse model

Group	Dose	Route	Schedule (days)
Vehicle	50µl Saline	IT	3, 4, 6, 8, 10
SB 11285	100µg/animal	IT	3, 4, 6, 8, 10
Cyclophosphamide	100mg/kg	IP	1 & 2
Cyclophosphamide + (SB 11285)	100mg/kg + (100µg/animal)	IP + (IT)	1 & 2 3, 4, 6, 8, 10

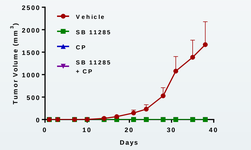


Durable anti-tumor response



In the A20 model, all animals were monitored for several months Following stoppage of treatment to evaluate durability of anti-tumor Response.

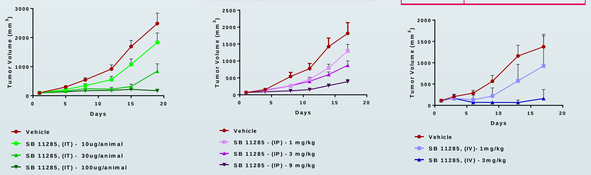
Induction of Immune memory



In the A20 model, mice that showed complete tumor regression were challenged with A20 cells and rejected the tumor

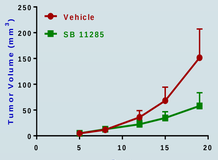
IT, IP, and IV administered SB 11285 shows dose-dependent and highly potent anti-tumor activity In the CT26 colon carcinoma mouse model

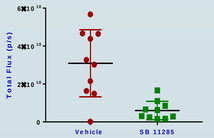
IT	IP	IV
days 1, 2, 4, 6, 8	days 1, 3, 5, 8, 11	days 1, 5, 9, 14



SB 11285 inhibits tumor growth and metastasis in the orthotopic 4T1 breast cancer model after intraperitoneal (IP) administration with induction of $CD8^+$ T cells in spleen, blood and lymph nodes

Group	Dose	Route	Schedule (days)
Vehicle	100 µl Saline	IP	5, 7, 9, 11, 13, 17, 19
SB 11285	10 mg/kg	IP	5, 7, 9, 11, 13, 17, 19

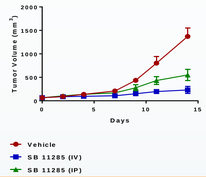




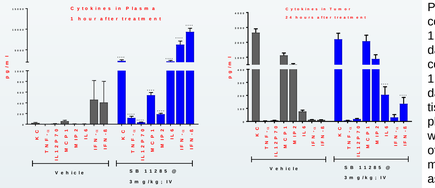
IP and IV administered SB 11285 shows highly potent anti-tumor activity In the B16 melanoma mouse model

B16 Melanoma

Group	Dose	Route	Schedule (days)
Vehicle	100µl Saline	IV	1, 3, 8
SB 11285	9mg/kg	IP	1, 3, 8
SB 11285	9mg/kg	IV	1, 3, 8

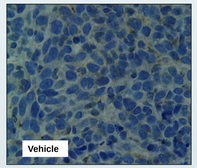


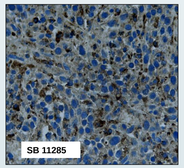
SB 11285 induced significant induction of cytokines associated with a Type I IFN signature in both plasma and TME



Plasma samples were collected 1 hour after SB 11285 IV administration on day 1. Tumor tissues were collected on day 6 after SB 11285 administration on day 1 & 5 to obtain tumor tissue lysates. Both plasma and tissue lysates were tested for presence of cytokines by using mouse magnetic Luminex assay.

SB 11285 induced significant $CD8^+$ T cells infiltration in tumor tissue





Tumor tissues on day 6 (after SB 11285 IV administration on day 1 & 5) were collected and fixed in 10% neutral buffered saline for further processing into paraffin embedded tissue sections. The sections were then stained with anti-CD8 antibody to detect $CD8^+$ T cells. The images shown are a representative pictures from a group of 6

Conclusions

We have discovered next generation CDNs as highly potent first-in-class STING agonists. These CDNs self-assemble into nanostructures that enable systemic administration and efficient cellular delivery. Administration of the lead compound SB 11285 by I.V., I.P. or I.T. routes in syngeneic mouse models of A20 lymphoma, CT26 colon carcinoma, B16 melanoma, 4T1 breast cancer models resulted in potent dose-dependent and durable anti-tumor response with the induction of immune memory and abscopal effects. Antitumor activity was associated with induction of Type I IFN and CD8+ T cells in the tumor and periphery. The lead compound is being advanced into human clinical trials.

Reference

1. S. Challa, et al. Pharmacodynamic studies of SB 11285, a systemically bioavailable STING agonist in orthotopic tumor models. AACR Tumor Immunology and Immuno-therapy, November 27-30, 2018.

Acknowledgements

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For more information, please contact: kiyer@springbankpharm.com