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MECHANISTIC INSIGHTS INTO THE ANTI-TUMOR ACTIVITY OF SB 11285 - A NOVEL STING AGONIST

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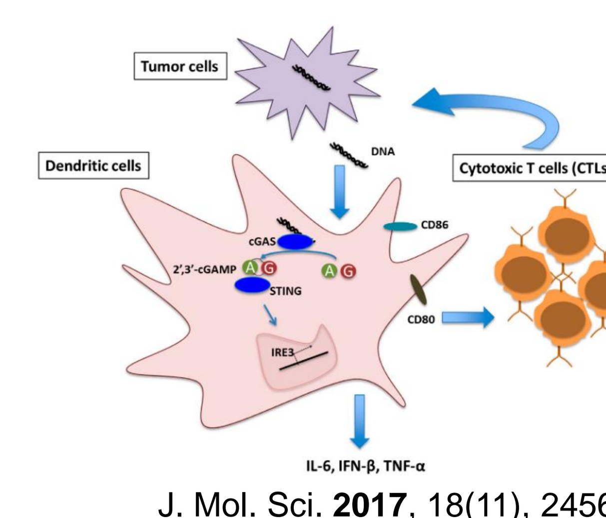
AACR 2018 -
Tumor Immunology and Immunotherapy
Poster # B87
November 27-30. Miami.

INTRODUCTION

Cancer immunotherapy has proven to be a highly effective therapeutic option for cancer patients. However, the overall response rate with check-point inhibitors and other related modalities has been modest due to various factors. Targeting innate immune signaling pathways that induce type I IFN production to re-program tumor microenvironment and restore anti-tumor immunity represents a novel immunotherapeutic approach. In this regard, agonists that activate the Stimulator of Interferon Genes (STING) pathway has emerged as an attractive strategy. We have previously reported that SB 11285 is a novel synthetic cyclic dinucleotide STING agonist, which has demonstrated highly potent antitumor activity, when used alone or in combination with other antitumor agents, in several syngeneic mouse tumor models when administered by intratumoral, intravenous or intraperitoneal routes. Presented here are recent studies that provide additional insights into the mechanism of action of SB 11285.

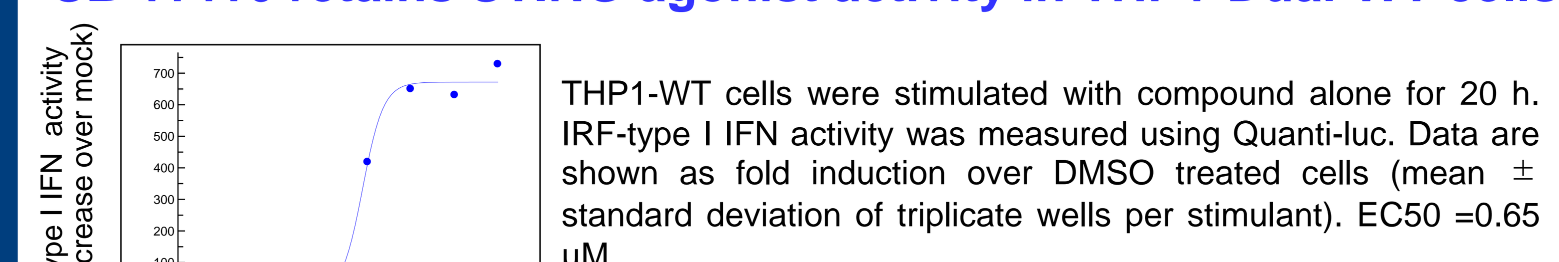


J. Mol. Sci. **2017**, 18(11), 2456

RESULTS

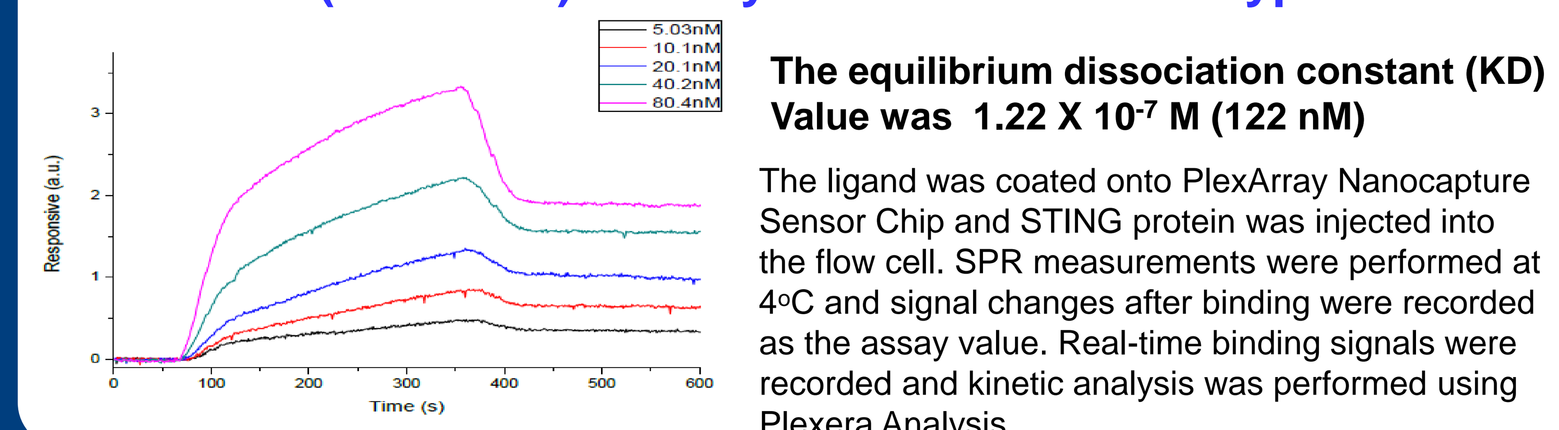
EVALUATION OF BINDING AFFINITY OF WT-STING-CTD (AA138-379) TO BIOTINYLATED SB 11285 ANALOG (SB 11416) BY SURFACE PLASMON RESONANCE (SPR)

SB 11416 retains STING agonist activity in THP1-Dual-WT cells



THP1-WT cells were stimulated with compound alone for 20 h. IRF-type I IFN activity was measured using Quanti-luc. Data are shown as fold induction over DMSO treated cells (mean ± standard deviation of triplicate wells per stimulant). EC50 =0.65 μM

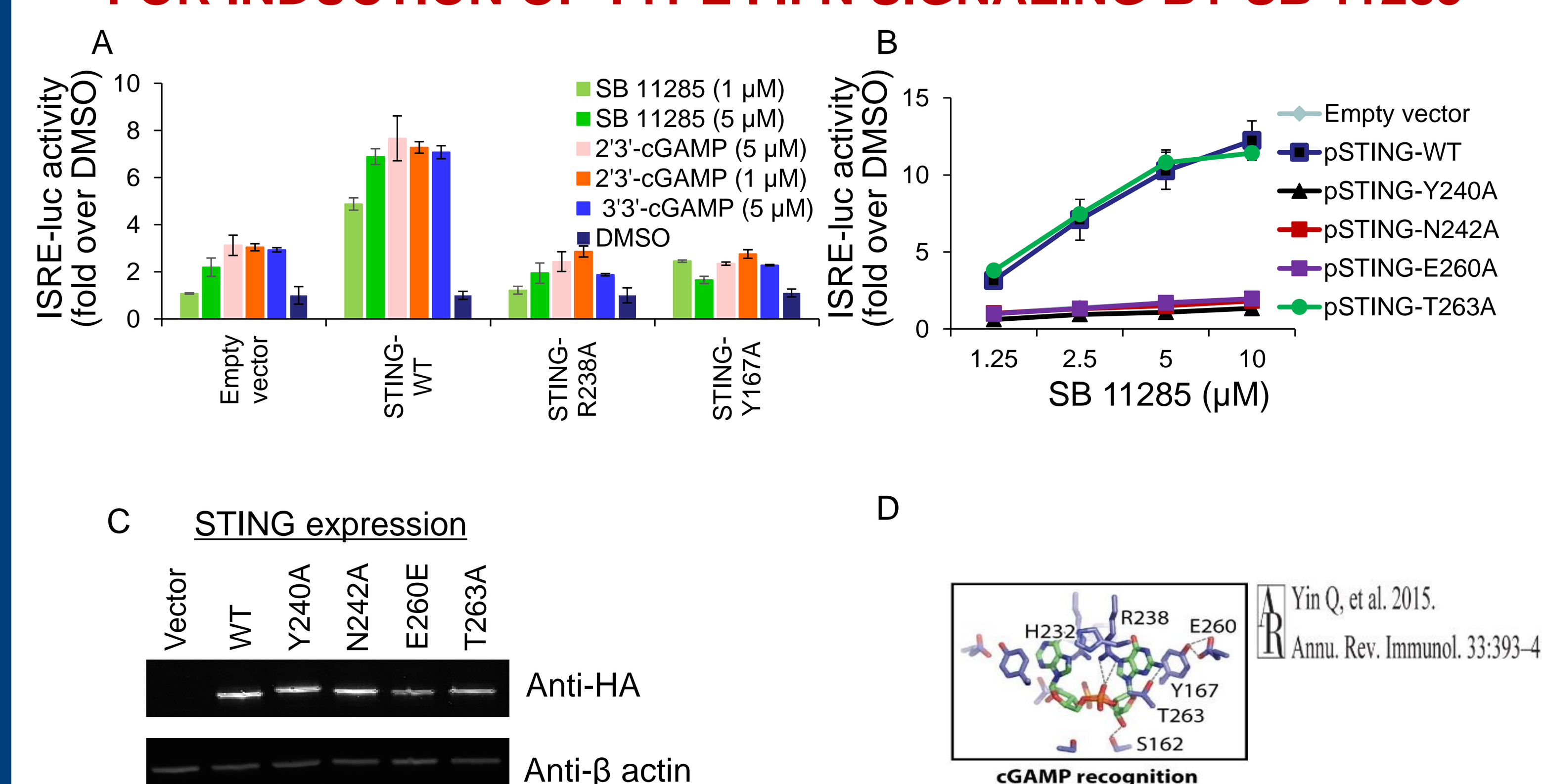
SB 11285 (SB 11416) directly binds human wild-type STING



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

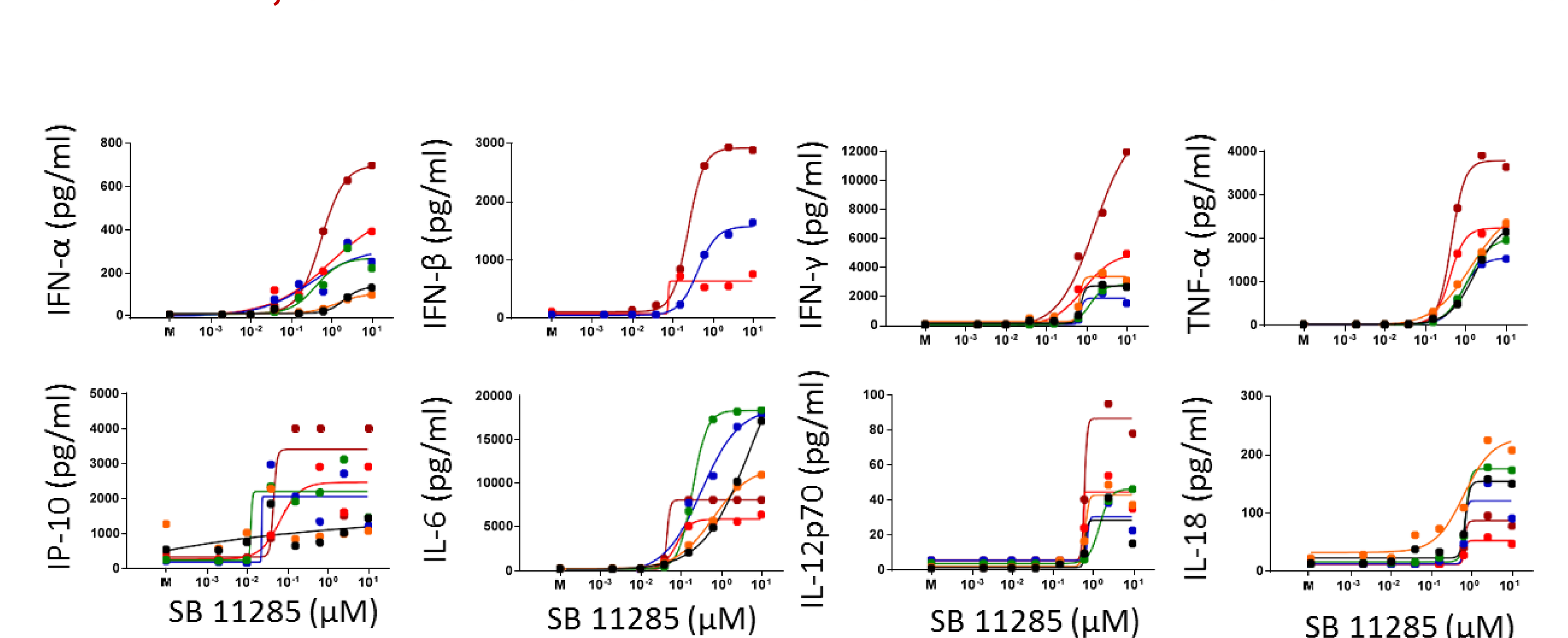
The ligand was coated onto PlexArray Nanocapture Sensor Chip and STING protein was injected into the flow cell. SPR measurements were performed at 4°C and signal changes after binding were recorded as the assay value. Real-time binding signals were recorded and kinetic analysis was performed using Plexera Analysis.

KEY RESIDUES IN STING BINDING POCKET ARE CRITICAL FOR INDUCTION OF TYPE I IFN SIGNALING BY SB 11285



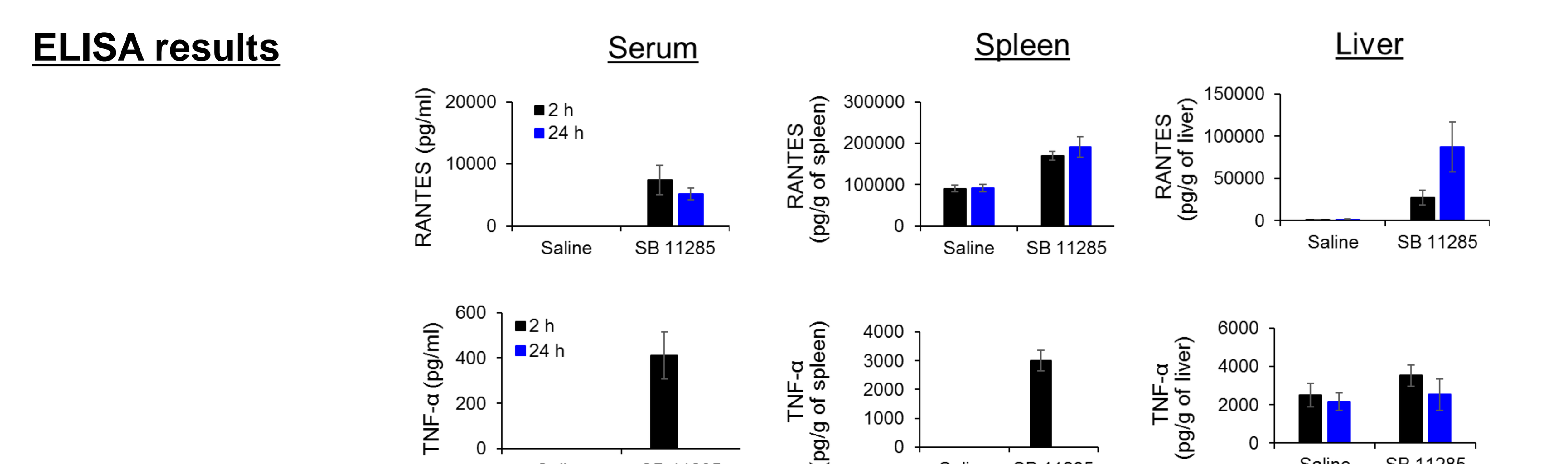
HEK293T cells were transfected with various STING expression plasmids together with plasmids encoding ISRE-luc reporter gene and Renilla-luc reporter gene for 18 hrs, followed by treatment with SB 11285 (A and B) or cGAMP (A) for 5 hrs. ISG54 ISRE-luciferase activity was determined. Results are shown as fold induction over DMSO-treated cells (mean ± standard deviation of triplicate wells per stimulant). (C) The expression of STING was determined by Western blot with anti-HA antibody. (D) Residues involved in binding of 2'3'-cGAMP.

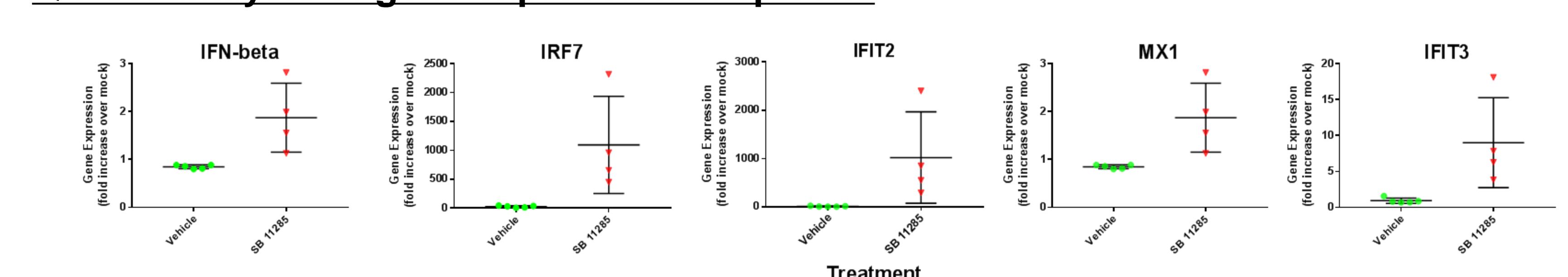
SB 11285 INDUCED DOSE-DEPENDENT PRODUCTION OF TYPE I IFNS, TYPE II IFN, OTHER CYTOKINES AND CHEMOKINES IN HUMAN PBMCS



Human PBMCs from healthy volunteers (n=3 to 6 donors) were stimulated with SB 11285 alone for 24 hours. Cytokine production in culture supernatants was monitored by multiplex analysis using a 45-plex luminex assay and ELISA (IFN-β).

PHARMACODYNAMIC STUDY OF SB 11285 IN NORMAL BALB/C MICE





Groups of 4-5 Balb/C mice (female, 8 weeks of age) were intravenously injected via tail vein with saline control or SB 11285 at 9 mg/kg. Serum, spleen, and liver samples were collected at 2 and 24 hrs post-treatment. (Top panels) Levels of RANTES (top) and TNF-α (bottom) were measured using ELISA and results are shown as pg/ml for serum samples and pg/g of tissue for spleen and liver samples. (Bottom panels) The expression levels of representative ISGs in spleen samples were quantified using Q-PCR. $p<0.05$ for all ISGs tested in SB 11285-treated mice as compared to mock-treated mice.

MATERIALS AND METHODS

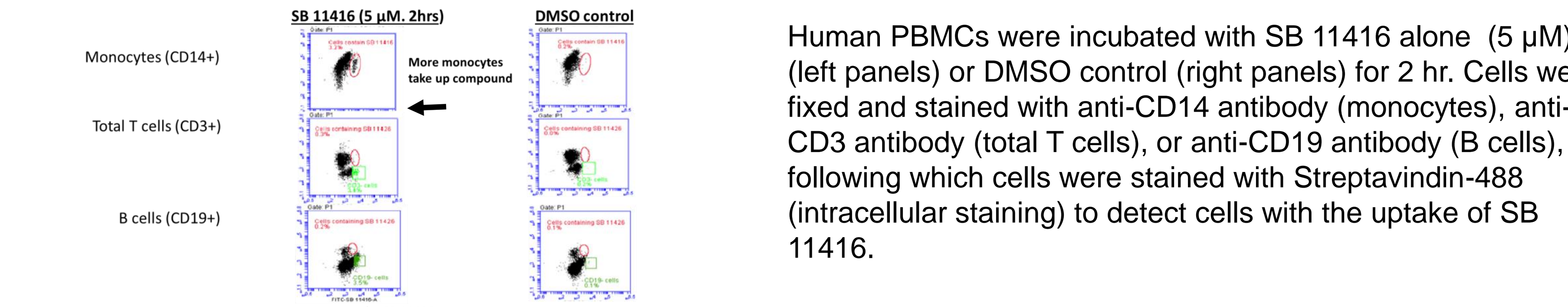
To address whether SB 11285 directly binds wild-type human STING, surface plasmon resonance assay was performed with a Biacore T200 device and a biotinylated SB 11285 analog (SB 11416). SB 11416's STING agonistic activity in inducing type I IFN response was evaluated using THP1-Dual-WT reporter cells (Invivogen). To determine the agonist activity of SB 11285 in human primary immune cells, cells were stimulated with SB 11285 and the production of IFN-β/α, cytokines and chemokines were assayed using regular and multiplex ELISA. Normal BALB/c mice were injected (i.v.) with compound. Serum, spleen, and liver samples were collected to quantify RANTES and TNF-α using ELISA. Gene expression in the spleen was monitored using RT-qPCR. The cellular uptake of biotinylated SB 11285 analog by human primary immune cells was evaluated using flow cytometry.

ACTIVATION OF STING POLYMORPHIC VARIANTS BY SB 11285

Compound	THP1 IRF reporter assay (Average EC50 (μM) ± SD				
	STING-**WT** (57.9% population)	STING-**HAQ** (20.4% population)	STING-**R232H** (13.7% population)	STING-**AQ** (5.2% population)	STING-**R293Q** (1.5% population)
SB 11285	0.54 ± 0.21	0.42 ± 0.20	Not calculated	0.55 ± 0.11	0.32 ± 45
2'3'-cGAMP	57 ± 14	~306 ± 268	Not calculated	~5599 ± 6726	~4590 ± 5203

THP1-Dual (WT) cells were stimulated with compound alone for 24 h. IRF-type I IFN activity was measured using Quanti-luc (Invivogen). Data are shown as fold induction over DMSO treated cells (mean ± standard deviation of triplicate wells per stimulant). EC50 values were calculated using XLfit.

SB 11285 IS TAKEN UP PREDOMINANTLY BY MONOCYTES AT EARLY TIME POINTS



Human PBMCs were incubated with SB 11416 alone (5 μM) (left panels) or DMSO control (right panels) for 2 hr. Cells were fixed and stained with anti-CD14 antibody (monocytes), anti-CD3 antibody (total T cells), or anti-CD19 antibody (B cells), following which cells were stained with Streptavidin-488 (intracellular staining) to detect cells with the uptake of SB 11416.

CONCLUSIONS

- **SB 11285** is a potent systemically bioavailable STING agonist:
  - SB 11285 directly binds human wild-type STING.
  - Key residues in STING binding pocket play an essential role in SB 11285-induction of type I IFN signaling.
- **SB 11285** induces type I IFN and other cytokines in human PBMCs and PBMC-derived monocytes.
- **SB 11285** administered by i.v. route in normal BALB/c mice induces type I IFN response.
- During early incubation, **SB 11285** is predominately taken up by monocytes and other innate immune cells.
- Previous pharmacodynamic studies (ref 3-5) in multiple syngeneic mouse tumor models have shown that **SB 11285** has potent and durable anti-tumor activity.
- **SB 11285** is being advanced to clinical trials.

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