SB 11312, an active metabolite of SB 11285, is a potent and systemically bioavailable STING agonist

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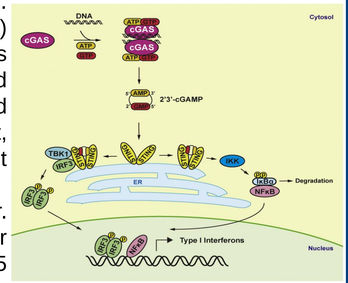
AACR Tumor Immunology and Immunotherapy, 2018 Poster # B88

INTRODUCTION

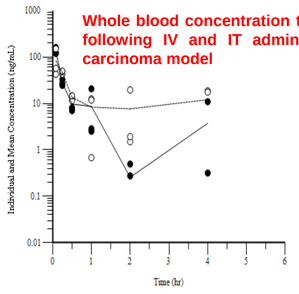
Immunotherapy has emerged as a transformative approach for the treatment of cancer. Recent work has highlighted a major role for Stimulator of Interferon Genes (**STING**) agonists in immunotherapy. Conceptually, the activation of STING pathway in immune cells and tumor cells in the tumor microenvironment could result in the induction of innate and adaptive immunity through the activation of cytotoxic T cells and NK cells for profound and durable anti-tumor response. We recently reported the discovery of SB 11285 as a potent, first-in-class, STING agonist. Herein, we describe the discovery of the highly potent metabolite of SB 11285, designated as SB 11312 for application in immuno-oncology.

SB 11312 is a mixture of two diastereomers of which SB 11312-A is the more active isomer. Both SB 11312 & SB 11312-A have potent immune-modulating, as well as, anti-tumor activities in tumor models. Comparative single-dose pharmacokinetic profiles of SB 11285 and SB 11312 following intravenous (IV) and intratumoral (IT) administration of SB 11285 in the CT26 tumor model is also described.

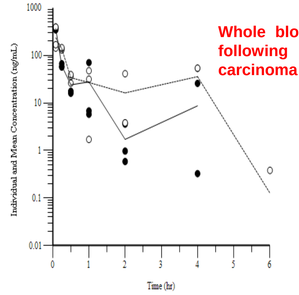


Molecular cell: Volume 54, Issue 2, 24 April 2014, Pages 289-296

SB 11312 is a metabolite of SB 11285



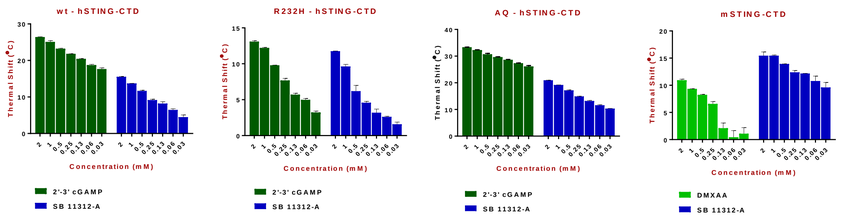
Whole blood concentration time profiles of SB 11285 following IV and IT administration in CT26 colon carcinoma model



Whole blood concentration time profiles of SB 11312 following IV and IT administration in CT26 colon carcinoma model

CT26 cells were implanted subcutaneously in right flank and when mean tumor volume reached 200-300mm^3 , SB 11285 was administered either IV @ 5mg/kg or IT @ 200ug/mouse. In both intravenous and intratumoral routes, SB 11285 was given only once. Following administration, whole blood samples were collected at 5, 15, 30, 60, 120, 240, and 360 mins and drug levels were quantified using LC/MS/MS.

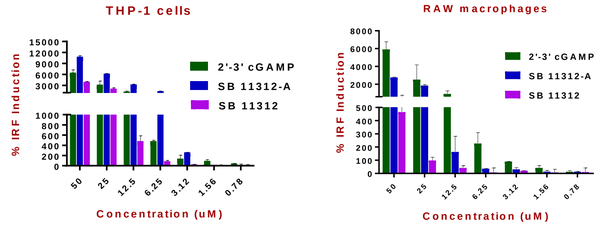
SB 11312 demonstrated high binding affinity to human wildtype and STING polymorphic variants as well as mouse STING



Thermal shift assay was conducted with 0.11mg/ml protein with different concentrations (2mM to 0.03mM) of compound in buffer containing 10mM HEPES (pH 7.5), 140mM NaCl and a 1× dilution of Protein Thermal Shift Dye *(Thermofisher)*. The fluorescence signal as a function of temperature was recorded using a Real Time PCR machine *(Step One Plus, Thermofisher)*. The temperature gradient is performed in the range of 25–99 °C with a ramp rate of 1% over the course of 60 min. Control assays were carried out with protein in the absence of compound. Data were analyzed in Protein Thermal Shift Software *(Thermofisher)*, and dTm by Derivative model was used to fit the fluorescence data to obtain the midpoint temperature for the thermal protein unfolding transition (T_m). Thermal shift © was calculated by subtracting T_m of protein with no ligand from T_m of protein with ligand.

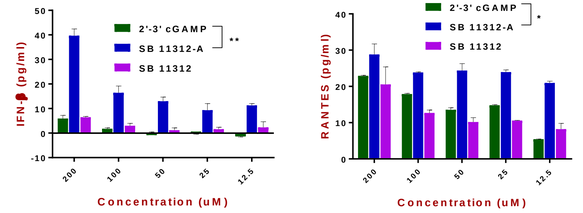
RESULTS

SB 11312 & SB 11312-A showed potent induction of IRF signaling in THP-1 & RAW macrophages



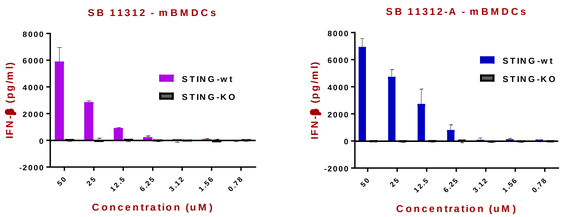
THP-1 dual cells and Raw-ISG cells were treated with various concentrations of SB 11312 or SB 11312-A or 2'-3'cGAMP. IRF activity was determined using QUANTI-luc. % induction was calculated from fold change in luminescence compared to DMSO treated sample.

SB 11312 & SB 11312-A showed potent IFN-β and RANTES from human PBMCs



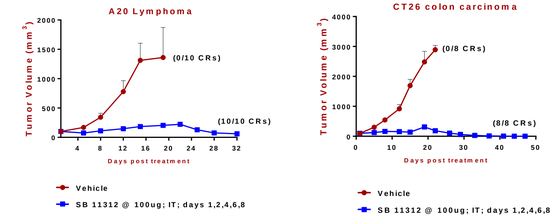
SB 11312 & SB 11312-A induced secretion of IFN-β and RANTES by human PBMCs treated with various concentrations of SB 11312 or SB 11312-A or 2'-3'cGAMP were measured by ELISA . For IFN-β, VeriKine-HS Human Interferon Beta Serum ELISA Kit (PBL Assay Science) and for RANTES, CCL5/RANTES Quantikine ELISA Kit (R & D Systems) were used. Representative data from one PBMCs donor is shown above.

SB 11312 & SB 11312-A showed potent STING dependent induction of IFN-β from mouse bone marrow derived dendritic cells (mBMDCs)



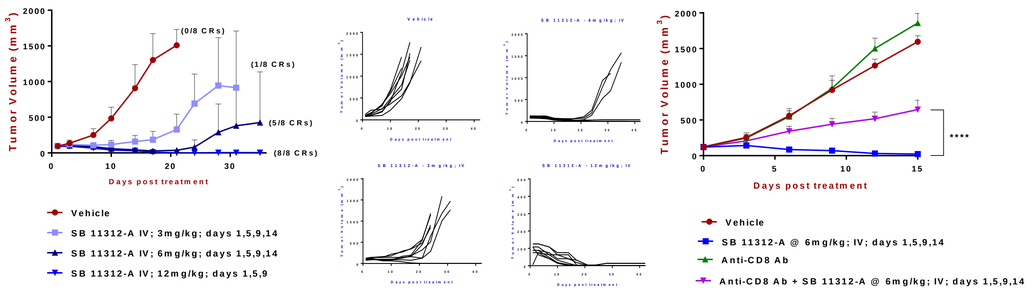
Bone marrow derived dendritic cells (BMDCs) differentiated from both STING-wt (C57BL/6J) and STING-KO (C57BL/6J-*Tmem173*^{gt}/J) mice were treated with various concentrations of SB 11312 or SB 11312-A or 2'-3'cGAMP. IFN-β secreted was measured by using mIFN-β lumikine kit (Invivogen).

Intratumorally administered SB 11312 in A20 Murine Lymphoma Model and CT26 Murine Colon Carcinoma Model show potent antitumor activity



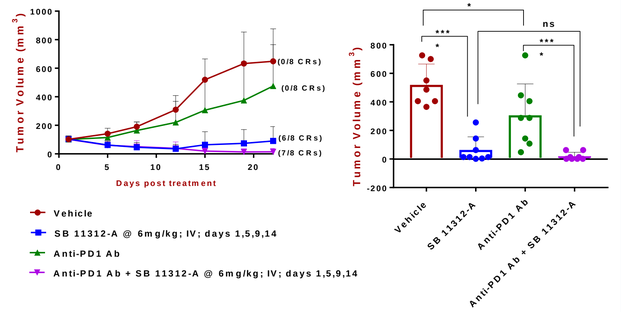
A20 or CT26 cells were implanted subcutaneously in right flank. When mean tumor volume reached 100mm^3, SB 11312 was administered IT @ 100µg/animal on days 1, 2, 4, 6 and 8. Mean tumor volumes were measured over the course of the study.

Intravenously administered SB 11312-A induced potent CD8$^+$ T cell dependent anti-tumor activity in CT26 colon carcinoma



CT26 cells were implanted subcutaneously in right flank and SB 11312-A treatment was started when mean tumor volume reached 100mm^3. SB 11312-A was administered IV @ 3/6/12 mg/kg on days 1,4,9,14. Anti-CD8 Ab (53-5.8) was administered IP on days -3,-1,0,7,14,21. Mean tumor volumes were measured over the course of the study

Intravenously administered SB 11312-A potently synergizes the weak anti-tumor activity of anti-PD1 antibody in the MC38 colon carcinoma mouse model



MC38 cells were implanted subcutaneously in right flank of mice (N=8) and SB 11312-A treatment was started when mean tumor volume reached 100mm^3. SB 11312-A was administered IV @ 6 mg/kg on days 1,4,9,14. Anti-PD1 RMPI-14 @ 5mg/kg was administered IP biwk X 2. Mean tumor volumes were measured over the course of the study.

Summary: We have discovered a highly potent first-in-class STING agonist SB 11285 that can be administered by systemic routes. SB 11312 (an active metabolite of SB 11285) and SB 11312-A (the single isomer of SB 11312) has demonstrated potent CD8$^+$ T cell dependent anti-tumor activity in multiple subcutaneous tumor models. As a novel agonist, SB 11312-A also potently enhanced anti-tumor activity of the anti-PD1 antibody.

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