The STING Agonist SB 11285 is a Broad-spectrum Antiviral Agent

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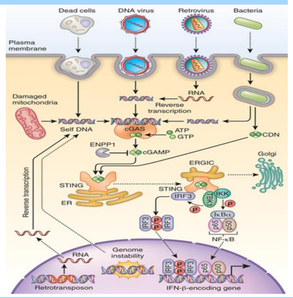
BACKGROUND

Cyclic guanylate adenylate synthase (cGAS), an important pattern recognition receptor (PRR) for viral genomes, uses Stimulator of Interferon Genes (STING) as the key adapter protein in the IRF3-IFN signaling axis to trigger innate and adaptive immune response for antiviral defense. We report here the antiviral evaluation of a novel nucleotide compound SB 11285, a potent STING agonist, that is being developed for immuno-oncology.



METHODS

In vitro antiviral evaluations of SB 11285 were conducted as follows:

1. **RSV:** We used RSVA2-infected (0.5 MOI) A549 cells (human lung epithelial cells) and viral titer was estimate aque assays
2. **Norovirus:** A replicon of Norovirus strain GI NoV in HG23 (hepatoma) cell line was used and activity assessed by RNA hybridization and quantitative PCR
3. **HCV:** Activity against HCV genotype 3 was assessed using the capture fusion assay. Briefly, THP-1 cells were exposed to donor serum, fused with Huh7 derivative cells and qPCR was used to assess HCV replication
4. **Hemorrhagic fever viruses:** Activity against JUNV strain 4454 and Dengue-2 strain NGC, was conducted respectively in A549 cells and extracellular yields were determined in Vero cells by plaque assays.

Cytotoxicity assays were done in parallel by neutral red, MTT or MTS methods.

RESULTS

RSV

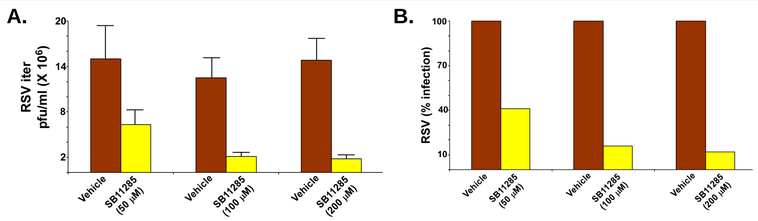


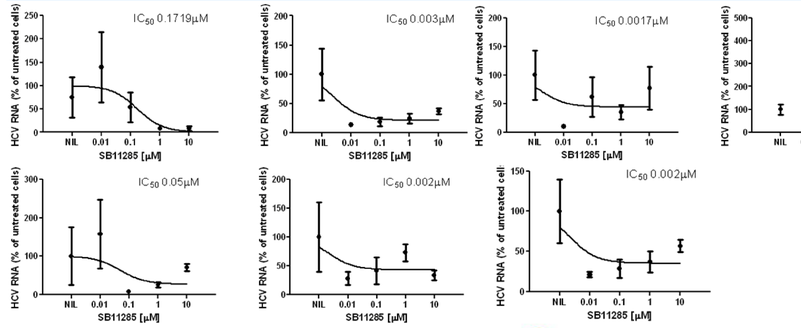
Figure 1: **(A)** RSV infection (RSV titer) was calculated by performing viral plaque assay. **(B)** RSV percentage infection was calculated based on the viral titer values shown in (A). 100% infection represents RSV infection in vehicle treated cells. For vehicle vs. SB11285 treated cells, $p \leq 0.05$ using Student's t test.

Norovirus

Compound	Assay Name	EC_{50}, μM	EC_{90}, μM	CC_{50}, μM	SI CC_{50}/ EC_{50}
2'-C-methylcytidine (Pos. control)	Primary assay, Replicon, RNA hybridization, neutral red for toxicity	6.8	18	>300	>17
SB 11285		0.342	1.0	>100	>292

Table 1: Antiviral activity of SB 11285 against Norovirus (strain GI NoV) in HG23 cell line. SB 11285 showed potent antiviral activity against norovirus with EC_{50} of 0.342 μM, and CC_{50} of 100 μM with a high selectivity index (>292). Cytotoxicity was measured through neutral red method.

HCV



Hemorrhagic Fever Viruses: JUNV and DENV

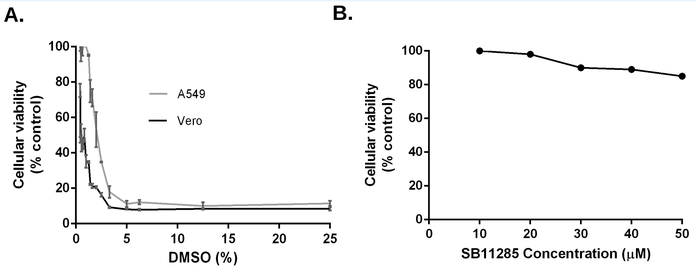


Figure 3: DMSO (A) and SB 11285 (B) cytotoxicity for Vero and A549 cells. Cytotoxicity was measured by MTT method. **(A)** SB 11285 was solubilized in DMSO at a non-toxic concentration for both cell lines. **(B)** SB 11285 CC50 could not be determined for the range of concentrations tested.

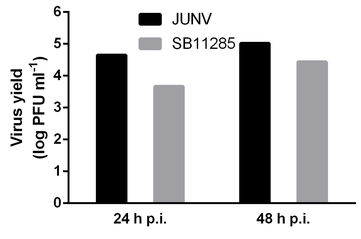


Figure 4: Antiviral activity of SB 11285 against Junín virus. Following pre-treatment with SB 11285, A549 cells were infected with JUNV and virus yield assessed. A 1 log reduction in viral yield was observed compared to untreated A549 infected cells, both at 24 and 48 hours post-infection (h p.i.)

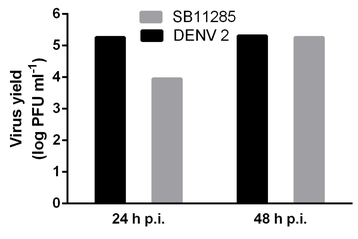


Figure 5: Antiviral activity of SB 11285 against Dengue virus serotype 2. Following pre-treatment with SB 11285, A549 cells were infected with DENV-2. SB 11285 caused reduction in virus yield of 1 log compared to untreated A549 infected cells at 24 h p.i. No significant antiviral activity was seen at 48 h p.i.

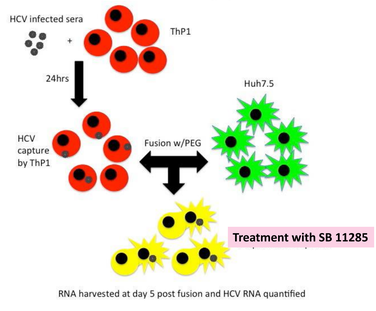


Figure 2: HCV Capture-Fusion Assay: Pre-stimulated THP-1 cells (PMA 200 ng/ml and IFNγ 10 ng/ml) were infected with serum from donors chronically infected with common HCV genotypes G3. Hybrid cells were treated with a range of concentrations of SB 11285. For comparison, fused cells infected with G3 sera were treated with Alisporivir. Cells were cultured for 5 days before quantification of HCV RNA by PCR. Dose-response curves were used to calculate IC_{50} values for each experiment.

CONCLUSION

SB 11285 elicited potent antiviral activity against all tested RNA viruses with EC50 ranging from 0.002 to 25 μM, and with high selectivity index.

Consistent with its mechanism of action, the STING agonist SB 11285 showed potent antiviral activity against several RNA viruses including hemorrhagic fever viruses. Additional preclinical studies are ongoing.

ACKNOWLEDGEMENTS

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