SR-8541A is a potent inhibitor of ENPP1 and exhibits dendritic cell mediated anti-tumor activity

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LB-118
AACR 2020

Abstract

Purpose: In recent years, programs targeting the innate immune pathways alone and alongside the adaptive immune pathways have become increasingly popular. A main area of interest in innate immunity is the STING (STimulator of INterferon Genes) pathway. STING plays an integral role by activating type 1 interferons in response to detection of cytosolic nucleic acid by cGAS (Cyclic GMP-AMP Synthase). cGAS detects cytosolic DNA and converts it into 2′3′ cGAMP, which is the direct ligand of STING. Targeting the direct activation of this pathway using synthetic, non-hydrolyzable forms of this ligand has shown to be effective in preclinical efficacy models, but has failed to impress in phase 1 clinical trials. Another method of activating this pathway is to inhibit the direct negative regulator, ENPP1 (Ectonucleotide pyrophosphatase/phosphodiesterase 1). ENPP1 constitutively hydrolyzes 2′3′-cGAMP, suppressing the pathway both in the cancer cells and in the surrounding tumor microenvironment. Previously, we reported that SR-8314, a highly selective and potent ENPP1 inhibitor, induces the activation of the STING pathway via type I interferon response in both *in vitro* and *in vivo* models. In this study, we continue this research and present our nominated clinical candidate, SR-8541A, which shows improved physiochemical characteristics and increased immune response.

Methods: Direct binding of SR-8541A to ENPP1 was evaluated using a thermal shift assay. Inhibition of ENPP1 enzymatic activity was shown using TMP or ATP as substrates in either cell free or cell based assays. RT-PCR, western blots, immune infiltration using peripheral blood mononuclear cells (PBMCs), and MesoScale Disovery (MSD) ELISA assays were performed to evaluate the effect of SR-8541A on the STING pathway. ENPP1 CRISPR knockout cells were generated to demonstrate on-target activity of SR-8541A. Pharmacokinetics, stability, and selectivity were completed with outside vendors.

Results: SR-8541A exhibits strong binding to ENPP1 and shows inhibitory activity at an IC_{50} of 1.4 nM. STING pathway activation was confirmed by a significant increase in gene expression of IFNβ, ISG15 and CXCL10 in SR-8541A treated cells. An increase in secreted type 1 interferon and other cytokines was also observed in these treated cells. Using immune infiltration assays, we show that SR-8541A stimulates the migration and infiltration of immune cells (PBMC) into cancer spheroids. Importantly, depletion of dendritic cells results in loss of infiltration of remaining PBMCs into cancer spheroids. Additionally, natural killer (NK) cells do not infiltrate into SR-8541A treated cancer spheroids unless co-cultured with dendritic cells. ENPP1 CRISPR knockout cell models confirmed that the drug effect was dependent on the presence of ENPP1. SR-8541A was stable for >60 mins in liver microsomes and S9 fractions from various species. Rodent pharmacokinetics showed a strong oral bioavailability of ~50% and tumor efficacy studies are ongoing.

Conclusions: In summary, we show that SR-8541A is a potent and selective inhibitor of ENPP1 that displayed strong immune response in 3D spheroid models. The results from these studies along with physiochemical properties and tumor model data will be presented. The promising physiochemical properties make it a strong candidate for clinical studies.

Introduction

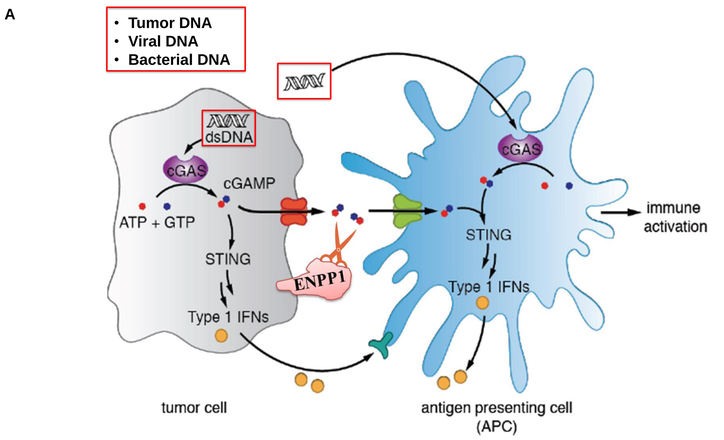

Fig 1A: A schematic model for activation of STING pathway in the innate immune response. ENPP1 negatively regulates the pathway by hydrolyzing 2′3′-cGAMP, the natural ligand of STING. The STING pathway responds to detection of foreign DNA, including DNA from leaky or dying tumor cells and bacterial/viral DNA.

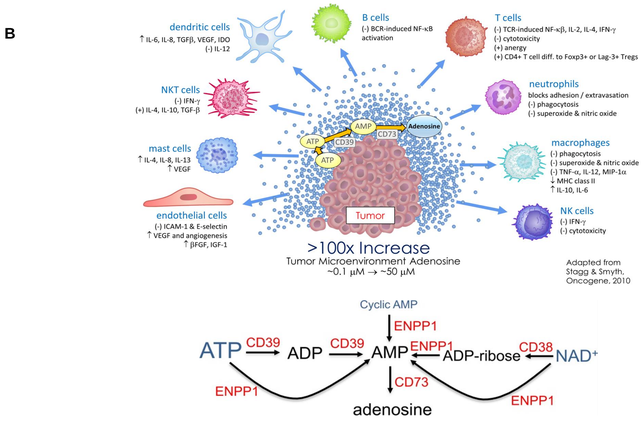

Fig 1B: A schematic model for production of adenosine, a major immune suppressor, in the tumor microenvironment. ENPP1 can hydrolyze ATP, cyclic AMP, and ADP-ribose to aid in the production of adenosine.

Results: SR-8541A selectively binds and inhibits ENPP1 activity

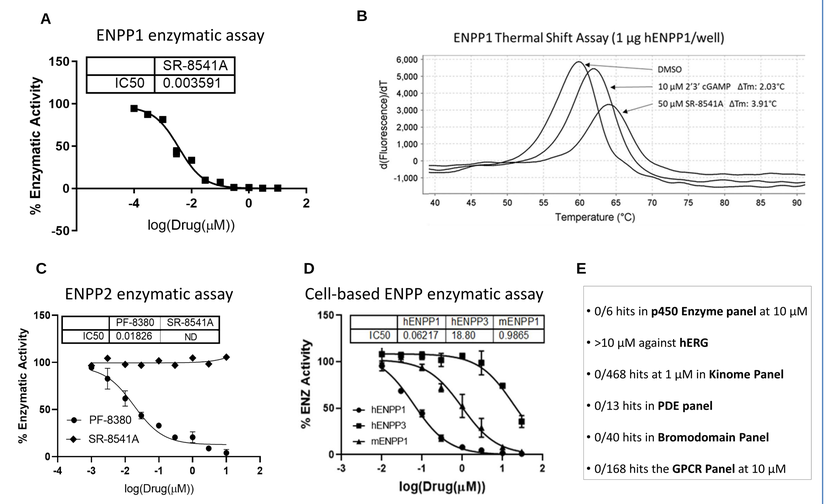

Fig 2: (**A**) Activity of recombinant hENPP1 protein was measured by incubating different concentrations of SR-8541A and thymidine 5'-monophosphate *p*-nitrophenyl ester as a substrate. (**B**) Protein melt assays were performed with recombinant hENPP1 protein and sypro orange dye in the presence or absence of indicated compounds. (**C**) Activity of recombinant hENPP2 protein was measured by incubating different concentrations of indicated compounds and Bis(*p*-Nitrophenyl) phosphate as substrate. (**D**) HEK293T cells were transfected with indicated ENPP constructs. After 24 hours, cell lysates were treated with SR-8541A for three hours at 4°C and ENPP activity was assessed as described in A. **(E)** SR-8541A activity was assessed against p450 (1A2, 2C19, 2C8, 2D6, 3A4), hERG, kinome, phosphodiesterase (PDE), bromodomain, and GPCR panels.

Results: SR-8541A model of catalytic binding

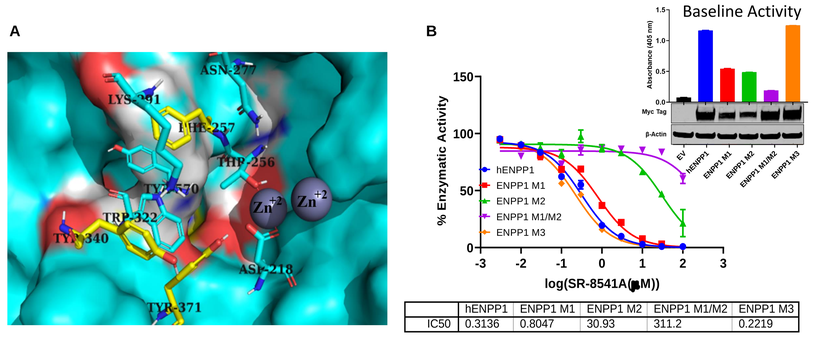

Fig 3: (**A**) Computational modeling of the catalytic pocket of hENPP1 showing critical residues. **(B)** HEK293T cells were transfected with wild type or mutant ENPP1 constructs. After 24 hours, cell lysates were treated with SR-8541A for three hours at 4°C and ENPP1 activity was assessed as described in fig 2.

Results: SR-8541A stimulates ENPP1 dependent lymphocyte infiltration

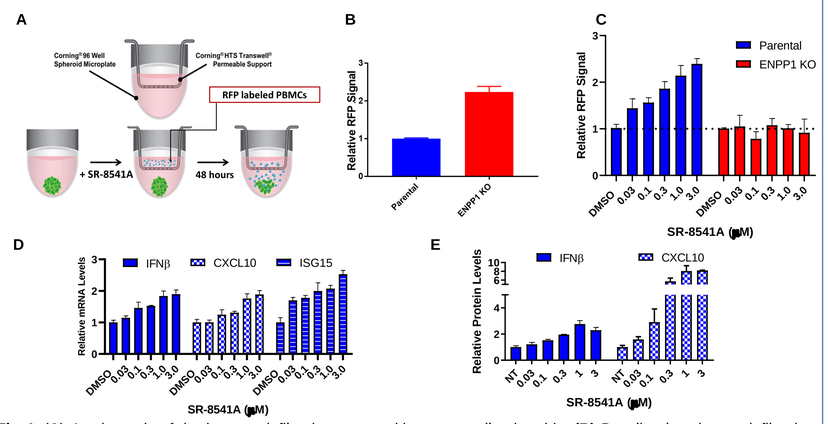

Fig 4: (**A**) A schematic of the immune infiltration assay with cancer cell spheroids. **(B)** Baseline lymphocyte infiltration into ENPP1 WT and knockout (KO) MDA-MB-231 spheroids. **(C)** Effect of SR-8541A treatment on lymphocyte infiltration into ENPP1 WT and KO MDA-MB-231 spheroids. (**D**) Expression of IFNβ, CXCL10, and ISG-15 were measured from MDA-MB-231 spheroids shown in C. **(E)** Secreted levels of IFNβ and CXCL10 were measured in the SR-8541A treated cell line media using the MSD platform.

Results: SR-8541A lymphocyte infiltration is mediated by dendritic cells

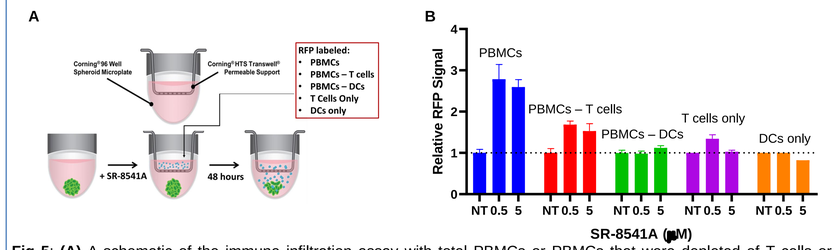

Fig 5: **(A)** A schematic of the immune infiltration assay with total PBMCs or PBMCs that were depleted of T cells or dendritic cells. **(B)** Lymphocyte infiltration in pancreatic cancer cells (HPAC) with or without T cells or dendritic cells in the presence of SR-8541A. T cells and dendritic cells alone were also tested in the assay. NT, not treated.

Results: SR-8541A exhibits single agent efficacy in murine colon cancer

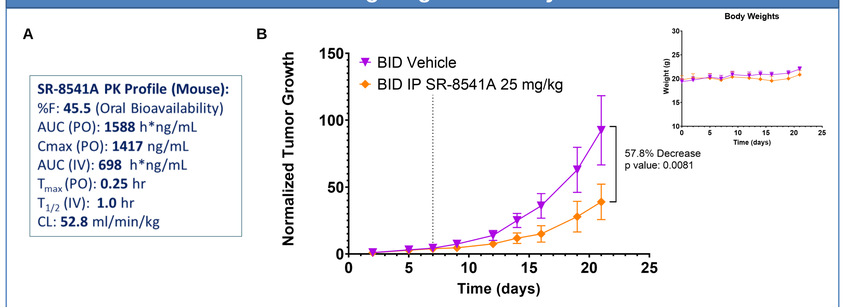

Fig 6: (**A**) Pharmacokinetic profile of SR-8541 in mice **(B)** Single agent effect of SR-8541A in a murine colon cancer model (CT-26). Mice were dosed at 25mg/kg BID for 14 days. Body weights of each mouse were measured every other day for the duration of the study.

Results: SR-8541A demonstrates synergy and abscopal response with radiation therapy in an MC38 model

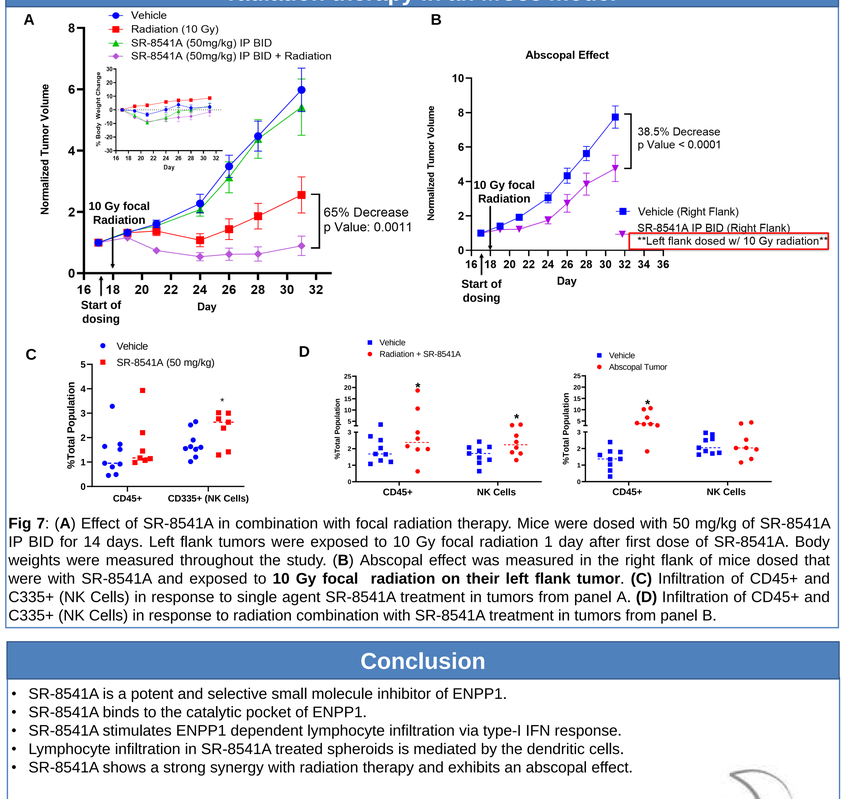

Fig 7: (**A**) Effect of SR-8541A in combination with focal radiation therapy. Mice were dosed with 50 mg/kg of SR-8541A IP BID for 14 days. Left flank tumors were exposed to 10 Gy focal radiation 1 day after first dose of SR-8541A. Body weights were measured throughout the study. (**B**) Abscopal effect was measured in the right flank of mice dosed that were with SR-8541A and exposed to **10 Gy focal radiation on their left flank tumor**. **(C)** Infiltration of CD45+ and C335+ (NK Cells) in response to single agent SR-8541A treatment in tumors from panel A. **(D)** Infiltration of CD45+ and C335+ (NK Cells) in response to radiation combination with SR-8541A treatment in tumors from panel B.

Conclusion

- SR-8541A is a potent and selective small molecule inhibitor of ENPP1.
- SR-8541A binds to the catalytic pocket of ENPP1.
- SR-8541A stimulates ENPP1 dependent lymphocyte infiltration via type-I IFN response.
- Lymphocyte infiltration in SR-8541A treated spheroids is mediated by the dendritic cells.
- SR-8541A shows a strong synergy with radiation therapy and exhibits an abscopal effect.

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COI: AW, TT, MK, SK, and SS own equity in Stingray Therapeutics

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