MV-626, A Potent and Selective Inhibitor of ENPP1, Enhances STING Activation and Augments T-cell Mediated Anti-tumor Activity in Vivo

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Abstract

Background

STING is an endogenous sensor of cGAMP, which is synthesized by cGAS following detection of cytoplasmic DNA. STING activation leads to interferon production and activation of inflammatory pathways that facilitate cytolytic T cell priming. STING agonists administered intratumorally show potent anti-tumor efficacy in a range of preclinical models; several agonists are in clinical development. Radiation therapy also increases cytoplasmic DNA levels in cancer cells, resulting in STING activation and secretion of inflammatory cytokines. Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) is the phosphodiesterase that negatively regulates STING by hydrolyzing cGAMP. MV-626, a highly potent and selective ENPP1 inhibitor with 100% oral bioavailability in rats and mice, blocks cGAMP hydrolysis and increases STING activation in cells where cGAS is active. We hypothesize that by conditionally enhancing STING activation, ENPP1 inhibitors will facilitate development of anti-tumor cellular immune responses, particularly following radiation therapy.

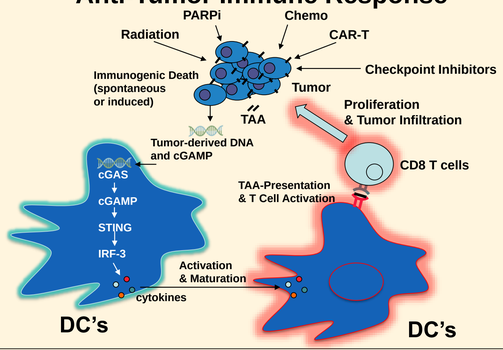
Methods

The effects of ENPP1 inhibition on STING activation using cGAMP or DNA treatment of cells were assessed in vitro. Panc02-SIY tumors were implanted in C57BL/6 mice and randomized to receive 20Gy CT-guided radiation therapy, 5 daily ip doses of MV-626, or both MV-626 and radiation. Mice were followed for outcome, tumor antigen specific T cell responses and changes in the tumor immune environment. Additional studies were conducted in C57BL/6 mice bearing MC38 tumors treated with anti-PDL1, MV-626 or MV-626 + anti-PDL1.

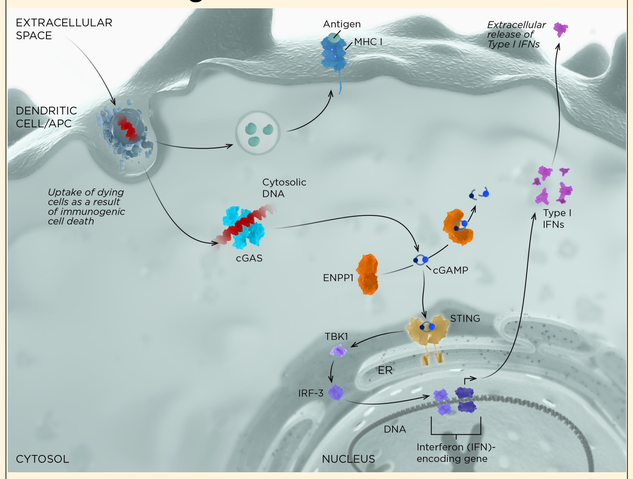
Results

In vitro, MV-626 blocks ENPP1-mediated hydrolysis of cGAMP and enhances STING activation by DNA-mediated cGAS activation or exogenous cGAMP. Therapeutic doses of MV-626 were well tolerated in mice, with no evidence of toxicity or clinically-significant increases in systemic cytokine levels. Systemic administration of MV-626 monotherapy caused tumor growth delay. MV-626 combined with radiation therapy significantly increased overall survival, and most animals achieved durable tumor cures. Additional studies in the MC38 model confirmed MV-626 activity. Studies characterizing effects of MV-626 in the tumor microenvironment are underway.

STING Pathway is a Central Mediator of Anti-Tumor Immune Response



MV-626 Inhibits ENPP1-Mediated Degradation of cGAMP

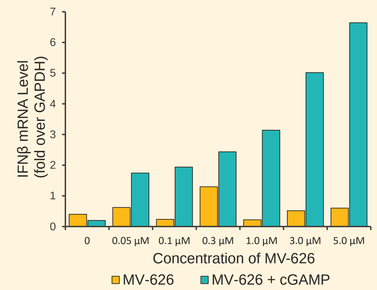


PARAMETER	MV-626
ENPP1 Ki (human)	~5 nM
ENPP1 Ki (mouse)	~18 nM
Solubility	11 μM
Mouse PK: Clearance	1.68 ml/min/kg
Mouse PK: terminal half life	8.8 hours
Rat PK: Clearance (mL/min/kg)	1.8 ml/min/kg
Rat PK: terminal half life	6.7 hours
Oral bioavailability	100% rat , 100% mouse
Selectivity vs canonical PDEs (1-12)	>500

MV-626 Enhances STING Responses

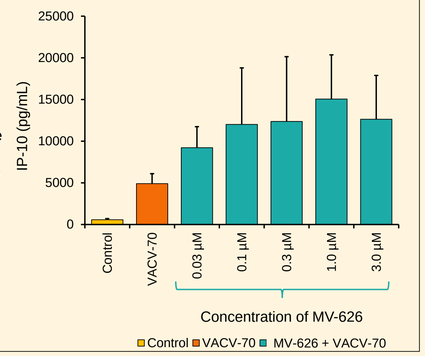
Human Fibroblast activation

- Cells incubated with increasing concentrations of MV-626, with or without cGAMP (12.5 μM) for partial STING pathway activation
- IFNβ mRNA measured after cGAMP addition (RT-PCR)

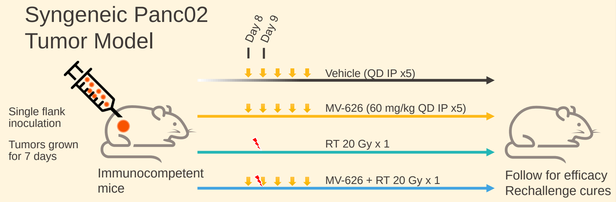


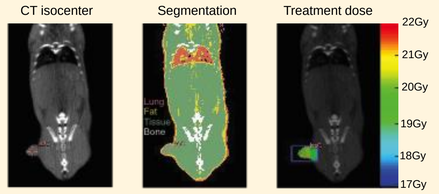
Human PBMC activation

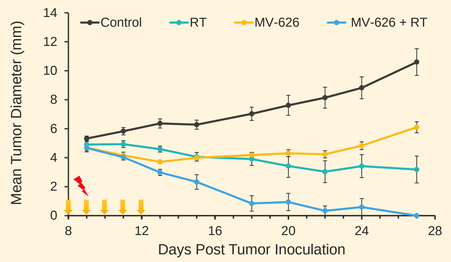
- VAC-70 DNA (5 μg/mL) added to activate cGAS, which produces cGAMP
- MV-626 added to enhance STING pathway signaling
- IP-10 measured by ELISA at 19 hrs



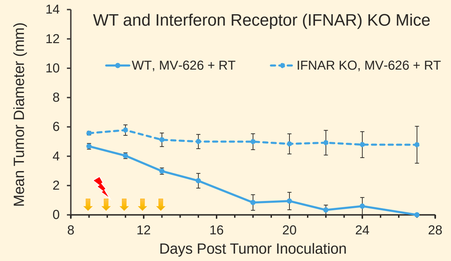
Combination of MV-626 with Radiation





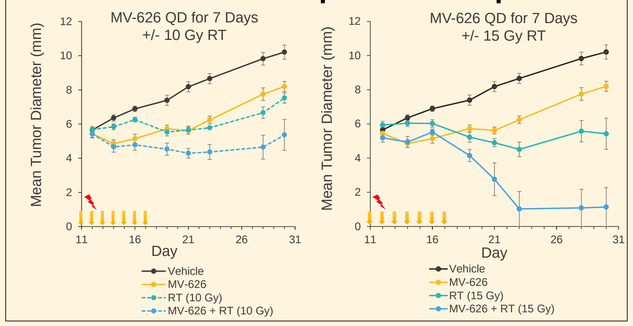


- 6/6 mice treated w/MV-626 + RT were cured by day 28
- All 6 cured mice were rechallenged at day 80
- 4/6 mice rejected the rechallenge, indicating **durable anti-tumor immunity**

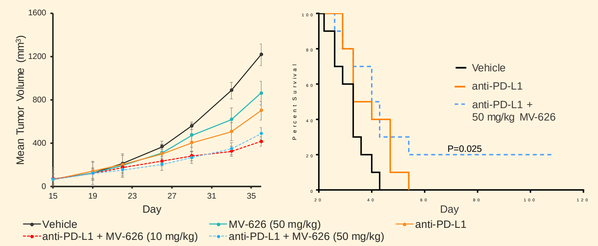


- Loss of MV-626 efficacy in IFNAR KO mice indicates a type 1 **IFN-dependent MOA**

Radiation Dose-Dependent Response



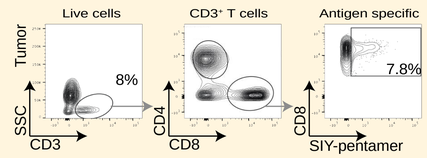
MV-626 Shows Monotherapy Activity and Enhances anti-PD-L1 Efficacy in MC38 Tumor Model

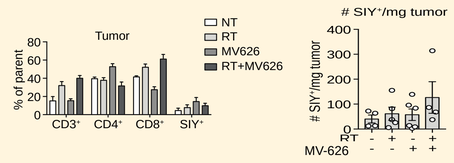


- MAVU-626 was dosed at 50 mg/kg PO QD on days 15 -36; data presented as mean +/- SEM
- Anti-PD-L1 mAb (10 mg/kg) was dosed twice weekly x 6 doses beginning on day 14
- Only anti-PD-L1 + 50 mg/kg MAVU-626 group had survival with p<0.05 vs vehicle (Wilcoxon; no animals were censored)

Immune Environment Changes

i) Identification of antigen-specific T cells





Conclusions

These data demonstrate the potent and selective ENPP1 inhibitor MV-626, when delivered orally or IP, augments STING activation in vitro and enhances immune responses to tumors in vivo. We demonstrate for the first time that, in combination with radiation therapy or anti-PDL1 mAb treatment, ENPP1 inhibition improves outcomes and cures tumors in preclinical models through changes in the tumor immune environment. The loss of this efficacy in IFN receptor knockout mice confirms STING-pathway mechanism of action, and the ability of the majority of MV-626 treated mice to reject tumor rechallenge indicates that MV-626 drives disseminated, durable, adaptive immune response. These translational studies demonstrate a novel approach to STING pathway modulation and form the foundation for clinical development of an ENPP1 inhibitor as a cancer immunotherapy.

Future Directions

- Role of host STING activation and CD8 T cells
- Additional profiling of tumor antigen specific responses
- Evaluate distal tumor responses
- Clinical translation – biomarker identification
- Nanostring profiling of immune changes in the tumor and LN
- Evaluation of ENPP1 inhibition with other anti-cancer agents that produce immunogenic tumor cell death

Acknowledgements

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