**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**

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**

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 clinical changes in the tumor immune environment. Additional studies were conducted in C57BL/6 mice bearing MC38 tumors treated with anti-PD-L1, MV-626 or MV-626 + anti-PD-L1.

**Results**

In vivo, 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 clinical changes in the tumor immune environment. Additional studies were conducted in C57BL/6 mice bearing MC38 tumors treated with anti-PD-L1, MV-626 or MV-626 + anti-PD-L1.

**Human Fibroblast activation**

- Cells incubated with increasing concentrations of MV-626, with or without cGAMP (125 μM) for partial STING pathway activation
- IFN-γ mRNA measured after cGAMP addition (RT-PCR)

**Human PBMC activation**

- VAC7 DNA (5 μg/mg), added to activate cGAS, which produces cGAMP
- MV-626 added to enhance STING pathway signaling
- IP-10 measured by ELISA at 19 hrs

**Conclusions**

These data demonstrate the potent and selective ENPP1 inhibitor MV-626, when delivered orally or IV, 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-PD-L1 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 in CD4 T cell responses
- Additional profiling of tumor antigen-specific responses
- Evaluate distant tumor responses
- Clinical translation – biomarker identification

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