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DISCOVERY OF A NOVEL, POTENT SMALL MOLECULE INHIBITORS OF ENPP1 ENHANCES ANTI-TUMOR EFFICACY IN COMBINATION WITH IMMUNE CHECK INHIBITORS

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Background The cGAS-STING (cyclic GMP-AMP synthase-stimulator of interferon genes) signaling pathway has led to significant innate immune responses in cancer immunotherapy. 2'3'-cGAMP, the second messenger in the cGAS-STING signaling pathway, binds to STING and promotes the production of various pro-inflammatory cytokines such as type I interferon (IFN). Therefore, activated STING pathway has been considered an important target for cancer immunotherapy. However, STING agonists excessively activate the STING pathway, causing side effect due to hyper-accumulation of cytokines. Here we show that targeting ENPP1 is emerging as a promising target of STING regulation in immunotherapy, because it indirectly activates the STING pathway. Ecto-nucleotide pyrophosphatase/phosphodiesterases 1 (ENPP1), highly expressed membrane-bound enzyme in cancer cells, modulates cGAMP levels by hydrolyzing 2',3'-cGAMP to alter the inflammatory milieu. In this study, we present our potent ENPP1 inhibitors, which show highly improved enzymatic activities, immune cell related immune responses in vitro and CT-26 syngeneic mouse model.

Methods We discovered potent ENPP1 inhibitors with highly potent activities in various cell-based assay systems. The in vivo antitumor efficacy was assessed as monotherapy and in combination with Anti-PD-L1 (immune checkpoint inhibitor) by monitoring tumor growth for 15 days, in a CT-26 syngeneic mouse model.

Results Our two candidates showed excellent inhibitory activity of nanomolar level in a non-cell-based in vitro ENPP1 inhibition assay using pNP-TMP (artificial substrate) and cGAMP as substrates, respectively. In vitro THP-1 dual reporter assay demonstrated the potency and efficacy of the compounds that induce STING-mediated type I IFN release. In addition, one of the two candidates exhibited good PDE and kinase panel selectivity as well as good in vivo pharmacokinetic properties.

Finally, both candidates exhibited good anticancer efficacy in the CT-26 colorectal cancer syngeneic mouse model. IV administration of one candidate at 25 mg/kg demonstrated a TGI of 53% as monotherapy and a TGI of 81% in combination with anti-PD-L1 treatment. In contrast, another candidate, when administered orally at 25 mg/kg, provided a TGI of 39% as monotherapy and a TGI of 86% in combination with anti-PD-L1 antibody treatment. Therefore, our candidates demonstrated a much better efficacy profile in combination with Anti-PD-L1 antibody.

Conclusions Taken together, our study demonstrates that a novel and potent small molecule inhibitor that inhibits extracellular ENPP1 has been designed. Our candidates with desirable drug properties, were evaluated for activity in the syngeneic mouse tumor model CT-26. As a result, these candidates were well tolerated and demonstrated significant antitumor activity when combined with anti-PD-L1 antibody.

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