

THE FIRST-IN-CLASS SMALL MOLECULE TREX1 INHIBITOR CPI-381 DEMONSTRATES TYPE I IFN INDUCTION AND SENSITIZATION OF TUMORS TO IMMUNE CHECKPOINT BLOCKADE<http://dx.doi.org/10.1136/jitc-2021-SITC2021.765>

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Background TREX1 is an exonuclease that functions as a negative regulator of innate immunity. TREX1 controls dsDNA sensing in tumor and immune cells by preventing aberrant dsDNA buildup that triggers STING-mediated Type 1 Interferon (IFN) induction leading to priming of the adaptive immune system. Loss of function mutations in TREX1 and genetic ablation of *trex1* in mice lead to induction of IFN-beta-driven autoimmunity. Thus, TREX1 is a promising target to elicit IFN-mediated anti-tumor immunity.

Methods To characterize TREX1 inhibitors we developed cell-based assays utilizing human HCT116 carcinoma and THP-1 monocytic Dual reporter cell lines to monitor IRF activity. Activation of cGAS was assessed by measuring cGAMP levels in B16F10 melanoma cells. The potency of TREX1 inhibitors in primary human dendritic cells (DC)s was analyzed by measuring IFNbeta induction by exogenous dsDNA. Analysis of tumor growth inhibition following TREX1 inhibitor treatment was conducted in mouse syngeneic tumor models. TREX1 activity was assessed by measuring degradation of a custom dsDNA substrate.

Results We report here the development of a small molecule TREX1 inhibitor, CPI-381, with nanomolar cellular potency, which translated into a robust induction of IRF reporter activity. We observed a significant increase in cGAMP production in B16F10 cells transfected with DNA in the presence of CPI-381, suggesting that CPI-381-mediated inhibition of TREX1 leads to the activation of dsDNA sensors, such as cGAS. Treatment of THP-1 cells with CPI-381 induced the expression of several key ISG involved in innate immunity. Moreover, inhibition of TREX1 with CPI-381 phenocopied the effect of TREX1 genetic deletion in primary human DCs by upregulating IFNbeta. To evaluate whether TREX1 negatively regulates IFNbeta production in syngeneic tumor models, we knocked down *trex1* in B16F10, MB49, MC38, and CT26 murine cells. Accumulation of cytosolic dsDNA resulted in a substantial increase in IFNbeta secretion by all four TREX1-KO cell lines. In vivo efficacy studies with CPI-381 demonstrated reduced tumor growth in the MC38 syngeneic tumor model either alone or in combination with anti-PD1. We observed a reduction of TREX1 activity in CPI-381 treated tumors, confirming an inverse relationship between TREX1 intra-tumor activity and tumor growth, and efficient target engagement after systemic (oral) delivery.

Conclusions We have developed a first-in-class, potent TREX1 inhibitor demonstrating excellent in vitro and in vivo potency via enhancement of cytosolic dsDNA sensing and induction of IFNbeta in cancer and immune cells. CPI-381-induced tumor-intrinsic TREX1 inhibition elicits antitumor immunity as a single agent and increases response to immune checkpoint blockade via mechanisms downstream of TREX1 that activate type I IFN signaling.

Ethics Approval All animal work was approved and conducted under the oversight of the Charles River Accelerator and Development Lab (CRADL, Cambridge, MA) Institutional Animal Care and Use Committee (protocol # 2021-1258).