1338 CRYSTALLOGRAPHY-GUIDED CHARACTERIZATION OF NOVEL CYTOSOLIC DNASE 3′ REPAIR EXONUCLEASE 1 (TREX1) INHIBITORS

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Background The stimulator of interferon genes (STING) protein is an innate immune sensor critical for the development of immunity. Tumor cells frequently inactivate the STING pathway to avoid immune recognition, emphasizing its importance in generating tumor-specific immunity. Broad tumor-specific activation of STING in advanced cancers may be required to initiate $CD8^+$ T cell priming against unique antigenic repertoires among distinct metastases and to reverse an immune-suppressive tumor microenvironment. Due to STING's ubiquitous expression, systemic delivery of untargeted STING agonists may not achieve a therapeutic index. In contrast, expression of the cytosolic DNAse 3' repair exonuclease (TREX1) is upregulated in tumor cells in response to genomic instability, inflammatory stimuli, and DNA replication, providing an opportunity for selective activation of the STING pathway. In addition to modulating cyclic GMP-AMP synthase (cGAS)/STING signaling, interaction with DNA replication enzymes that generate immunogenic DNA waste highlight a facet of TREX1 biology that may inform clinical development of targeted inhibitors.

Methods Using a structure-based drug design strategy, we designed and optimized small-molecule inhibitors of TREX1 with drug-like physicochemical properties that were profiled in biochemical and cell-based assays. X-ray crystallography studies, thermal shift, and biochemical assays were employed to determine mechanism of action. We evaluated the in vivo profile of select compounds.

Results Inhibitors of TREX1 with < 100 μM potency were optimized into two distinct chemical series with nanomolar potency against purified, recombinant murine and human TREX1 protein in biochemical assays. Inhibitors had similar IC_{50} values against TREX1 nuclease in a cell-lysate based assay. Thermal shift analysis demonstrated that inhibitor interactions with TREX1 required magnesium. To facilitate understanding of structure-activity relationship, we produced the first high-resolution co-crystal structures of inhibitor-bound human and mouse TREX1. We used these structures to dissect mouse- and human-specific interactions, identifying key residues driving species specificity of a lead series in biochemical and cellular assays. Systemic administration of lead inhibitors achieved exposures necessary for target engagement in tumors of mice, as shown by a tumor lysate TREX1 nuclease assay. Pharmacologic inhibition of TREX1 nuclease activity resulted in tumor growth inhibition.

Conclusions We present the identification and characterization of potential first-in-class TREX1 inhibitors with nanomolar potency against human and mouse TREX1 and provide a mechanism to explain species specificity. Treatment with TREX1 inhibitors conferred anti-tumor activity in mouse models demonstrating that targeting TREX1 can specifically and locally engage the STING pathway in the tumor microenvironment, enhance tumor-specific immunity, and provide therapeutic benefit.

Ethics Approval This study is a non-GLP study. All the procedures related to animal handling, care, and the treatment in this study will be performed according to guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of Pharmaron following the guidance of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

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