IMMUNOPEPTIDOME CHANGES MEDIATED BY A NOVEL ERAP1 INHIBITOR LEADS TO TUMOR GROWTH INHIBITION

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Background Clinical data demonstrates increased antigen presentation diversity is a key factor in determining response rates to checkpoint inhibitors.1 In addition to tumour mutational burden/microsatellite instability, increased HLA heterozygosity and HLA evolutionary diversity are non-overlapping factors recently identified to further diversify the immunopeptidome and improve clinical response to checkpoint therapies.2,3 Endoplasmic reticulum aminopeptidase 1 (ERAP1) is an enzyme that trims peptides loaded into classical and nonclassical I MHC molecules.4,5 Ablation of mouse ERAP modifies the immunopeptidome, resulting in improved immunogenicity, generation of CD8 T cell responses and tumor growth inhibition.6,7 Recently identified selective small molecules potently inhibit ERAP1 across key species and haplotypes.8 We report the further profiling of lead candidate ERAP1 inhibitors in human primary T cell in vitro assays and in vivo tumor models in mice.

Methods Human cancer cell lines treated with ERAP1 inhibitors in vitro or in vivo in xenograft mouse models were assessed by immunopeptidomics9 to profile peptide repertoire changes. Novel or upregulated peptides were also tested in human immunogenicity assays. FACS analysis of T cells stimulated with Tyrosinase mRNA transfected human dendritic cells ± ERAP1 inhibition was to assess T cell repertoire changes. ERAP1 inhibitor and anti PD-1 mAb combination was assessed in syngeneic mouse tumor models to investigate tumour growth inhibition and PD end-points (eg. IHC).

Results Analysis of human cervical, lung, colorectal and melanoma cell lines carrying distinct HLA haplotypes demonstrates a consistent and profound effect of ERAP1 inhibition on the immunopeptidome. Novel and upregulated cancer associated antigens identified in association with multiple different HLA-A and B alleles stimulate IFNγ production in primary naïve human T cell immunogenicity assays. The impact of ERAP1 inhibition on the T cell repertoire to the melanoma antigen tyrosinase is ongoing. The combination of ERAP1 inhibitor and anti PD-1 mAb led to significant tumour growth inhibition in the CT26 syngeneic mouse tumor model that correlated with increased infiltration of T cells to the tumor. Further PD end-points to be analysed include immune gene array and TCR Vbeta repertoire.

Conclusions Grey Wolf ERAP1 inhibitors significantly modify the immunopeptidome both in vitro and in vivo across a broad range of HLA and tumor types. Combination of these inhibitors with anti PD-1 leads to significant T cell infiltration and tumor growth inhibition. Thus, ERAP1 mediated modulation of the immunopeptidome has the potential to drive anti tumor T cell responses and be a transformative immunotherapy.

REFERENCES
overcome the checkpoint resistance seen in contemporary treatments involving PD-1.

Ethics Approval All mouse studies described in this work were carried out in accordance with the principles of the Guide for the Care and Use of Animals and were approved by the Institutional Animal Care and Use Committee of Dartmouth College, NH, USA (protocol 2012).

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**DISCOVERY OF CLINICAL CANDIDATE IK-175, A SELECTIVE ORALLY ACTIVE AHR ANTAGONIST**

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**Background** Aryl Hydrocarbon Receptor (AHR) is a transcription factor that regulates the activity of multiple innate and adaptive immune cells subsequent to binding to a diverse set of endogenous and exogenous ligands. One such endogenous AHR ligand is kynurenine, generated from the precursor tryptophan by indoleamine-pyrrole 2,3-dioxygenase 1 (IDO1) and tryptophan 2,3-dioxygenase 2 (TDO2). Binding of kynurenine to AHR leads to a net immunosuppressive tumor microenvironment. In addition, increased levels of serum kynurenine are associated with resistance to checkpoint inhibitors. Given that kynurenine can be generated by both IDO1 and TDO2 and that AHR is activated by multiple other endogenous ligands, AHR inhibition provides a novel and ideal approach to overcome immunosuppression in a broad range of cancers.

**Methods** We sought to identify an orally active AHR antagonist as an immunomodulatory agent for the treatment of solid tumors. Lead optimization efforts identified IK-175 as an AHR antagonist with a favorable ADME and pharmacokinetic profile in preclinical species.

**Results** IK-175 inhibits AHR activity in rodent and human cancer cell lines as well as human and nonhuman primate primary immune cells, with concentration dependent effects on AHR target gene expression and cytokine release. IK-175 is inactive in a broad panel of kinases, receptors, and transporters. Orally administered IK-175 dose-dependently blocks ligand-stimulated-AHR activation of Cyp1a1 transcription in liver and spleen, demonstrating on-target in vivo activity in mice. IK-175 alone and in combination with an anti-PD-1 antibody demonstrates significant antitumor activity in syngeneic mouse models of colorectal cancer (CT26.WT) and melanoma (B16-IDO1). In addition, IK-175 in combination with liposomal doxorubicin demonstrates antitumor activity in syngeneic mouse models of colorectal cancer (CT26.WT and MC38).

**Conclusions** These studies provide rationale for targeting AHR in cancer patients. Ikena will evaluate the anti-tumor activity of IK-175 as a single agent in cancers with activated AHR and in combination with other therapies. Overall, our data demonstrates that IK-175 is a selective orally active AHR antagonist that inhibits tumor growth and reverses immune suppression in mouse tumors models. IK-175 is currently being evaluated in a Phase 1 clinical trial in patients with advanced solid tumors and urothelial carcinoma (Clinicaltrials.gov NCT04200963).

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**NEOADJUVANT CYCLIC DINUCLEOTIDES COMBINED WITH INTERLEUKIN-2 AND ANTI-PD-1 ANTIBODY LIMIT LUNG METASTASIS OF ORTHOTOPIC BREAST TUMORS THROUGH PROLONGED NK CELL ACTIVATION**

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**Background** Cyclic dinucleotides (CDN) – agonists of stimulator of interferon genes – can initiate potent anti-tumor immunity by activating antigen presenting cells which prime CD8+ T cells.1 Recent studies have also highlighted CDN activation of NK cells via IL-15 in T cell-resistant tumors.2 Thus far, limited analysis has been made of the impact of CDN-based therapies on cancer metastasis. We employed a surgical resection model of metastatic mammary carcinoma to examine the effects of surgery – a predominant breast cancer intervention – and lung metastasis on neoadjuvant therapy with CDNs combined with other clinically-relevant immunotherapies including IL-2 and anti-PD-1.1

**Methods** 4T1-luciferase cells were inoculated in the mammary fat pad, palpable tumors were treated with immunotherapy starting eight days later, any remaining primary tumor was surgically resected on day 17, and metastases were monitored by luciferase imaging. Combinations of intratumoral biphosphonothioate 2′,3′-c-di-AMP (CDN), intraperitoneal (i.p.) albumin-IL2 fusion protein (Alb-IL2), and i.p. anti-PD-1 were tested in this model by measuring primary tumor growth and monitoring overall survival. CD8+ T cells, CD4+ T cells, or NK cells were depleted using anti-CD8 (2.43), anti-CD4 (GK1.5), and anti-asialo-GM1 antibodies, respectively, administered i.p. every 3 days beginning one day prior to treatment initiation. Immunophenotyping of primary tumors and lungs was conducted at several timepoints after starting therapy.

**Results** In mice bearing orthotopic 4T1-luciferase tumors, administration of three doses of CDN resulted in no cures in the absence of surgical resection. When administered prior to surgical resection CDN monotherapy yields a 20% cure rate and enhanced median overall survival compared to untreated mice (median survival 44.5 days vs 38 days, p=0.0026). Combination of CDN with Alb-IL2 and anti-PD-1 substantially improved survival, with 60% of mice surviving long-term. Through cellular depletions we determined that neither CD8+ nor CD4+ T cells were required for efficacy in this neoadjuvant therapy model, while NK cell depletion decreased survival rate by approximately 50%. Lung immunophenotyping of CDN/Alb-IL2/anti-PD-1-treated mice revealed a near doubling of the absolute NK cell count compared to untreated controls. More strikingly, lung infiltrating NK cells in the CDN/Alb-IL2/anti-PD-1 cohort exhibited prolonged granzyme B production compared to CDN monotherapy – examine the effects of surgery – a predominant breast cancer intervention – and lung metastasis on neoadjuvant therapy with CDNs combined with other clinically-relevant immunotherapies including IL-2 and anti-PD-1.1

**Conclusions** Our findings suggest that combining intratumoral CDN with systemic Alb-IL2 and anti-PD-1 can delay the growth of primary breast tumors and limit metastatic outgrowth in the lungs. Efficacy is attributed to sustained cytotoxicity of NK cells.

**Ethics Approval** All mouse experiments were approved by MIT’s Committee on Animal Care, protocol #0720-070-23.

**REFERENCES**