

bead arrays and the peptidome library generated above will be used to assess anti-cancer B and T cell responses.

**Trial Registration** NCT04470024

**Ethics Approval** The original clinical trial was approved by the Providence Portland Medical Center IRB, approval # 13-046. The proposed clinical trial has not yet been reviewed by the IRB.

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#### MHC-I SKEWING IN MUTANT CALRETICULIN-POSITIVE MYELOPROLIFERATIVE NEOPLASMS IS COUNTERED BY HETEROCLITIC PEPTIDE CANCER VACCINATION

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**Background** The majority of JAK2V617F-negative myeloproliferative neoplasms (MPN) have disease-initiating frameshift mutations in calreticulin (CALR) resulting in a common novel C-terminal mutant fragment (CALRMUT), representing an attractive source of neoantigens for cancer vaccines. However, studies have shown that CALRMUT-specific T cells are rare in CALRMUT MPN patients, but the underlying reasons for this phenomenon are unknown.

**Methods** In this study, we examine class-I major histocompatibility complex (MHC-I) allele frequency in CALRMUT MPN patients from two independent cohorts and observed that MHC-I alleles that present CALRMUT neopeptides with high affinity are under-represented in CALRMUT MPN patients. We speculate that this is due to an increased chance of immune-mediated tumor rejection by individuals expressing one of these MHC-I alleles such that the disease never clinically manifests. As a consequence of this MHC-I allele restriction, we reasoned that CALRMUT MPN patients would not efficiently respond to cancer vaccines composed of the CALRMUT fragment, but could do so when immunized with a properly modified CALRMUT heteroclitic peptide vaccine approach.

**Results** We found that heteroclitic CALRMUT peptides specifically designed for CALRMUT MPN patient MHC-I alleles efficiently elicited a cross-reactive CD8+ T cell response in human PBMC samples otherwise unable to respond to the matched weakly immunogenic CALRMUT native peptides. We also modeled this effect in mice and observed that C57BL/6J mice, which are unable to mount an immune response to the human CALRMUT fragment, can mount a cross-reactive CD8+ T cell response against a CALRMUT-derived peptide upon heteroclitic peptide immunization and this was further amplified by combining the heteroclitic peptide vaccine with blockade of the immune checkpoint molecule PD-1.

**Conclusions** Together, our data underscore the therapeutic potential of heteroclitic peptide-based cancer vaccines in CALRMUT MPN patients.

**Ethics Approval** Approval was obtained for the use of patient-derived specimens and access to clinical data extracted from patient charts by the Institutional Review Boards at Memorial Sloan Kettering Cancer Center, the Dana-Farber Cancer Institute and the Massachusetts General Hospital, as well as by the Danish Regional Science Ethics Committee. Mouse experiments were performed in accordance with institutional guidelines under a protocol approved by the Memorial Sloan-Kettering Cancer Center Institutional Animal Care and Use Committee.

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#### BLOCKADE OF THE INHIBITORY COLLAGEN RECEPTOR LAIR-1 WITH NC410, A LAIR2-FC FUSION PROTEIN, ENHANCES ANTI-TUMOR ACTIVITY OF THE BIFUNCTIONAL FUSION PROTEIN BINTRAFUSP ALFA

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**Background** LAIR-1 is an immune inhibitory receptor expressed on several immune cell types including activated T cells, B cells, NK cells, macrophages, and dendritic cells. The ligands for LAIR-1 contain collagen-like domains which are commonly found in extracellular matrix collagens and complement component C1q. In numerous cancer types, including gastric, colon, ovarian, bladder, and others, upregulation of collagens has been shown to enhance tumor growth, metastases, and invasion while actively suppressing antitumor immunity. Although humans produce a natural, soluble decoy, LAIR-2, that competes with LAIR-1 for binding of collagen domains, excess LAIR ligands in the tumor often result in an immune suppressive environment.

**Methods** Here, we report on a novel immunotherapy approach which combined NC410, a LAIR-2-Fc fusion protein capable of blocking LAIR-1 signaling, and bintrafusp alfa, a first-in-class bifunctional fusion protein composed of the extracellular domain of the human transforming growth factor  $\beta$  receptor II (TGF- $\beta$ R2 or TGF- $\beta$  'trap') fused via a flexible linker to the C-terminus of each heavy chain of an IgG1 antibody blocking programmed death ligand 1 (anti-PD-L1).

**Results** We demonstrate that the combination of NC410 and bintrafusp alfa more effectively controls in vivo tumor growth of the collagen rich MC38 colon carcinoma compared to either monotherapy. We hypothesize that this potent anti-tumor immune response is propagated through the synergy of activated tumor infiltrating lymphocytes and a repolarization of macrophages towards a tumoricidal phenotype. MC38 tumors treated with the combination of NC410/Bintrafusp alfa contained higher numbers of infiltrating CD4+ and CD8+ T cells and higher numbers of CD38+ and MHCII+ M1 polarized macrophages.

**Conclusions** This study highlights the synergy of reshaping the large suppressive myeloid cell populations often present in tumors with activation of adaptive T-cell immune responses dampened by checkpoint inhibition. The results also provide the rationale for the future evaluation of this combination therapy in the clinic.

**Acknowledgements** Bintrafusp alfa was kindly provided by EMD Serono under a CRADA with the NCI.

**Trial Registration** N/A

**Ethics Approval** Mice were maintained under pathogen-free conditions in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care guidelines. All animal studies were approved by the NIH Intramural Animal Care and Use Committee under protocol LTIB-038.

**Consent** N/A

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#### 446 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.<sup>1</sup> 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.<sup>2</sup> <sup>3</sup> Endoplasmic reticulum aminopeptidase 1 (ERAP1) is an enzyme that trims peptides loaded into classical and nonclassical class I MHC molecules.<sup>4</sup> <sup>5</sup> Ablation of mouse ERAAP modifies the immunopeptidome, resulting in improved immunogenicity, generation of CD8 T cell responses and tumor growth inhibition.<sup>6</sup> <sup>7</sup> Recently identified selective small molecules potently inhibit ERAP1 across key species and haplotypes.<sup>8</sup> 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 immunopeptidomics<sup>9</sup> 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 (e.g. 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 IFN $\gamma$  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 tumor 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.

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#### 447 VISTA TARGETING REMODELS THE TUMOR MICROENVIRONMENT TO OVERCOME ADAPTIVE RESISTANCE

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**Background** VISTA is a negative checkpoint regulator prominently expressed in the TME of a wide variety of cancers. In a preclinical model of colorectal cancer, monotherapy of small tumors (40 mm<sup>3</sup>) with anti-VISTA results in markedly slowed tumor growth. Mice bearing significantly larger tumors (600 mm<sup>3</sup>) are resistant to anti-PD-1 and anti-CTLA4 treatment and all mice die following treatment, indicating checkpoint resistance. Inclusion of anti-VISTA leads to complete rejection of 50% of tumors.

**Methods** The underlying therapeutic mechanisms of leading to enhanced anti-tumor immunity in both models was investigated by high-dimensional scRNAseq of the CD45+ immune infiltrate of tumors 10 days after treatment initiation.

**Results** In both modes, anti-VISTA treatment stimulated several pathways involving myeloid activation and antigen-presentation. Multi-spectral imaging of anti-VISTA treated tumors supported increased antigen presentation, and suppression assays showed that the myeloid infiltrate was less suppressive to T cells. Transcriptional analysis of tumor-specific CD8 T cells showed that anti-VISTA therapy induced T cell pathways highly distinct from the anti-exhaustion effects of anti-PD-1 therapy.

**Conclusions** These data document the unique and complementary impact of targeting VISTA in contrast to PD-1 and CTLA-4 in both the myeloid and T cell lineages. These mechanistic insights strongly support the use of anti-VISTA to