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

**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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**Abstracts**

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

1Mathieu Gigoux, 1Robert Zappasodi, 2Joseph Park, 3Stephane Pourpe, 1Anab Ghosh, 4Cansu Cimen Bozkus, 1Levi Margarin, 3David Redmond, 2Svena Verma, 1Sara Schad, 1William Duke, 1Max Jan, 5Matthew Levenshal, 1Vincent Ho, 1Gabriela Hobbs, 6Trine Alma Knudsen, 1Vibe Skov, 1Lasse Kjer, 1Thomas Stauffer Larsen, 1Dennis Lund Hansen, 4R Coleman Lindsley, 6Hans Hasselbalch, 1Jacob Grauslund, 1Mads Andersen, 6Morten Holmström, 1Timothy Chan, 1Raajit Rampal, 1Omar Abdel-Wahab, 3Nina Bhaward, 1Jedd Wolchok, 1Ann Mullally, 1Taha Menghoub.

**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 neoeptopes 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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**445** 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

1Lucas Horn, 1Linjie Tian, 2Dallas Flies, 2Linda Liu, 3Solomon Langermann, 2Jeffrey Schlom, 1Claudia Palena.

1National Cancer Institute, Bethesda, MD, USA; 2NextCure, Beltsville, MD, USA

**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 β receptor II (TGF-βRII or TGF-β ‘trap’) fused via a flexible linker to the C-terminus of an 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 antitumor 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


1Claudia Palena.

1National Cancer Institute, Bethesda, MD, USA; 2NextCure, Beltsville, MD, USA

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**Trial Registration** N/A

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