cytokine secretion activities, and the association between tumor infiltration and positive prognosis.[1 2] ImCheck Therapeutics is developing ICT01, an anti-human butyrophilin-3A (BTN3A/CD277) mAb specifically activating g9d2 T-cells in a phosphoantigen (pAg)-independent manner. ICT01 is currently in a Phase 1/2a study in solid and hematologic tumors (NCT04243499).IL-2 has been shown to expand g9d2 T-cells in vitro and in non-human primates in presence of pAgs.[3 4 5] We wanted to characterize the proliferative effects of combining ICT01 with IL-2 on γ9δ2 T-cells as an approach to potentiate g9d2 T-cell mediated cancer immunotherapy.

Methods g9d2 T-cell activation and expansion was assessed in vitro in human PBMCs treated with ICT01±IL-2, and in vivo, in the blood of immunocompromised NCG mice engrafted with 20 × 106 human PBMCs and treated with ICT01 (single IV dose, 5 mg/kg on Day 1) ±IL-2 (0.3MIU/kg IP on Day 1–4). A dose-ranging ICT01 (single IV dose, 1 or 5 mg/kg on Day 1)+IL-2 combination (1 MIU SC QD on Days 1–5) study was conducted in cynomolgus monkeys.

Results In PBMCs cultures in vitro, ICT01 selectively activated g9d2 T-cells and IL-2 significantly enhanced ICT01-mediated g9d2 T-cell proliferation, this compartment reaching >50% of T-cells after 8 days of treatment versus ~10% with ICT01 alone. This was confirmed in vivo in mice models. Flow cytometry analysis of mice blood revealed a 5.5-fold increase in human g9d2 T-cell number in the combination groups compared to ICT01 or IL-2 alone treated animals, with g9d2 T-cell frequency reaching ~35% of the CD3+ T-cell compartment. In Cynomolgus, a specific expansion and activation of peripheral g9d2 T-cells from ~1–2% at baseline to up to 30% of T cells 7 days post ICT01 administration was observed. No ICT01 effect was observed on other immune cells. Histopathological examinations revealed a trend towards higher numbers of g9d2 T-cells in several organs in ICT01+IL-2 treated monkeys. There was no evidence for a systemic cytokine release syndrome at any time point. Adverse effects with variable severity were observed, most of them being reversible and commonly associated with IL-2 alone, and not reported in the IND-enabling GLP toxicity study with ICT01 monotherapy at doses up to 100 mg/kg.

Conclusions These results demonstrate the ability of ICT01 +IL-2 combination to trigger profound γ9δ2 T-cell activation and expansion, suggesting that the clinical combination of ICT01 with a lymphoproliferative cytokine (e.g., IL-2) may be a novel therapeutic approach for cancer patients.

Ethics Approval Pseudonymized samples isolated from healthy volunteers: whole blood by ImCheck Therapeutics under the agreement n° 7173 between ImCheck Therapeutic SAS and EFS PACA (Etablissement Français du Sang Provence-Alpes-cote d'Azur)

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443 AN IMMUNOTHERAPY TRIO IN ADVANCED HNSCC FOR COORDINATED B AND T CELL ANTIGEN RESPONSE

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Background Outcomes for recurrent or metastatic (R/M) head and neck squamous cell carcinoma (HNSCC) are dismal and responses to anti-PD-1 appear best in tumors with PD-1+ T cells in proximity to PD-L1+ cells, arguing that improved outcome is associated with a pre-existing anti-cancer immune response. Based on this, we hypothesize that vaccines which prime and/or expand T cells to a spectrum of antigens overexpressed by HNSCC combined with T cell agonists, like anti-GITR, that provide costimulatory signals will improve the anti-PD-1 response rates. We have developed a cancer vaccine, DPV-001, that contains more than 300 proteins for genes overexpressed by HNSCC, encapsulated in a CLEC9A-targeted microvesicle and containing TLR/NOD agonists and DAMPs. Recently, we reported that combining anti-GITR + vaccine + anti-PD-1 augmented therapeutic efficacy in a preclinical model and now plan a phase 1b trial of this combination in patients with advanced HNSCC.

Methods Sera from patients receiving DPV-001 as adjuvant therapy for definitively treated NSCLC, were analyzed for IgG responses to human proteins by MAP bead arrays and results compared to TCGA gene expression data sets for HNSCC. HNSCC cell lines were evaluated by RNASeq and peptides were eluted from HLA, analyzed by mass spectroscopy and correlated against MAP bead arrays and TCGA data sets. Tumor-reactive T cells from a vaccinated patient were enriched and expanded, and used in cytokine release assay (CRA) against autologous NSCLC and partially HLA matched allogeneic HNSCC cell lines.

Results Patients receiving DPV-001 (N=13) made 147 IgG responses to at least 70 proteins for genes overexpressed by HNSCC. Preliminary evaluation of the HNSCC peptidome against the results of MAP bead array identify antigens that are target of a humoral immune response. Additionally, tumor-reactive T cells from DPV-001 vaccinated patient recognize two partially HLA-matched HNSCC targets, but not a mismatched target.

Conclusions Recent observations from our lab and others have correlated IgG Ab responses with T cell responses to epitopes of the same protein. Based on the data summarized above, we hypothesize that we have induced T cell responses against a broad spectrum of shared cancer antigens that are common among adenocarcinomas and squamous cell cancers. Our planned clinical trial will vaccinate and boost the induced responses by costimulation with anti-GITR and then sequence in delayed anti-PD-1 to relieve checkpoint inhibition. MAP

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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444 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 neoepitopes 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

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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 β receptor II (TGF-βRII or TGF-β '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.

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Trial Registration N/A