who achieved objective response assessed by RECIST v1.1/iRECIST or progression free survival more than 24 weeks. Vactosertib responsive gene signature (VRGS) that showed significantly different expression among previously identified TGF-β responsive gene signature and IFN-γ signature in responders than in non-responders was identified and VRGS score was calculated by a mean value of VRGS filtered-in gene expressions divided by 6 house-keeping gene expressions.

Results As of July 1, 2020, of the total evaluable 24 patients, 71% were CMS4 subtype and 33% were with high TMB (≥10 mut/Mb). Clinical benefit rate was 33.3% including 3 PR and 1 iPR patients. No significant associations in response rate were observed with CMS subtypes or TMB status. VRGS score was significantly enriched in responders than in non-responders (P value = 0.006; AUC = 0.836). A preliminary cut-off value of 2.179 resulted in 94% specificity and 75% sensitivity with 85.7% patients correctly classifying as a responder. After treatment of vactosertib plus pembrolizumab, TGF-β-related VRGS was significantly decreased and the extent of decrease was greater in responders, compared to non-responders.

Ethics Approval The study was approved by Ethics Board of Asan Medical Center, Yonsei University College of Medicine, Samsung Medical Center, and Seoul National University Bundang Hospital with approval number 2018-1215, 4-2018-0728, SMC 2018-07-146-006, and B-1808/487-003, respectively.

Conclusions Development of VRGS as a predictive biomarker for this combination treatment with vactosertib and pembrolizumab is ongoing and its potential clinical utility for patient selection will be explored.

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333 TARGETING THE APICAL INTRACELLULAR CHECKPOINT CISH UNLEASHES T CELL NEOANTIGEN REACTIVITY AND EFFECTOR PROGRAM

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Background Neoantigen-specific T cells isolated from tumors have shown promise clinically but fail to consistently elicit durable tumor regression. Expression of the intracellular checkpoint CISH is elevated in human tumor infiltrating lymphocytes (TIL) and has been shown to inhibit neoantigen reactivity in murine TIL.

Methods To explore CISH function in human T cells we developed a CRISPR/Cas9-based strategy to knockout (KO) CISH in human T cells with high-efficiency (>90%) and without detectable off-target editing.

Results CISH KO in peripheral blood T cells enhanced proliferation, cytokine polyfunctionality, and cytotoxicity in vitro. To determine if CISH KO similarly enhances TIL function, we developed a clinical-scale, GMP-compliant manufacturing process for CISH disruption in primary human TIL. In process validation runs we achieved CISH KO efficiencies >90% without detectable off-target editing while maintaining high viability and expansion. Compared to WT controls, CISH KO in patient-derived TIL demonstrated increased proliferation, T cell receptor (TCR) avidity, neoantigen recognition, and unmasked reactivity to common p53 mutations. Hyperactivation in CISH KO TIL did not increase differentiation, suggesting that CISH KO may uncouple activation and differentiation pathways. Single cell profiling identifies a pattern of CISH expression inverse to key regulators of activation, and CISH KO in human TIL increases PD1 expression. Adoptive transfer of Cish KO T cells synergistically combines with PD1 inhibition resulting in durable tumor regression in mice, highlighting orthogonal dual cell surface and intracellular checkpoint inhibition as a novel combinatorial approach for T cell immunotherapy.

Conclusions These pre-clinical data offer new insight into neoantigen recognition and serve as the basis for a recently initiated human clinical trial at the University of Minnesota (NCT04426669) evaluating inhibition of the novel intracellular immune checkpoint CISH in a CRISPR-engineered, neoantigen-specific T cell therapy for solid tumors. Updates from the clinical trial will be highlighted.

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334 PHASE II STUDY EVALUATING A CHEMOKINE-MODULATORY (CKM) REGIMEN IN PATIENTS WITH COLORECTAL CANCER (CRC) METASTATIC TO THE LIVER

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Background CRC remains the 2nd most common cause of cancer-related death in the US. Hepatic metastases develop in 20–50% of CRC patients.[1] Median overall survival (OS) of patients with metastatic CRC is poor, even with the advent of biologics. A high density of CRC-infiltrating effector cytotoxic T lymphocytes (Teff; CTL) is known to predict long-term outcomes and the responsiveness of tumors to immune checkpoint inhibitors (ICI). In our ex vivo tumor explant models and CRC-bearing experimental animals, the combination of toll-like receptor-3 (TLR3) ligands with interferon (IFN)-α with cyclooxygenase (COX)-2 inhibitors resulted in increased production of Teff attracting chemokines CXCL10 and CCL5, along with suppression of regulatory T cells (Treg) attracting chemokine, CCL22 in the tumor microenvironment.[2,3] A combination of all three factors was needed to uniformly elevate the desirable chemokines and counteract CCL22 induction. Based on these studies and on prior clinical safety data, we developed this phase IIa study combining IFNα2b, celecoxib (COX-2 inhibitor) and rintatolimod (selective TLR3 agonist) as a chemokine-modulating (CKM) regimen for CRC patients with unresectable liver-metastatic disease. We aim to study the immunological impact, potential clinical efficacy and safety