

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.

Trial Registration NCT03724851

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0332>

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.

Trial Registration NCT04426669

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0333>

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 IFN α 2b, 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

of this CKM regimen in a non-randomized, single-arm prospective phase II trial.

Methods Eligible patients have recurrent/metastatic unresectable CRC with hepatic metastases that are amenable to biopsy. Enrolled patients have prior treatment with or contra-indication to a fluoropyrimidine, irinotecan, oxaliplatin, anti-VEGF treatment, and an anti-EGFR targeted therapy (if RAS wt), as well as a PD-1 or PD-L1 targeted drug if MSI-H/dMMR. Patients receive celecoxib (200 mg orally PO BID), IFN α 2b IV (20 million units/m² IV QD), and rintatolimod (200 mg IV QD) on days 1, 2, 3, 8, 9, 10, 15, 16 and 17 in the absence of disease progression or unacceptable toxicity. Response assessment via liver biopsies (pre-treatment and on D20) and CT imaging (RECIST v1.1) on D46. If stable disease/response is demonstrated during repeat CT imaging, patients will continue to follow-up with CT imaging q8 weeks until progression, clinical deterioration, or withdrawal from the study. Primary endpoint assessment compares the change in CD8+ T-cells before treatment, with that seen post-treatment (measured by quantitative RT-PCR and expressed as a ratio of CD8 α to a housekeeping gene). Secondary endpoints include objective response rate and safety profile. Subjects are monitored continuously for safety, based on Bayesian analysis. Exploratory endpoints include progression-free survival and overall survival. With a sample size of n=12 evaluable pts, the study design has a 90% power to detect a 0.77 standard deviation increase (pre- to post treatment) at a significance level of 0.1.

Results N/A

Conclusions N/A

Trial Registration ClinicalTrials.gov Identifier: NCT03403634.

Ethics Approval The study was approved by Roswell Park Comprehensive Cancer Center's Institutional Review Board, approval number: MOD00006722/I-52917.

Consent N/A

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<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0334>

335

NOVEL COUPLED CARTM TECHNOLOGY FOR TREATING COLORECTAL CANCER

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Background Chimeric antigen receptor (CAR) T cell therapy has made significant progress in the treatment of blood cancers such as leukemia, lymphoma, and myeloma. However, the therapy faces many challenges in treating solid tumors. These challenges include physical barriers, tumor microenvironment immunosuppression, tumor heterogeneity, target

specificity, and limited reactive cell expansion in vivo. Conventional CAR T cell therapy has thus far shown weak cell expansion in solid tumor patients and achieved little or no therapeutic responses. Here, we developed CAR T cells based on a novel CoupledCAR[®] technology to treat solid tumors. In contrast to conventional CAR T cells, CoupledCAR T cells significantly improved the expansion of the CAR T cells in vivo and enhanced the CAR T cells' migration ability and resistance to immunosuppression by the tumor microenvironment. The enhanced migration ability and resistance allow the CAR T cells to infiltrate to tumor tissue sites and increase anti-tumor activities.

Methods We designed a 'CoupledCAR' lentivirus vector containing a single-chain variable fragment (scFv) targeting human TSHR. The lentivirus was produced by transfecting HEK-293T cells with 'CoupledCAR' lentiviral vectors and viral packaging plasmids. Patient's CD3 T cells were cultured in X-VIVO medium containing 125U/mL interleukin-2 (IL-2), and transduced with 'CoupledCAR' lentivirus at certain MOI. Transduction efficiency and was evaluated at 7 to 9 days after 'CoupledCAR' lentivirus transduction, and quality controls for fungi, bacteria, mycoplasma, chlamydia, and endotoxin were performed. After infusion, serial peripheral blood samples were collected, and the expansion and the cytokine release of CART cells were detected by FACS and QPCR. The evaluation of response level for patients were performed at month 1, month 3, and month 6 by PET/CT.

Results Specifically, we engineered CoupledCAR T cells with lentiviral vectors encoding an anti-GCC (guanylate cyclase 2C) CAR molecule. Furthermore, anti-GCC CAR T cells showed anti-tumor activities in vitro and in vivo experiments. To verify the safety and efficacy of CoupledCAR T cells for treating solid tumors, we conducted several clinical trials for different solid tumors, including seven patients with colorectal cancer. These seven patients failed multiple rounds of chemotherapy and radiotherapy. In the clinical trial, the patients were infused with autologous anti-GCC CoupledCAR T cells range from 4.9×10^5 /kg to 2.9×10^6 /kg. All patients using anti-GCC CoupledCAR T cells showed rapid expansion of CoupledCAR T cells and killing of tumor cells. Specifically, we observed that CoupledCAR T cells expanded significantly in the patients and infiltrated tumor tissue sites, demonstrating enhanced anti-tumor activities. PET/CT showed significant tumor shrinkage and SUV max declined, and the ongoing responses were monitored. Patient 3 achieved complete response and the best overall response rate (ORR, include complete remission, complete metabolic response, partial response, and partial metabolic response.) was 71.4% (5/7), complete remission (CR) rate was 14.3% (1/7).

Conclusions The clinical data demonstrated that CoupledCAR T cells effectively expanded, infiltrated tumor tissue sites, and kill tumor cells in patients with colorectal cancer. We used immunotherapy to achieve complete remission in patients with advanced colorectal cancer for the first time. We are recruiting more colorectal cancer patients to further test the safety and efficacy of anti-GCC CoupledCAR T cells. Since our CoupledCAR[®] technology is a platform technology, we are expanding it to treat other solid tumors using different target tumor markers.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0335>