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Activity of Mesothelin-specific Chimeric Antigen Receptor T cells Against Pancreatic Carcinoma Metastases in a Phase 1 Trial

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is resistant to T-cell mediated immunotherapy. We engineered T cells to transiently express an mRNA encoding a chimeric antigen receptor (CAR) specific for mesothelin—a protein that is over-expressed by PDAC cells. We performed a phase 1

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Conflict of Interest Statement: B.L.L., C.H.J., G.L.B., G.P., S.F.L., and J.J.M. are inventors of intellectual property related to CAR T cells that is licensed by the University of Pennsylvania to Novartis. All other authors declare no conflict.

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This manuscript presents original results of a phase I study of autologous mesothelin-specific chimeric antigen receptor modified T cells in patients with chemotherapy-refractory metastatic pancreatic ductal adenocarcinoma. Preliminary findings have been reported at the 51st Annual Meeting of the American Society of Clinical Oncology, May 29-June 2, 2015, Chicago, IL.

study to evaluate the safety and efficacy of adoptive cell therapy with autologous mesothelin-specific CAR T cells (CARTmeso cells) in 6 patients with chemotherapy-refractory metastatic PDAC. Patients were given intravenous CARTmeso cells 3 times weekly for 3 weeks. None of the patients developed cytokine release syndrome or neurologic symptoms and there were no dose limiting toxicities. Disease stabilized in 2 patients, with progression-free survival times of 3.8 and 5.4 months. We used FDG-positron emission tomography/computed tomography imaging to monitor the metabolic active volume (MAV) of individual tumor lesions. The total MAV remained stable in 3 patients and decreased by 69.2% in 1 patient with biopsy-proven mesothelin expression; in this patient, all liver lesions had a complete reduction in FDG uptake at 1 month compared to baseline, although there was no effect on the primary PDAC. Transient CAR expression was detected in patients' blood after infusion and led to expansion of new immunoglobulin G proteins. Our results provide evidence for the potential anti-tumor activity of mRNA CARTmeso cells, as well as PDAC resistance to the immune response. Keywords: immune response heterogeneity, immune therapy, anti-tumor immunity, pancreatic cancer treatment

MAIN TEXT

Pancreatic ductal adenocarcinoma (PDAC) has demonstrated striking resistance to T cell immunotherapy [1–3]. This resistance may reflect PDAC's lack of strong immunogenic neoantigens [4] or ineffective priming of endogenous T cells *in vivo* [5,6]. To circumvent these issues, T cells can be synthetically modified to express a chimeric antigen receptor (CAR) which activates T cells upon engagement with its cognate cell surface target protein [7]. However, the translation of CAR T cells to solid malignancies has been hampered by on-target off-tumor toxicities as a result of target antigen expression by normal healthy tissues [8,9]. To address this concern, we have used mRNA electroporation to engineer T cells to transiently express a CAR, so as to limit exposure and potential for toxicity. Here, we conducted a phase I study to evaluate adoptive cell therapy with autologous T lymphocytes (CARTmeso cells) genetically modified with mRNA to express a CAR recognizing mesothelin [10], a protein that is over-expressed in most surgically-resected PDACs [11,12] but also found on some normal tissues including the lining of the peritoneum, pleura, and pericardium.

Six patients with chemotherapy-refractory metastatic PDAC received CARTmeso cells administered intravenously three times weekly for three weeks (Figure 1A, Suppl Figure 1 and Suppl Methods). 53 of 54 planned CARTmeso cell infusions were administered. None of the patients experienced cytokine release syndrome or neurologic symptoms (Supplementary Table 1). There were no dose limiting toxicities. Safety and tolerability of the infusions are described in Supplementary Methods.

The best overall response achieved with mRNA CARTmeso cells was stable disease by RECIST v1.1, with two patients (08212-100 and 08212-108) demonstrating progression-free survival lasting 3.8 and 5.4 months, respectively (Figure 1B and Supplementary Figure 1). We used ^{18}F-2-fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography (FDG-PET/CT) imaging to monitor the metabolically active volume (MAV) and maximum standardized uptake value (SUV_{max}) of individual tumor lesions (Figure 1C, D).

We found that the total MAV remained stable in three patients but decreased by 69.2% in one patient (08212- 113). For 08212-113, SUV_{max} for all lesions was found to decrease with a complete reduction in FDG uptake seen in all liver lesions at Month 1 compared to baseline, despite an increase in MAV within the primary pancreatic lesion (Figure 1E, F). This dramatic reduction in FDG uptake is atypical for the course of this disease and thus, this observation is highly suggestive of the potential anti-tumor activity of mRNA CARTmeso cells.

CAR expression is transient in T cells when introduced as mRNA [13]. Consistent with this, we detected CAR transcripts transiently in the blood after each infusion in all patients (Figure 2A). To determine the impact of CARTmeso cell infusion on endogenous immune responses, we first examined serum cytokine levels within the peripheral blood after infusion. We found elevated levels of several inflammatory cytokines, including IL-6, HGF, IL-1RA, and IL-8, after starting therapy in patient 08212-113, but not in any of the other patients (Figure 2B). We also detected no significant levels of human anti-mouse antibodies (HAMA) in patients at baseline or at multiple defined time points after CARTmeso infusion. In contrast, human anti-chimeric antibodies (HACA) were seen in some patients (Supplementary Figure 2A). We next analyzed serum from patients at baseline and 1–2 months after beginning CARTmeso therapy for the presence of IgG antibodies reactive to an array of >9000 proteins. For all but one patient (08212-108), antibodies levels against multiple protein targets were found to increase, with some against previously unrecognized proteins (Figure 2C, Supplementary Tables 2 and 3). Gene ontology analysis of differentially recognized proteins from all patients identified oxidative stress and leukocyte activation and proliferation as the most significantly upregulated protein function groups (Supplementary Figure 2C). Increases in IgG antibodies reactive against six proteins, including three immune-related proteins (BCMA, PD-1, and PD-L1), were detected in multiple patients (Supplementary Figure 2D).

We next considered the possibility that exclusion of T cells from the tumor microenvironment, lack of mesothelin expression on malignant cells, or both might limit the efficacy of CARTmeso cell therapy. Tumor biopsies at 3–7 days after the last infusion were available from patients 08212-104 and 08212-108 who demonstrated disease progression and prolonged stable disease, respectively. CAR transcripts were not detected in either biopsy, excluding the presence of CARTmeso cells within tumors at this time point. Baseline tumor biopsies were available for patients 08212-108 and 08212-113 and showed $CD3^+$ T cells amongst malignant cells in both specimens suggesting the potential of T cells to penetrate the tumor microenvironment in these patients (Figure 2D). For patient 08212-108, mesothelin expression was confined to the cytoplasm of malignant cells, whereas for patient 08212-113, malignant cells showed mesothelin expression on the cell surface, a pre-requisite for CARTmeso effector activity (Figure 2D).

Our findings reveal the safety, feasibility and therapeutic potential of autologous T cells redirected with a CAR recognizing mesothelin for the treatment of PDAC. The mixed metabolic response detected in one patient with biopsy-proven cell surface expression of mesothelin implies distinct biology associated with primary and metastatic lesions in this patient. We hypothesized that CARTmeso cells may produce a vaccine effect by inducing

tumor cell death and the release of tumor-associated antigens. Consistent with this hypothesis, we found that therapy induced a spreading of antibody responses against multiple proteins, including immunoregulatory molecules (e.g. PD-1, PD-L1, and BCMA). Further, we have recently reported that mRNA CARTmeso cell infusion stimulates clonal expansion of T cell clones, which may contribute to the anti-tumor potential of CAR T cells [14]. The ultimate fate of tumor-infiltrating T cells, though, is determined by signals received within the tumor microenvironment. In our study, we were unable to evaluate the fate of adoptively transferred CARTmeso cells due to transient CAR expression *in vivo.* In conclusion, we propose that CARTmeso adoptive cell therapy can serve as a novel tool for probing the immunobiology of PDAC, without issues of T cell priming, and in doing so, guide the development of effective T cell immunotherapies in this disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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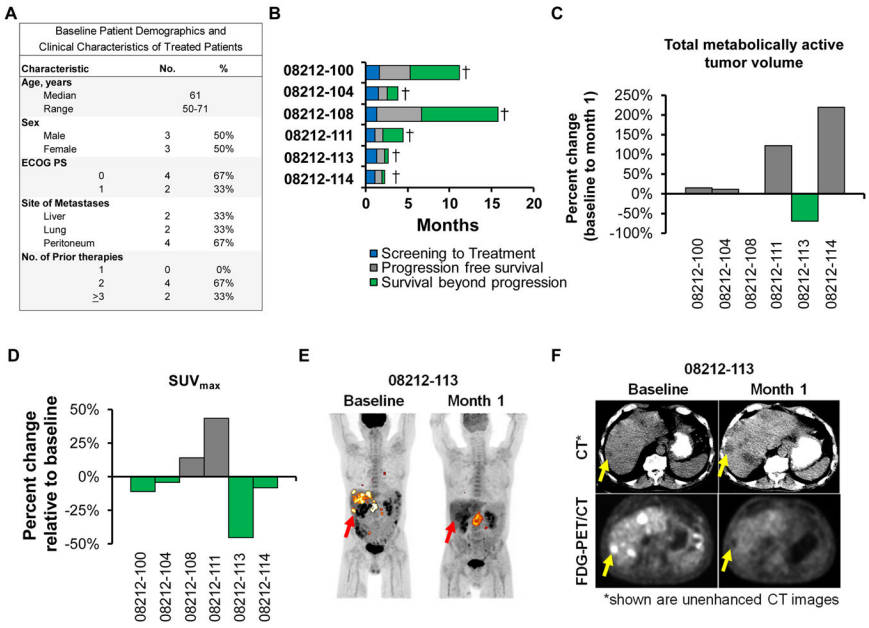

Figure 1. Clinical responses

(A) Baseline patient demographics. **(B)** Swimmer plot showing patient outcomes. Percent change at 1 month relative to baseline in **(C)** total MAV and **(D)** SUV_{max} for tumor lesions detected using FDG-PET/CT imaging. **(E)** Sequential coronal maximum intensity projection PET images and **(F)** sequential unenhanced CT and FDG-PET images for patient 08212-113. In **(E)**, red and yellow arrows indicate liver and primary pancreatic lesions, respectively. In **(F)**, yellow arrows mark a representative liver lesion.

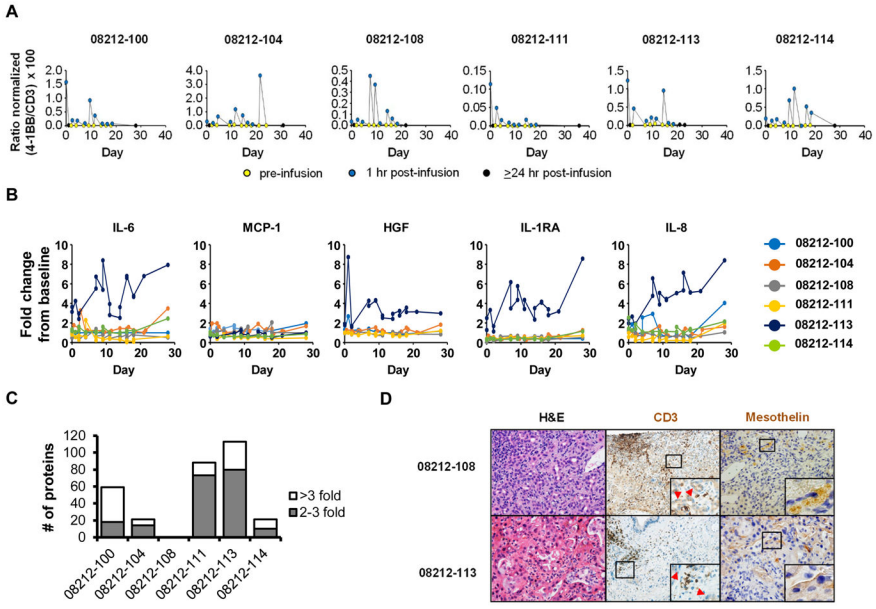

Figure 2. Pharmacodynamic analyses

(A) RT-qPCR analysis to detect CAR expression in whole blood after CARTmeso cell infusion. **(B)** Longitudinal measurements of serum soluble factors after CARTmeso cell infusion. **(C)** Differentially recognized proteins detected by self-reactive IgG antibodies in the serum of each patient at 1-2 months after CARTmeso cell infusion compared to baseline. **(D)** Representative images of H&E staining (40x) and IHC staining to detect CD3 (20x) and mesothelin (40x) expression in metastatic liver lesions analyzed from patient 08212-108 and 08212-113. Red arrowheads indicate malignant cells adjacent to $CD3^+$ cells.