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Original Article

Phase I Study of Lentiviral-Transduced Chimeric Antigen Receptor-Modified T Cells Recognizing Mesothelin in Advanced Solid Cancers

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This phase I study investigated the safety and activity of lentiviral-transduced chimeric antigen receptor (CAR)-modified autologous T cells redirected against mesothelin (CART-meso) in patients with malignant pleural mesothelioma, ovarian carcinoma, and pancreatic ductal adenocarcinoma. Fifteen patients with chemotherapy-refractory cancer (n = 5 per indication) were treated with a single CART-meso cell infusion. CART-meso cells were engineered by lentiviral transduction with a construct composed of the anti-mesothelin single-chain variable fragment derived from the mouse monoclonal antibody SS1 fused to intracellular signaling domains of 4-1BB and CD3zeta. Patients received 1–3 $\times$ 10^7 or 1–3 $\times$ 10^8 CART-meso cells/m^2 with or without 1.5 g/m^2 cyclophosphamide. Lentiviral-transduced CART-meso cells were well tolerated; one dose-limiting toxicity (grade 4, sepsis) occurred at 1–3 $\times$ 10^7/m^2 CART-meso without cyclophosphamide. The best overall response was stable disease (11/15 patients). CART-meso cells expanded in the blood and reached peak levels by days 6–14 but persisted transiently. Cyclophosphamide pre-treatment enhanced CART-meso expansion but did not improve persistence beyond 28 days. CART-meso DNA was detected in 7/10 tumor biopsies. Human anti-chimeric antibodies (HACA) were detected in the blood of 8/14 patients. CART-meso cells were well tolerated and expanded in the blood of all patients but showed limited clinical activity. Studies evaluating a fully human anti-mesothelin CAR are ongoing.

INTRODUCTION

Adoptive cell transfer of engineered autologous chimeric antigen receptor (CAR) T cells has shown remarkable success in hematologic malignancies and led to US Food and Drug Administration approval of CAR T cell products for treatment of pediatric B cell acute lymphoblastic leukemia and adult non-Hodgkin's lymphoma.[1,2] However, the application of CAR T cells to solid tumors has been challenging. Reasons for this differential efficacy seen between hematologic and solid malignancy patients remain ill-defined. Proposed barriers include impaired CAR T cell proliferation after adoptive transfer, limited CAR T cell trafficking and infiltration of tumors, the immune-suppressive tumor microenvironment, rapid acquisition of CAR T cell hypofunction in tumors, and immunological elimination of CAR T cells leading to poor persistence.[3–5] Successful translation of CAR T cells to solid malignancies will need to overcome these immune-regulatory mechanisms.

Applying CAR T cells to solid malignancies has also been hampered by the need for suitable protein targets, which must be expressed on the surface of malignant cells, yet limited in expression on normal tissues to avoid off-tumor, on-target toxicity.[6,7] To date, several potential antigens have been evaluated as CAR T cell targets in clinical trials, including HER2, EGFR, CEACAM-5, CEA, IL13Rα2, cMET, and EGFRvIII.[8–15] Mesothelin, a cell surface antigen, has also been evaluated.[16] Although mesothelin is expressed at low levels on mesothelial tissues (pleura, pericardium, and peritoneal mesothelial cells), it is overexpressed on several solid tumors including malignant pleural mesothelioma (MPM), ovarian adenocarcinoma (OVCA),

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pancreatic ductal adenocarcinoma (PDAC), and some lung cancers.[17]

Based on preclinical data,[18] we investigated mesothelin as a CAR T cell target using a "second generation" CAR that expresses the murine anti-mesothelin SS1 single-chain variable fragment (scFv)[16] coupled to the CD3ζ and 4-1BB cytoplasmic signaling domains. To minimize toxicity concerns, we conducted our first phase 1 trial using autologous T cells engineered with mRNA electroporation to transiently express this mesothelin-specific CAR (RNA CART-meso cells).[19,20] RNA CART-meso cells were infused intravenously with no on-target toxicities (i.e., pleuritis, pericarditis, or peritonitis) observed in patients with PDAC[20] or MPM (Figure S1; Table S1). However, one patient with MPM developed an anaphylactic reaction during the second infusion of CART-meso cells after prolonged treatment interruption, likely a result of an immune response to the murine scFv in the CAR.[21] In the remainder of patients, we found that RNA CART-meso cells were safe, although limited in efficacy in the treatment of both PDAC[19,20] and MPM (Figure S1). By design to maximize safety in these studies, the persistence of RNA CART-meso cells was short, and thus levels of CAR T cells achieved in the blood were far less[20] than those seen with CD19-targeting CAR T cells (CART19) generated through transduction with a lentiviral CAR-expression system.[22]

Given the lack of on-target toxicity with RNA CART-meso cells, we subsequently designed a phase 1 trial using a comparable CAR incorporating the same anti-mesothelin scFv, CD3ζ, and 4-1BB chains as used for manufacturing RNA CART-meso cells, but transduced with a lentiviral vector. In this study, we included patients with three types of cancers that commonly express mesothelin: (1) MPM, (2) OVCA, and (3) PDAC. Here, we report a final analysis with biological correlatives of treatment with lentiviral CART-meso cells with and without cyclophosphamide pre-treatment as a lymphoreduction strategy to improve CAR T cell persistence and efficacy.[23,24] The primary objective of this study was to define the feasibility and safety of lentiviral-transduced CART-meso cells in mesothelin-expressing solid malignancies. Secondary objectives were to evaluate clinical activity, as well as the immunogenicity, persistence, and trafficking of lentiviral-transduced CART-meso cells.

RESULTS

Patient Characteristics

Between June 2014 and October 2015, 19 patients signed an informed consent (Figure 1B). Four patients were screening failures and did not receive CART-meso cell infusion because of inadequate organ function (n = 2, PDAC), lack of measurable disease (n = 1, MPM), or failure to complete screening (n = 1, PDAC). The full analysis set included 15 patients (n = 5, MPM; n = 5, OVCA; n = 5, PDAC) who were treated with lentiviral CART-meso cells. Demographics and baseline characteristics are summarized in Table 1. The mesothelin expression level in tumor tissue was not an inclusion criterion but was evaluated in pre-treatment biopsy specimens; patients with OVCA had the most consistently elevated levels (Figure 1C).

Manufacturing Feasibility and Characteristics of Infused CART-Meso Cells

For all patients, CART-meso cells were successfully manufactured to achieve the target dose of $1–3 \times 10^7$ CART-meso cells/m^2 (cohorts 1 and 2) or $1–3 \times 10^8$ CART-meso cells/m^2 (cohorts 3 and 4). CART-meso cell product characteristics are summarized in Table S2. The average transduction efficiency rate was 24.7% (range of 15.5%–35.7%). The infused cell products were an average of 94.7% $CD3^+$ with an average CD4/CD8 ratio of 2.8. The average cell viability was 85.6% (range of 71.9%–95.5%).

Safety of Lentiviral-Transduced CART-Meso Cellular Therapy

Adverse events (AEs) related to study treatment are summarized in Table 2. The most common AEs were low-grade fatigue and nausea observed in 47% (7 out of 15) and 40% (6 out of 15) of patients, respectively. One patient (patient 1-34), a 69-year-old male with metastatic PDAC, experienced a dose-limiting toxicity (DLT) characterized by worsening abdominal pain, jaundice, and fatigue on day 34 after CART-meso cell infusion. At this time point, CART-meso transcripts were undetectable in the blood, with peak levels seen on day 10 after infusion. Ferritin (15,668 ng/mL) and interleukin-6 (IL-6; 782 ng/mL) were elevated in the serum, but interferon (IFN)-γ (7.59 pg/mL) was unchanged from baseline. A mild transaminitis with increased aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was observed (Figure S2), and the patient received 1 mg/kg prednisone for concern of CAR T cell-related cytokine release syndrome (CRS). Subsequent workup revealed *Klebsiella* bacteremia that was managed with broad-spectrum antibiotics. Computed tomography (CT) imaging showed marked progression of lesions in the liver, which on magnetic resonance imaging (MRI) were poorly enhancing and necrotic. The patient's clinical course deteriorated rapidly with development of refractory ascites and *Candida albicans* peritonitis. The patient eventually died on day 62 after CART-meso cell infusion. An autopsy was performed and showed multiple foci of metastatic adenocarcinoma in the abdominal mesentery, peritoneum, gastric wall, right lung, spleen, and paraaortic lymph nodes. Foci of viable and focally necrotic metastatic disease accounted for roughly 50% of liver volume with intrahepatic Candidal microabscesses seen. CAR-meso T cells were undetectable by qPCR analysis in all autopsy-collected tissues except a necrotic spleen sample, which showed 49 CAR copies/μg of genomic DNA (lower limit of detection was 25 copies). This DLT resulted in expansion of cohort 1 to six patients. No other patients experienced a DLT, and the trial was completed without additional safety events.

Clinical Activity of CART-Meso Cells

The best overall response (BOR) based on Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 (for PDAC and OVCA) and on modified RECIST (for MPM) was stable disease observed in 11 of 15 patients at 28 days and in 3 of 8 patients on follow-up at months 2–3 (Figure 2A; Table S3). Response based on immune-related response criteria (irRC) was similar to RECIST (for PDAC and OVCA) (Table S3). Median progression-free survival (PFS) was 2.1 months (Figure 2B). Only one patient (patient 2-68),

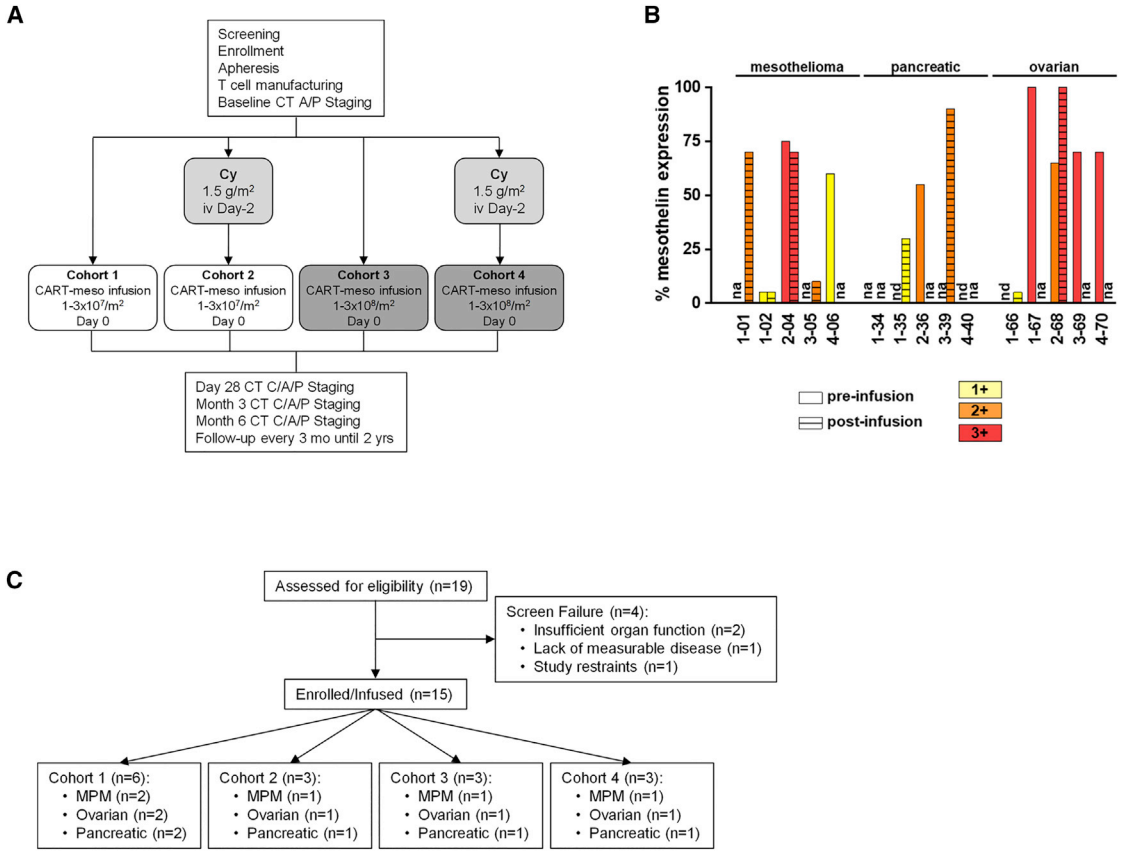

Figure 1. Study Schema and Consort Diagram

(A) Shown is the study schema. Subjects were screened for enrollment and then underwent leukapheresis for manufacturing of CAR T cells. Four treatment cohorts were defined in which subjects received CART-meso cells (1–3 × 10^7/m^2 or 1–3 × 10^8/m^2) on day 0 with or without cyclophosphamide (cy) administered 3 (±1) days prior to CART-meso cell infusion. Tumor assessments included computed tomography (CT) scans of the chest, abdomen, and pelvis (C/A/P) performed at baseline and 1, 3, and 6 months after CART-meso infusion, with additional staging every 3 months through 2 years. (B) Consort flow diagram shows the number of patients at each stage of the trial. (C) Mesothelin expression in tumor biopsies collected at baseline and post-infusion. na, not assessed; nd, not detected.

a 51-year-old female with OVCA diagnosed 4 years prior to study entry, demonstrated an appreciable reduction in target tumor burden, although this did not meet RECIST 1.1 criteria for partial response. Prior to enrollment, this patient had been treated with anastrozole monotherapy, with the last dose administered 6 weeks prior to receiving CART-meso cells. At trial entry, radiologic imaging showed metastatic foci, including capsular tumor deposits detected anterior to the superior aspect of the left hepatic lobe, paraceliac region, and left superior posterior pelvis (Figure 2C). The patient received 3 × 10^7 CART-meso cells/m^2 after lymphodepleting chemotherapy (cohort 2) without complications, and imaging performed on day 28 showed a 26% decrease in tumor burden (Figure 2C) with a corresponding decrease in CA125 from 340 to 151 U/mL. The patient subsequently developed a malignant pleural effusion by month 3 with progressive disease seen on CT imaging (Figure 2C) and an increase in CA125 to 565 U/mL.

We have previously reported differential anti-tumor activity with CART-meso cells among lesions within the same patient.[20] Therefore, we examined for changes in individual target lesions in each patient. The majority of target lesions remained stable on CT imaging through day 28 post-infusion (Figure 2D). Patients with PDAC showed the greatest percentage of lesions progressing within 28 days. After 2 months, an increased percentage of lesions had progressed for each malignancy group, with only a minority of lesions showing a >30% decrease in size. Blood tumor markers largely remained stable after infusion for MPM and OVCA but showed marked increases in patients with PDAC within 28 days (Figure S3). Together, these findings indicated that although some anti-tumor activity may be produced by a single CART-meso cell infusion, particularly in patients with OVCA during the first month, responses were transient, and most lesions remained unresponsive.

CART-Meso Cell Expansion and Persistence *In Vivo* and Impact of the Conditioning Regimen

Based on the limited anti-tumor activity seen with CART-meso cells, we next determined CART-meso cell persistence *in vivo*. We found that CART-meso cells initially expanded within the peripheral blood

Table 1. Patient Demographics and Disease Characteristics

Characteristics	Total (N = 15)
Age, Years	
Median	69
Min, max	48, 75
Gender, n (%)	
Male	10 (67.0)
Female	5 (33.0)
Race, n (%)	
Caucasian	14 (93.0)
Asian	1 (7.0)
ECOG Status, n (%)	
0	10 (66.7)
1	5 (33.3)
Tumor Site,[a] n (%)	
Mesothelioma	5 (33.3)
Ovarian carcinoma	5 (33.3)
Pancreatic ductal adenocarcinoma	5 (33.3)
Number of Prior Anti-cancer Regimens	
Median	5
Min, max	1, 11

ECOG, Eastern Cooperative Oncology Group; max, maximum; min, minimum.
[a]Confirmed pathology for each tumor site: mesothelioma, malignant pleural mesothelioma; ovarian carcinoma, persistent or recurrent serous ovarian cancer or primary peritoneal carcinoma; pancreatic ductal adenocarcinoma, metastatic pancreatic adenocarcinoma.

Table 2. Summary of Reported Adverse Events Related to CART-Meso Cells by Grade Reported in More Than One Subject (Unless ≥ Grade 3)

All Subjects (n = 15)	Grade 1, n	Grade 2, n	Grade 3, n	Grade 4, n	Total, n
Clinical Events					
Fatigue	1	5	1	0	7
Nausea	5	1	0	0	6
Ascites	0	0	4	0	4
Vomiting	4	0	0	0	4
Confusion	3	0	0	0	3
Diarrhea	3	0	0	0	3
Dysgeusia	3	0	0	0	3
Fever	3	0	0	0	3
Abdominal pain	0	1	1	0	2
Anorexia	1	1	0	0	2
Anxiety	2	0	0	0	2
Chills	1	1	0	0	2
Constipation	1	1	0	0	2
Dizziness	2	0	0	0	2
Myalgia	2	0	0	0	2
Paroxysmal atrial tachycardia	1	1	0	0	2
Pleural effusion	1	1	0	0	2
Sore throat	2	0	0	0	2
Abdominal distension	0	0	1	0	1
Acute kidney injury	0	0	1	0	1
Bacteremia	0	0	1	0	1
Hepatic failure	0	0	1	0	1
Hepatitis	0	0	1	0	1
Dyspnea	0	0	1	0	1
Sepsis	0	0	0	1	1
Hematologic Events					
Anemia	0	0	0	1	1
DIC	0	0	1	0	1
Lymphocyte count decreased	0	0	1	0	1
Nonhematologic Events					
Alkaline phosphatase increased	0	0	1	0	1
ALT increased	0	0	1	0	1
AST increased	0	0	1	0	1
Blood bilirubin increased	0	0	1	0	1
Total	35	12	18	2	67

ALT, alanine aminotransferase; AST, aspartate aminotransferase; DIC, disseminated intravascular coagulation.

of all patients, reaching peak levels by days 6–14 (Figure 3). However, a marked contraction in CART-meso levels was seen thereafter with undetectable levels observed by month 2 in 9 of 15 patients. For two patients (patients 3-69 and 4-06), detectable CAR levels were seen through month 6. A higher cell infusion dose produced an approximate 10-fold increase in peak levels of CART-meso cells (cohort 3/cohort 1 ratio = 11.9, $p = 0.048$; cohort 4/cohort 2 ratio = 9.2, $p = 0.100$). In addition, lymphoreduction was associated with increased expansion of CART-meso cells in the peripheral blood (ratio cohort 2/cohort 1 ratio = 11.0, $p = 0.095$; cohort 4/cohort 3 ratio = 8.5, $p = 0.100$).

We next examined whether CART-meso cells infiltrated tumor lesions. Although post-treatment biopsies were not mandated, samples from 10 patients were available at various time points for qPCR analysis, as detailed in Figure S4. CART-meso cells were detectable in tumor biopsy samples specifically from three of five patients after CART-meso cell infusion, suggesting successful trafficking. CAR levels detected in tumor tissue, though, were generally quite low. CART-meso cells were also detected in ascites fluid from two of five patients and persisted for up to 29 days. For one of these patients (2-36) with PDAC, CART-meso DNA copies detected in peritoneal fluid on day 14 (2,125.3 copies/μg DNA) were higher than that

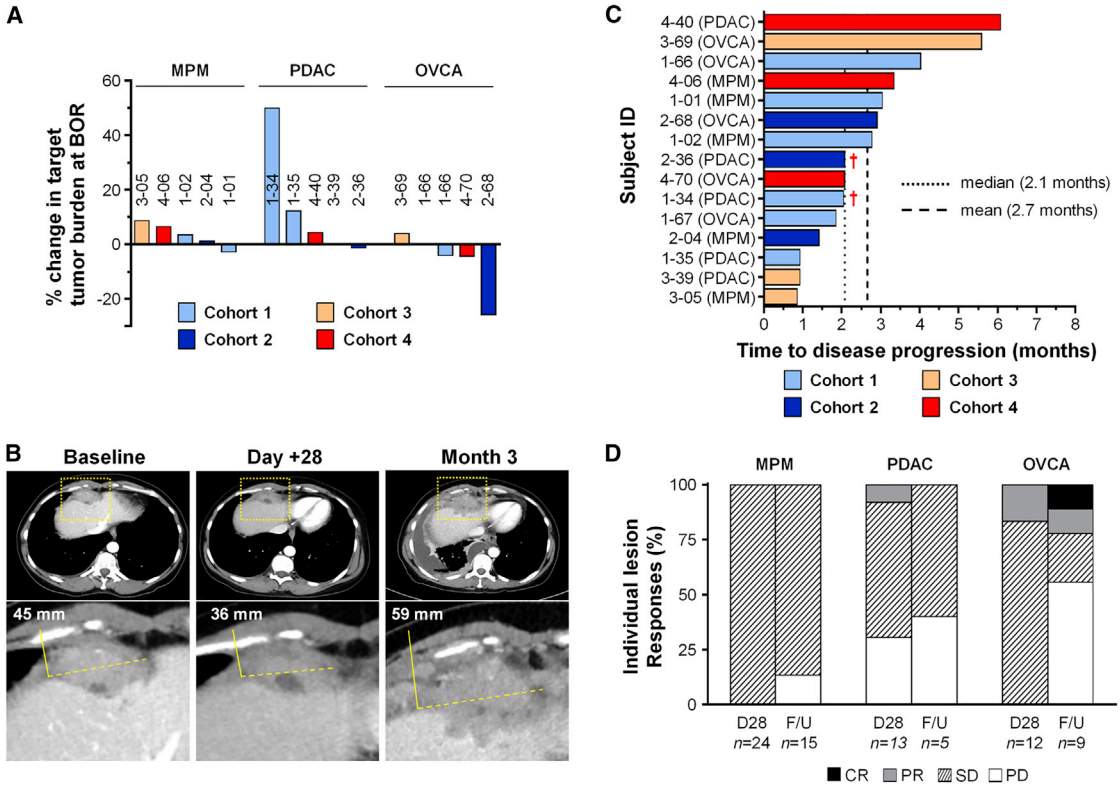

Figure 2. Tumor Response

(A) Waterfall plot showing percent change in tumor burden determined as the best overall response (BOR) based on RECIST 1.1 or modified RECIST criteria. Patients are grouped by histology. (B) Swimmer plot showing time to disease progression in months. (C) CT images (top row) with higher-magnification images (bottom row) of liver lesion (dotted line box) for patient 2-68. Shown is the longest dimension of an initially responding tumor lesion in the liver at baseline, 28 days, and 3 months after receiving CART-meso cell infusion. (D) Response based on RECIST criteria for individual target lesions for each histology at day 28 and on follow-up (F/U) assessments performed at months 2–6.

seen in the blood on day 10 (316.05 copies/μg DNA) but lower than peak levels detected in blood on day 6 (7,013.0 copies/μg DNA).

HAMA/HACA

We hypothesized that limited persistence of CART-meso cells may be related to a humoral response against the mouse anti-mesothelin scFv. However, we detected no appreciable changes in the levels of human anti-mouse antibody (HAMA) responses in any patient post-infusion (Table S4). In contrast, three patients (1-01, 3-69, and 4-70) showed high levels of human anti-CAR antibodies (HACAs) at baseline that increased by day 28 (Table S5). For the remaining 11 patients evaluated, 5 developed HACA detected by day 28. Lymphodepletion did not impact the subsequent development of HACA responses. Specifically, four of six patients who received the lymphodepleting regimen (cohorts 2 and 4) developed HACA in serum by day 28. However, six patients showed no serum HACA responses by day 28 in the blood. For one of these patients (2-36), a HACA response was detectable in ascites fluid at day 28, and for another patient (1-66), a HACA response became detectable by day 74 in the blood. However, despite detection of HACA responses in some, but not all, patients, we observed no correlation between a HACA response and CART-meso cell persistence. There were no clinical symptoms associated with the development of HACA.

Bioactivity of CART-Meso Cells by Luminex

We have previously observed increased serum cytokines in some patients receiving CART-meso cells. Therefore, we evaluated patients in cohort 4 who demonstrated the maximum peak expansion of CART-meso cells in the blood after infusion for serum cytokines by Luminex as a marker of CART-meso cell bioactivity and toxicity. For this cohort, we found that samples from day 1 through day 28 did not show appreciable changes in cytokine levels from baseline (data not shown). However, we did detect changes in serum cytokines, including IL-6, in patient 1-34, who experienced a DLT (Figure S3). The interpretation of this finding, though, was complicated by the aforementioned concomitant infection in this patient.

DISCUSSION

In this study, we assessed the feasibility, safety, persistence, and efficacy of CAR T cells lentivirally transduced with a second-generation CAR that targets mesothelin. CART-meso cells were administered intravenously to patients with MPM, OVCA, and PDAC with or

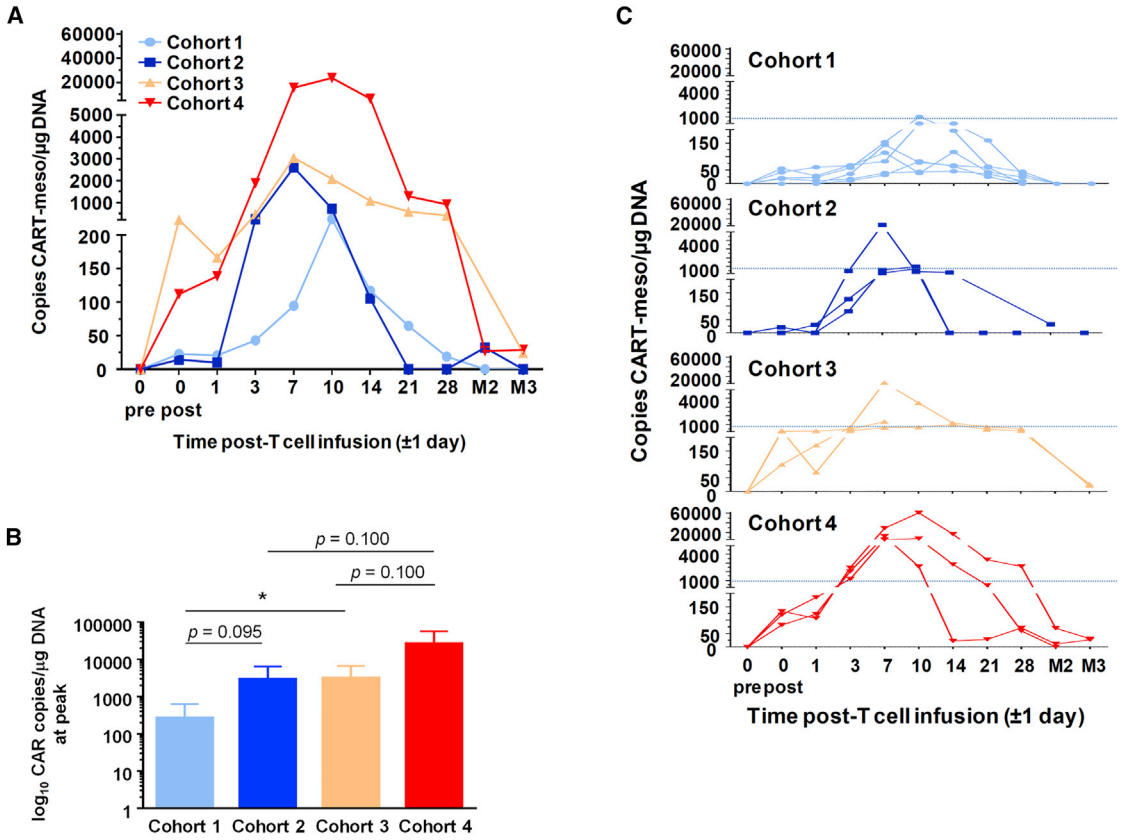

Figure 3. Lenti-CART-Meso Cell Persistence in Peripheral Blood

(A) CART-meso expansion and persistence in the peripheral blood was detected by qPCR. Shown is the mean copy number of CART-meso cells per microgram of DNA for each treatment cohort sampled prior to (pre) and at defined time points after CART-meso cell infusion. M, month. (B) Peripheral blood expansion of CART-meso cells is shown for each patient and grouped by cohort. Peak CART-meso levels observed among patients in cohort 1 (991.76 copies/µg, patient 1-02) are depicted by a blue line in each plot for reference. (C) Shown are mean peak expansion levels of CART-meso in peripheral blood within each cohort. Statistical significance was determined by Mann-Whitney test. *$p < 0.05$.

without lymphodepletion using cyclophosphamide. Overall, CART-meso cells were successfully manufactured for all patients and found to be safe at doses up to 3×10^8 CAR T cells/m^2 without evidence for on-target toxicities including pleuritis, peritonitis, or pericarditis. CART-meso cells showed modest expansion with limited persistence in the blood. Lymphodepletion improved the initial expansion of CART-meso cells but did not impact CART-meso cell persistence. Despite this, we did detect CAR DNA in tumor biopsies and ascites from several patients, suggesting CART-meso cells traffic to tumors. In summary, a single infusion of CART-meso cells was safe in this first-in-human study but produced minimal anti-tumor activity.

Our prior investigations with CAR T cells targeting mesothelin involved transient expression of a CAR using mRNA electroporation.[19,20] In contrast, in the current study, we engineered autologous T cells to stably express a CAR using lentiviral technology with the goal to improve CART-meso cell persistence *in vivo*. CART-meso DNA was consistently detectable in the blood of patients in all cohorts, with peak levels occurring 6–10 days after infusion. We observed no clear instances of CRS. Serum cytokines were serially evaluated as a marker of CART-meso cell bioactivity and toxicity, but showed no appreciable changes during the first month after infusion. This finding contrasts observations in hematological malignancies where CRS induced by CAR T cell therapy is associated with rapid proliferation of infused CAR T cells and increased tumor burden.[25,26] The lack of CRS seen in solid malignancies with CAR T cells, including our study, may reflect the anatomical location of malignant cells that are largely confined to solid tissues rather than lymphoid organs (e.g., bone marrow and lymph nodes) and blood.

Our study provides new insights into CAR T cell persistence *in vivo* and the effects of lymphodepletion on CAR T cell expansion and persistence in solid tumors (Figure 3). In contrast with CART19 cells, which can expand 1,000-fold in hematologic malignancies,[27,28] CART-meso cell expansion was 10-fold less. In addition, unlike CART19 cells, which can persist in patients for years after infusion,[29] CART-meso cells became undetectable in peripheral blood in most patients by 28 days after infusion. We observed a dose response

with patients in cohort 3 ($1–3 \times 10^8$ cells/m^2) compared with cohort 1 ($1–3 \times 10^7$ cells/m^2) demonstrating a 10-fold higher peak level (C_{max}) of CART-meso DNA in the blood. In addition, lymphodepletion with cyclophosphamide prior to CART-meso cell infusion produced a near-10-fold increased expansion of CART-meso cells. The lymphodepletion regimen (cyclophosphamide) was administered intravenously as an outpatient regimen, was inexpensive, and was well tolerated.

The relatively low levels and short persistence of CART-meso cells in the blood are consistent with reports from other CAR T cell trials in solid tumors.[12–14] However, the mechanism underlying this biology remains unclear. We hypothesized that lymphodepletion might improve CAR T cell expansion and persistence. We selected cyclophosphamide as a conditioning regimen that has been used in other adoptive T cell protocols.[30] Because cyclophosphamide has limited activity in pancreatic cancer and in mesothelioma, and doses greater than 3 g/m^2 are significantly myelosuppressive, we selected a dose of 1.5 g/m^2 to achieve transient lymphodepletion without significant neutropenia or prolonged myelosuppression that would put patients at risk for infection. Although lymphodepletion increased CAR T cell expansion, it did not significantly augment CAR T cell persistence. Further, lymphodepletion may diminish the potential for CAR T cells to provide a vaccine effect because of depletion of endogenous T cells.[31] In hematological malignancies, lymphodepleting chemotherapy is not an absolute requirement for CART19 cell efficacy.[27] Thus, it remains unclear whether lymphodepletion will be necessary and beneficial for improving CAR T cell efficacy in solid malignancies.

We considered the possibility that short persistence of CART-meso cells might reflect immune-mediated elimination because the scFv of the CAR is murine derived. As such, humoral or cellular immune responses directed against the murine portion of the CAR could eliminate CART-meso cells. We did not detect appreciable levels of HAMA in any of the patients (Figure S3) but did detect HACA that reacted against the SS1 mesothelin-specific CAR in 10 of 14 patients evaluated (Table S5). There was no correlative evidence that HACA impacted peak CART-meso cell levels or persistence. However, we did not evaluate for CAR-reactive T cell responses, and so immune-mediated elimination of CAR T cells remains a possible contributing factor to poor *in vivo* persistence of CART-meso cells. To address this possibility, we are conducting a phase 1 study evaluating a mesothelin-specific CAR containing a fully human scFv (ClinicalTrials.gov: NCT03054298 and NCT03323944).

Autologous CAR T cells recognizing mesothelin can effectively recognize and lyse mesothelin-expressing human tumor cells *in vitro* and in immunocompromised mouse models.[18,19] However, despite this anti-tumor potential, CART-meso cells did not produce significant clinical activity beyond stable disease in any of the treatment cohorts in our study. Multiple mechanisms may underlie this suboptimal efficacy. Specifically, we found that infused CAR T cells demonstrated poor persistence in the peripheral blood. In addition, we detected only low levels of CAR T cells within tumors using PCR, suggesting poor infiltration, lack of expansion within tumors, or both. In preclinical models conducted in immunodeficient mice, CAR T cells rapidly traffic to tumors, proliferate locally, and exert anti-tumor activity when injected intravenously. However, they eventually acquire a state of hypofunction within the tumor microenvironment and lose the ability to control tumor outgrowth.[4] Similar findings were seen for tumor-specific T cells adoptively transferred into a genetic mouse model of spontaneous PDAC.[32] Studying this biology in patients whereby T cells become dysfunctional in tumors has been challenging given the need for sufficient numbers of tumor-infiltrating CAR T cells to assess their functional status. Monitoring CAR T cell trafficking by labeling cells with positron emission tomography (PET)-avid markers could inform CAR T cell biodistribution after injection.[33] Ongoing studies to understand CAR T cell fate are investigating intratumoral injection of cells and collection of pleural or peritoneal fluid to isolate CAR T cells for functional studies. Finally, several patients lacked or showed limited expression of mesothelin within tumors. Only 3 of 15 patients had expression of mesothelin on >75% of tumor cells. Tumor mesothelin expression was not an inclusion criterion for our study. However, given these data, future trials focused on efficacy will need to screen prospectively for mesothelin expression. To this end, it remains unclear to what degree CAR T cells can elicit "bystander" anti-tumor activity capable of eliminating tumor-antigen loss variants. In hematological malignancies, loss of CD19 on malignant cells is a well-defined mechanism of immune escape.[34,35] In glioblastoma, loss of CAR target was also suggested as a mechanism of immune escape.[8,15] However, the percentage of tumor cells needed to express a CAR target for an effective clinical response to occur is an important unanswered question for patient selection.

Improving outcomes with CAR T cells in solid malignancies will require a multi-faceted approach that addresses T cell persistence, trafficking to tumors, effector activity, and mechanisms of immunosuppression within tumors. CAR T cells can be administered repeatedly to support their persistence or delivered intratumorally to circumvent issues of trafficking. In solid tumors, clinical activity has been observed with intratumoral (i.e., intra-cranial or intra-ventricular) administration of CAR T cells in glioblastoma.[9,15] Alternative strategies to improve CAR T cell trafficking might involve introducing chemokine receptors into CAR T cells,[36,37] engineering adhesion ligands on CAR T cells,[38] stimulating chemokine production in tumors,[39] combining CAR T cells with therapeutics that disrupt the extracellular matrix,[40,41] or modulating immune-suppressive elements within tumors.[3] Similarly, the persistence of adoptively transferred T cells can be enhanced through immune-stimulating agents such as CD40 agonists.[42] If CAR T cell hypofunction emerges as a mechanism of immune resistance, intrinsic T cell alterations can be incorporated including enhancement of CAR designs[43,44] or introducing chimeric switch-receptors targeting PD1 or transforming growth factor-β (TGF-β).[45,46] It is possible that enhancing CAR T cell effector activity within tumors may also require immune checkpoint blockade because of induction of mechanisms of adaptive

immune resistance. Similarly, CAR T cell therapy may need to address the emergence of antigen-loss variants.[8,15] To do this, CAR T cells could be engineered with two types of CAR T cells, CAR T cells with dual specificities or CAR T cells that deliver additional payloads.[47,48] Our study provides the framework for beginning these investigations to systematically define key elements for establishing productive T cell immunosurveillance in patients with solid malignancies.

The application of CAR T cells to solid tumors is in its earliest stages of development. In this study, we demonstrate safety and feasibility of administering mesothelin-specific lentiviral CAR T cells to patients with solid tumors. Based on our findings, we have initiated studies to determine whether a fully humanized anti-mesothelin scFv can improve CAR T cell persistence by preventing immunologic rejection and enhance anti-tumor activity (ClinicalTrials.gov: NCT0354298 and NCT03323944). Mesothelin expression on tumor cells is an inclusion criterion for these new studies. The selected humanized CAR was based on improved anti-tumor activity seen in preclinical models with the goal to optimize effector activity of tumor-infiltrating CAR T cells. Finally, we have added a cohort of patients that will allow for testing of local delivery of CAR T cells into pleural and peritoneal cavities. A trial using intra-pleural delivery of mesothelin CAR T cells for mesothelioma is already underway at Memorial Sloan Kettering Cancer Center (ClinicalTrials.gov: NCT02414269). In conclusion, our findings from this first-in-human clinical trial provide the framework for subsequent studies testing next generation CAR T cells targeting mesothelin in solid tumors.

MATERIALS AND METHODS

Patients

Patients with MPM (histologically confirmed epithelial type), persistent or recurrent serous OVCA, or PDAC were enrolled into a phase I clinical trial (ClinicalTrials.gov: NCT02159716) at the Abramson Cancer Center, University of Pennsylvania (Philadelphia, PA, USA). Inclusion criteria were age >18 years, Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, life expectancy >3 months, advanced disease, failure of $\geq$1 prior standard of care chemotherapy, measurable disease as defined by RECIST 1.1 criteria or modified RECIST criteria, satisfactory organ and bone marrow function, adequate blood coagulation parameters (international normalized ratio [INR] < 1.5 and partial thromboplastin time [PTT] < 1.2× upper limit of normal [ULN]), and agreement to use approved contraceptive methods and also abstain from other methods of conception during the study and for 6 months following study cell infusion in the absence of proof of sterility. Exclusion criteria included biphasic MPM or sarcomatoid MPM histology (known to express low levels of mesothelin); participation in a therapeutic investigational study within 4 weeks of enrollment; anticipated need for systemic chemotherapy within 2 weeks before apheresis and infusion of CART-meso cells; active invasive cancer other than MPM, OVCA, or PDAC; active autoimmune disease requiring immunosuppressive therapy; ongoing or active infection; planned concurrent treatment with systemic high-dose corticosteroids; requirement for supplemental oxygen therapy; prior gene therapy or therapy with murine or chimeric antibodies; prior therapy with gene-modified cells; previous experimental therapy with SS1 moiety or chimeric antibodies; history of allergy to murine proteins or study product excipients (human serum albumin, DMSO, and Dextran-40); viral infection with HIV, hepatitis C virus (HCV), or hepatitis B virus (HBV); pregnancy or breast-feeding; and pleural or peritoneal effusion that could not be drained with standard approaches. Patients with any clinically significant pericardial effusion, congestive heart failure (NY Heart Association grades II–IV), or cardiovascular condition that would preclude assessment of pericarditis were also excluded. Because this was a safety and feasibility trial, a specified level of mesothelin expression on tumors was not required for eligibility; however, it was assessed by histological evaluation. All patients provided written informed consent, and the study was approved by the local institutional review board of the University of Pennsylvania.

Study Design and Treatment Plan

This was a phase I trial (ClinicalTrials.gov: NCT02159716) using a classic 3+3 study design. The primary objective was to test the safety and manufacturing feasibility of a single infusion of autologous CAR T cells transduced with a lentiviral construct expressing an anti-mesothelin (SS1) single-chain antibody variable fragment (scFv) linked to the intracellular CD3ζ T cell receptor domain and the 4-1BB co-stimulatory domain (CART-meso). Secondary objectives were to assess the clinical anti-tumor effect of CART-meso cells by standard criteria (RECIST 1.1 and irRC for OVCA and PDAC, and modified RECIST for MPM) for each tumor type. PFS and overall survival (OS) were evaluated for up to 2 years post-infusion or until subjects initiated a subsequent cancer-related therapy.

Patients were screened for eligibility prior to undergoing a large-volume leukapheresis for collection of peripheral blood mononuclear cells (PBMCs) used for CAR T cell manufacturing (Figure 1A). Patients (n = 3–6 per cohort) were enrolled into one of four cohorts beginning with cohort 1 in which patients received CART-meso cells ($1–3 \times 10^7/m^2$) infused intravenously on day 0 without lymphodepletion. Upon completion of cohort 1, patients were enrolled into cohort 2 to receive CART-meso cells ($1–3 \times 10^7/m^2$) plus lymphodepletion with 1.5 g/m^2 cyclophosphamide administered intravenously 3 ± 1 days prior to CART-meso cell infusion. Based on safety observed in cohorts 1 and 2, patients were then enrolled into cohort 3 (without lymphodepletion) and then into cohort 4 (with lymphodepletion) using CART-meso cells ($1–3 \times 10^8/m^2$) infused on day 0. Dose escalation to cohorts 3 and 4 was not dependent on tumor histology. Optional tumor biopsies of accessible lesions were collected at baseline and 14 ± 7 days after infusion. Peripheral blood samples were obtained at defined time points to monitor for measures of safety, efficacy, and CART persistence. In some patients, pleural fluid and ascites samples were obtained and analyzed.

Safety Assessments

All patients administered CART-meso cells were evaluated for safety. Safety assessments included incidence of treatment-related AEs,

according to National Cancer Institute (NCI) Common Terminology Criteria of Adverse Events (CTCAE) version 4.0. AEs, including laboratory toxicities and clinical events, were defined as possibly, likely, or definitely related to study participation. Study-related AEs included chemotherapy toxicity, infusional toxicity, and any toxicity at least possibly related to CART-meso cells. A DLT was defined as grade 3 or higher hematologic or non-hematologic toxicity that developed after dosing through day 28 of the safety follow-up visit and was new (not existent before infusion) and at least possibly related to T cells. DLTs included: (1) grade 3 or higher non-hematological toxicity, except asymptomatic grade 3 electrolytes, grade 3 nausea, vomiting, diarrhea, or fatigue; (2) grade 3 or higher hematologic toxicity reported as an AE, except asymptomatic lymphopenia or other blood counts that were pre-existing regardless of grading; and (3) grade 3 or higher allergic reaction, hypersensitivity reactions, and autoimmune reactions, including pericarditis, peritonitis, and pleuritis.

Tumor Response Assessment

Tumor response was assessed by CT scans and disease-specific serum biomarkers (i.e., SMRP, CA19-9, CEA, CA125). Radiographic responses were measured using RECIST 1.1 and irRC (for OVCA and PDAC) and modified RECIST criteria (for MPM).

Sample Collection and Processing

Peripheral blood samples were collected in lavender top (K2EDTA) tubes for isolation of PBMC and red top (no additive) Vacutainer tubes (Becton Dickinson) for serum collection. Research tubes were delivered to the laboratory within 2 h of blood draw, and samples were generally processed within 30 min of receipt according to established laboratory standard operating procedures. PBMCs were purified, processed, and stored at −140°C. Serum was isolated from red top tubes by centrifugation, aliquoted in single-use 100- to 200-μL aliquots, and stored at −80°C.

CART-Meso Design and Cell Manufacturing

Mesothelin-specific CAR-modified T cells were manufactured in accordance with a US FDA investigational new drug (IND). Leukocytes were collected from a large-volume (~10 L) apheresis procedure performed at the Hospital of the University of Pennsylvania Apheresis Center approximately 4 weeks prior to the planned first dose of CART-meso cells. Manufacture and release testing of CART-meso cells was performed by the Clinical Cell and Vaccine Production (CVPF) at the University of Pennsylvania. Elutriated lymphocytes isolated from the leukopacks were transduced with a self-inactivating lentiviral vector expressing the mesothelin-specific CAR incorporating CD3ζ and 4-1BB signal domains. Transduced lymphocytes were expanded *in vitro* in cell culture medium supplemented with human serum for 9–10 days using bead-immobilized anti-CD3 and anti-CD28 antibodies.[25] The CAR-transduced autologous T cell product was cryopreserved in an infusible cryoprotectant-supplemented solution, and quality-control testing was performed prior to release of the cell product for infusion. Total T cell dose was based on the number of CAR-transduced cells. A pre-defined cutoff for cell viability prior to infusion was set at ≥70%. At the time of cell infusion, CART-meso cells were thawed at the bedside and administered to patients in the Clinical Trials Research Center at the Hospital of the University of Pennsylvania.

qPCR Analysis to Detect CART-Meso Transcripts

Total genomic DNA was isolated directly from whole blood, ascites, and tumor tissue, and CART-meso levels were measured by qPCR, using transgene-specific primers recognizing the 4-1BB-CD3ζ junctional fragment in the signaling domain of the CAR as previously described.[26] CART-meso levels are reported as transgene copies per microgram of genomic DNA.

Analysis of Serum-Soluble Factors

At baseline, day 0, and defined time points after CART-meso cell infusion, serum was collected from whole blood and analyzed by Luminex bead array technology using 30-plex kits (catalog no. [Cat#] LHC6003M; Life Technologies, Carlsbad, CA, USA) according to manufacturer's instructions.

Human Anti-Mouse and Anti-CAR Antibodies

The presence of HAMAs and HACAs was determined using ELISA and a flow cytometry-based assay,[19] respectively.

Tumor Biopsy and Mesothelin Expression

Tissues from tumor biopsies obtained at baseline and following CART-meso infusion were fixed in 10% formalin and paraffin embedded. Sections containing tumor were stained using mesothelin Ab-1 (clone 5B2, prediluted RTU; Thermo Fisher Scientific, Fremont, CA, USA) on a Bond III Autostainer (Leica Biosystem) for 20-min primary incubation in a ready-to-use EDTA-based (pH 9.0) epitope retrieval solution (ER2; Leica Biosystem). Membranous mesothelin immunoreactivity was scored under the microscope for its intensity (0–3 scale) and percentage of positive tumor cells by a designated board-certified pathologist. Post-infusion biopsies were collected as follows for each patient: 1-01, day 28; 1-02, day 21; 2-04, day 9; 3-05, day 24; 2-35, day 14; 3-39, day 15; 4-40, day 3; 1-66, day 14; and 2-68, day 21.

Statistics

Statistical analyses were conducted using Prism (GraphPad Software, version 7). Unpaired group comparison testing was performed using Mann-Whitney test. p values less than 0.05 were treated as statistically significant. Time to disease progression was defined as time from CART-meso cell infusion to disease progression or patient death, whichever occurred first.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.ymthe.2019.07.015.

AUTHOR CONTRIBUTIONS

A.R.H., J.L.T., G.P., C.H.J., and G.L.B conceived and designed the research. S.F.L., D.A.T., L.T., and J.J.M. performed the laboratory

correlative experiments. A.R.H., J.L.T., M.H.O., M.C.S., M.M., A.M.N., C.S.F., and G.L.B. conducted the clinical trial and clinical studies. A.R.H., J.L.T., M.H.O., W.L.G., S.F.L., D.A.T., E.M., B.L.L., J.J.M., S.M.A., and G.L.B. analyzed data. W.L.G., G.P., S.M.A., and G.L.B. wrote the manuscript, and all authors reviewed and edited the manuscript to its final version. The academic authors were fully responsible for the design, conduct, and analysis of the trial.

CONFLICT OF INTERESTS

B.L.L., C.H.J., G.L.B., G.P., S.F.L., S.M.A., and J.J.M. are inventors of intellectual property related to CAR T cells that is licensed by the University of Pennsylvania to Novartis.

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YMTHE, Volume *27*

Supplemental Information

Phase I Study of Lentiviral-Transduced Chimeric Antigen Receptor-Modified T Cells Recognizing Mesothelin in Advanced Solid Cancers

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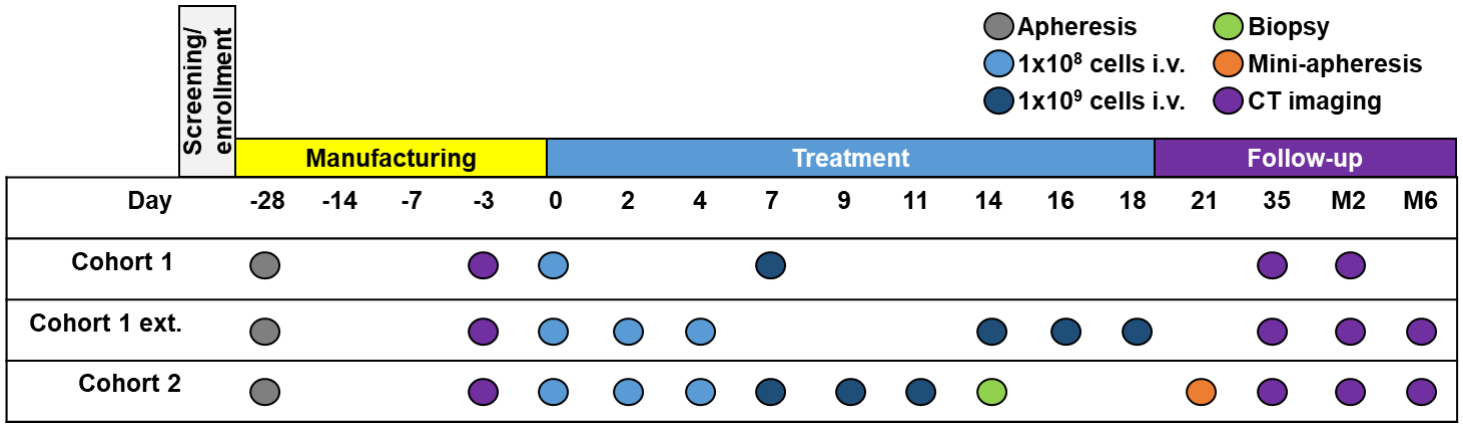
Supplementary Figure 1

A



B

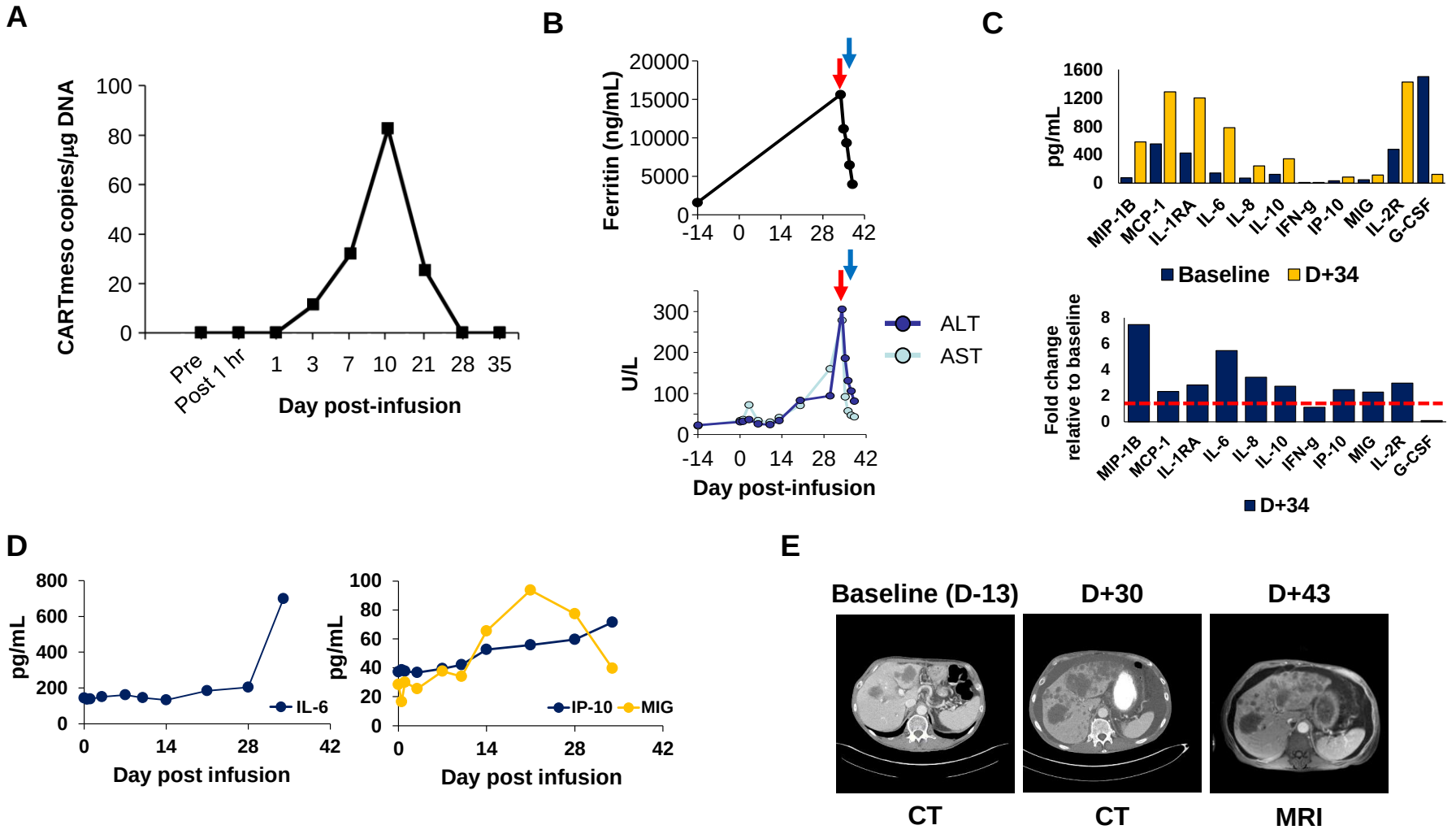
Subject ID	Cohort	No. of CART-meso infusions	Month 2 Response*	Month 6 Response
17510-101	1→1 ext.	8	PD	PD (Month 3)
17510-102	1	2	PD (Day 35)	N/A
17510-105	1→1 ext.	3	SD**	PD
17510-200	2	6	SD	N/A
17510-201	2	6	SD	SD
17510-202	2	6	SD	N/A
17510-205	2	6	SD	N/A

SD – Stable Disease
PD – Progressive Disease
N/A – Not Assessed

*Response assessed by modified RECIST criteria for mesothelioma.
**PR at Month 3 (Day 84) as reported in Haas et al., 2015.

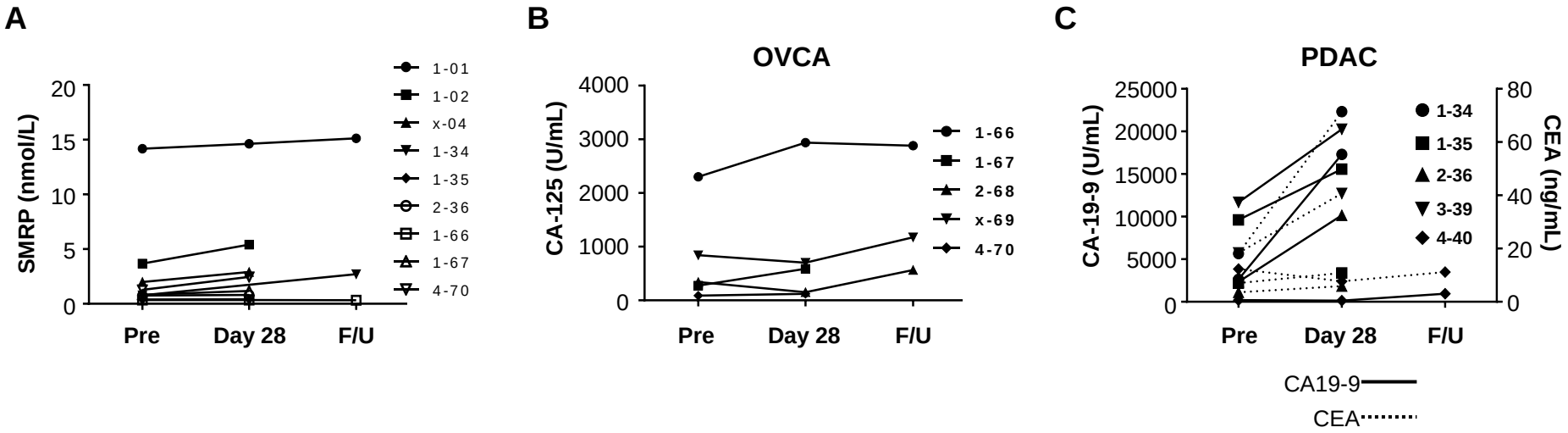
Supplementary Figure 1. Phase 1 study (UPCC17510) of RNA CART-meso in mesothelioma subjects. A, Protocol schema for screening, leukapheresis, manufacturing, CAR T cell infusions and subsequent follow up. **B**, Cohort assignment and clinical response for each subject at Months (M) 2 and 6 after initial CARTmeso cell infusion. Subjects received multiple infusions of RNA CART-meso cells, as indicated. CT, computed tomography; i.v., intravenous.

Supplementary Figure 2



Supplementary Figure 2. Correlative data on patient 01-34 with dose-limiting toxicity to Lenti CART-meso cell infusion. A. CARTmeso expansion and persistence in the peripheral blood was detected by qPCR. Shown is the copy number of CART-meso/µg DNA prior to CART-meso cell infusion (pre), 1 hr after infusion (post 1hr) and on the indicated days. **B.** Ferritin, AST and ALT levels detected in peripheral blood. The patient received 1 mg/kg prednisone on day 34 (red arrow). Blood cultures from day +34 revealed Klebsiella bacteremia and broad spectrum antibiotics were started on day 35 (blue arrow). **C.** Cytokine levels (top) and fold change in levels (bottom) detected by Luminex at baseline and on day +34. Red line indicates 1 for unchanged relative to baseline. **D.** Cytokine levels for IL-6 (left) and interferon-response proteins including IP-10 and MIG (right) over time after CARTmeso cell infusion on day 0. **E.** Cross-sectional images at baseline (CT imaging), day +30 (CT imaging) and day +43 (MRI imaging) after CARTmeso cell infusion.

Supplementary Figure 3



Supplementary Figure 3. Blood biomarkers of tumor response. Blood levels of circulating tumor markers were determined prior to CARTmeso cell infusion (pre) and on day 28 and subsequent follow-up (F/U) at month 3 when available. **A.** SMRP levels in subjects with MPM. **B.** CA-125 levels in subjects with OVCA. **C.** CA19-9 levels in subjects with PDAC.

Supplementary Figure 4

	Sample Type	Tumor Biopsy	Ascites/ Ascites cells	Other
Cohort	**Subject ID**	**time points evaluated# (copies/μg detected)**	**time points evaluated# (copies/μg detected)**	**time points evaluated# (copies/μg detected)**
Cohort 1	1-01 (MPM)	day 28		
	1-02 (MPM)	day 21	day 77	
	1-34 (PDAC)		day 44 | day 46	Month 2-autopsy[b] (49.0)
	1-35 (PDAC)	day 14 (45.3)		
	1-66 (OVCA)	day 14 (9.7) | Day 64[a]	day 70 | day 73 | day 74	
Cohort 2	2-04 (MPM)		day 9 (135.2) | day 17 (52.1)	
	2-36 (PDAC)		day 14 (2125.3) | day 29 (353.3)	
	2-68 (OVCA)			Month 3[c]
Cohort 3	3-39 (PDAC)			day 29[d] (964.4)
	3-69 (OVCA)	day 45 (65.3) | day 50 (14.4)		
Cohort 4				

KEY: No sample | Not detectable | Detectable

[#] Time points are in reference to day of CART-meso infusion (day 0)
[a] Five different tumor samples were evaluated at this time-point
[b] Necrotic spleen tissue from autopsy
[c] Pleural fluid
[d] CART-meso cells were detected in 4 arthrocentesis samples from left knee nodule. Shown is average of samples.

Supplementary Figure 4. Detection of CARTmeso in tumor tissue. CART-meso DNA was quantified by qPCR in tissues and fluid of subjects after CARTmeso cell infusion. Shown is the day of sample collection after CART-meso cell infusion. Blue indicates samples in which CAR T cells were detectable, whereas green indicates no CAR was detected and grey indicates that no sample was available. CART-meso cell quantification is shown in parenthesis as copies/μg genomic DNA.

Supplementary Table 1. Summary of reported adverse events related to RNA CART-meso cells by grade

All subjects N=7	Grade 1 *n*	Grade 2 *n*	Grade 3 *n*	Grade 4 *n*	Total *n*
Clinical events					
Fatigue (N=4)	2	1	1	0	4
Dysgeusia (N=3)	3	0	0	0	3
Non-cardiac chest pain (N=3)	1	2	0	0	3
Back pain (N=2)	0	2	0	0	3
Chills (N=2)	2	0	0	0	2
Cough (N=2)	2	0	0	0	2
Cytokine release syndrome (N=2)	1	0	0	1	2
Dyspnea (N=2)	2	0	0	0	2
Fever (N=2)	2	0	0	0	2
Headache (N=2)	2	0	0	0	2
Pain (N=2)	1	1	0	0	2
Nausea (N=2)	2	0	0	0	2
Tingling sensation in chest and other areas (N=2)	2	0	0	0	2
Cardiac arrest (N=1)	0	0	0	1	1
Respiratory failure (N=1)	0	0	0	1	1
Hematologic events					
Anemia (N=3)	0	2	1	0	3
Lymphocyte count decreased (N=2)	0	1	1	0	2
DIC (N=1)	0	0	0	1	1
Totals	22	9	3	4	38

Abbreviations: DIC, Disseminated intravascular coagulation

Supplementary Table 2. CART-meso cell product characteristics

Subject	CART-meso cells/m^2	%CD3$^+$ CD45$^+$	%CD3$^+$ CD4$^+$	%CD3$^+$ CD8$^+$	CD4:CD8 ratio	%scFv
31213-1-01	3.00E+07	96.2	76.7	24.2	3.2	35.1%
31213-1-02	3.00E+07	95.9	85.2	15.7	5.4	21.7%
31213-1-34	3.00E+07	98.3	85.5	15.4	5.6	18.7%
31213-1-35	3.00E+07	92.0	69.6	31.2	2.2	35.7%
31213-1-66	3.00E+07	98.9	66.0	35.7	1.8	27.9%
31213-1-67	3.00E+07	98.8	62.6	40.6	1.5	18.2%
31213-2-04	3.00E+07	97.6	43.7	57.0	0.8	17.9%
31213-2-36	3.00E+07	91.3	81.3	22.8	3.6	20.8%
31213-2-68	3.00E+07	97.3	53.5	46.5	1.2	35.7%
31213-3-05	3.00E+08	94.4	87	14.7	5.9	28.6%
31213-3-39	3.00E+08	92.4	69.9	33.4	2.1	17.0%
31213-3-69	3.00E+08	90.4	74.3	29.2	2.5	33.5%
31213-4-06	3.00E+08	87.7	69.6	32.3	2.2	24.2%
31213-4-40	3.00E+08	97.4	75.3	29.3	2.6	20.1%
31213-4-70	3.00E+08	92.5	61.5	40.3	1.5	15.5%

Supplementary Table 3. RECIST and irRC tumor responses per subject

Subject ID	Day 28 follow-up		Additional follow-up	
	RECIST/ modified RECIST*	irRC	RECIST/ modified RECIST*	irRC
Mesothelioma				
31213-1-01	SD	na	PD (Month 3)	na
31213-1-02	SD	na	PD (Month 3)	na
31213-2-04	SD	na	-	-
31213-3-05	PD (Day 12)	na	-	-
31213-4-06	SD	na	PD (Month 3)	na
Pancreatic ductal adenocarcinoma				
31213-1-34	PD	PD	-	-
31213-1-35	PD	PD	-	-
31213-2-36	SD	SD	SD (Month 2)	SD (Month 2)
31213-3-39	PD	SD	-	-
31213-4-40	SD	SD	SD (Month 3)	SD (Month 3)
Ovarian carcinoma				
31213-1-66	SD	SD	SD (Month 3)	SD (Month 3)
31213-1-67	SD	SD	-	PD (Month 2)
31213-2-68	SD	SD	PD (Month 3)	PD (Month 3)
31213-3-69	SD	SD	PD (Month 6)	PD (Month 6)
31213-4-70	SD	SD	-	-

RECIST – Response Evaluation Criteria in Solid Tumors
irRC – Immune-related response criteria
SD – Stable Disease
PD – Progressive Disease
na – not assessed

*RECIST 1.1 criteria. Mesothelioma response assessed by modified RECIST criteria for mesothelioma.

Supplementary Table 4. Human anti-mouse antibody quantification in patient sera

Cohort	Subject	Pre-infusion	Day 28	Month 2-3
Cohort 1	31213-1-01	nd	nd	15.13
	31213-1-02	nd	nd	nd
	31213-1-34	nd	nd	4.07
	31213-1-35	nd	nd	na
	31213-1-66	nd	nd (Day 21)	na
	31213-1-67	nd	nd	nd
Cohort 2	31213-2-04	nd	nd	nd
	31213-2-36	nd	nd	na
	31213-2-68	nd	nd	nd
Cohort 3	31213-3-05	nd	na	na
	31213-3-39	5.48	7.81	na
	31213-3-69	nd	5.21	na
Cohort 4	31213-4-06	6.39	6.08	na
	31213-4-40	5.48	5.68	na
	31213-4-70	na	na	na

HAMA=ng/mL
na, not assessed; nd, not detected.

Supplementary Table 5. Human anti-CAR antibody (IgG) in patient sera

Cohort	Subject	% cell staining		MFI		IgG response at Day 28?
		Pre-infusion	Day 28	Pre-infusion	Day 28	
Cohort 1	31213-1-01	55	72	503	889	Yes*
	31213-1-02	6.6	78	167	541	Yes
	31213-1-34	0.7	52	257	1062	Yes
	31213-1-35	13	13	463	501	No
	31213-1-66	3.4	14	166	224	No[#]
	31213-1-67	21	26	247	267	No
Cohort 2	31213-2-04	8.3	98	225	1689	Yes
	31213-2-36	0.6	0.6	258	228	No^
	31213-2-68	10	18	188	210	No
Cohort 3	31213-3-05	samples not available				
	31213-3-39	0.6	0.02	667	456	No
	31213-3-69	51	96	409	1131	Yes
Cohort 4	31213-4-06	17	50	259	402	Yes
	31213-4-40	3.2	65	331	1048	Yes
	31213-4-70	66	100	511	6258	Yes*

MFI – mean fluorescence intensity

*Present at baseline
[#]Positive at Day 74
^Positive in ascites at Day 28

Note: Serum samples diluted 1:50 in FACS buffer