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Phase I Hepatic Immunotherapy for Metastases study of intra-arterial chimeric antigen receptor modified T cell therapy for CEA+ liver metastases

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Abstract

Purpose—Chimeric antigen receptor modified T cells (CAR-T) have demonstrated encouraging results in early-phase clinical trials. Successful adaptation of CAR-T technology for CEA-expressing adenocarcinoma liver metastases (LM), a major cause of death in patients with gastrointestinal cancers, has yet to be achieved. We sought to test intrahepatic delivery of anti-CEA CAR-T through percutaneous hepatic artery infusions (HAI).

Experimental Design—We conducted a phase I trial to test HAI of CAR-T in patients with CEA+ LM. Six patients completed the protocol, and 3 received anti-CEA CAR-T HAIs alone in dose-escalation fashion (10^8, 10^9, and 10^{10} cells). We treated an additional 3 patients with the maximum planned CAR-T HAI dose (10^{10} cells X 3) along with systemic IL2 support.

Results—Four patients had more than 10 LM and patients received a mean of 2.5 lines of conventional systemic therapy prior to enrollment. No patient suffered a grade 3 or 4 adverse event related to the CAR-T HAIs. One patient remains alive with stable disease at 23 months following CAR-T HAI and 5 patients died of progressive disease. Among the patients in the cohort that received systemic IL2 support, CEA levels decreased 37% (range 19–48%) from baseline. Biopsies demonstrated an increase in LM necrosis or fibrosis in 4 of 6 patients. Elevated serum IFNγ levels correlated with IL2 administration and CEA decreases.

Conclusions—We have demonstrated the safety of anti-CEA CAR-T HAIs with encouraging signals of clinical activity in a heavily pre-treated population with large tumor burdens. Further clinical testing of CAR-T HAIs for LM is warranted.

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Conflicts of Interest: The authors have no relevant disclosures.

INTRODUCTION

Liver metastases (LM) are a significant cause of morbidity and mortality in patients with gastrointestinal adenocarcinoma. Tumor infiltrating lymphocyte (TIL) studies have revealed that host T cell responses to LM are significant correlates of survival (1–5). While those who mount effective immune responses to LM tend to have prolonged survival, the vast majority of patients succumb to their disease in the context of endogenous immune failure. The immunosuppressive nature of the intrahepatic milieu (6–9) may promote the development of LM and contribute to aggressive disease biology. Given the favorable effects of robust liver TIL responses and the inherent suppressive nature of the intrahepatic space, delivery of highly specific T cell products for the treatment of LM is a rational approach.

T cells engineered with chimeric antigen receptors (CAR) to enable highly specific tumor recognition and killing have gained considerable attention (10–12). Reprogramming T cells with CAR genes provides an MHC-independent mechanism for docking with and lysing tumor cells. Such modified T cells have been alternatively termed “designer T cells”, “T-bodies”, or “CAR-T cells” (13–15). Carcinoembryonic antigen (CEA) is an attractive target for CAR-T treatment of adenocarcinoma LM given high levels of CEA expression and the ability to measure CEA in serum (16, 17). Upon antigen recognition, anti-CEA CAR-Ts proliferate, produce cytokines, and kill target cells (18). Previous clinical studies, which evaluated systemic delivery of anti-CEA T cells for metastatic adenocarcinoma, demonstrated both promise and dose limiting toxicity (19). To improve the tolerability of anti-CEA CAR-Ts for LM in addition to enhancing tumor killing within the liver, we studied a regional delivery strategy.

Regional intra-arterial delivery of chemotherapy for LM has been demonstrated to yield superior response rates and limited systemic morbidity (20). Prior reports of regionally infused adoptive cell therapy products have demonstrated the safety of this approach (21–25). We propose that hepatic artery infusion (HAI) of anti-CEA CAR-Ts will limit extrahepatic toxicity while optimizing efficacy for treatment of LM. To test the safety and *in vivo* activity of anti-CEA CAR-Ts in patients with LM, we conducted the phase I Hepatic Immunotherapy for Metastases (HITM) trial (NCT01373047). We utilized a second-generation anti-CEA CAR (18), containing the CD28 co-stimulatory and CD3ζ signaling domains. We treated an initial cohort with CAR-T HAI intra-patient dose escalations without IL2 support and a second cohort that received fixed CAR-T doses with continuous IL2 infusions.

Six patients with LM completed our protocol and we demonstrated that HAIs of anti-CEA CAR-Ts were well tolerated with and without IL2 infusion. We also demonstrated *in vivo* activity of CAR-T HAIs in patients with large volume LM refractory to conventional treatment. In addition to exploring the safety and efficacy of CAR-T HAIs, we examined immunologic correlates of intrahepatic and systemic responses. Our findings support testing of CAR-T HAIs for LM in future trials.

METHODS

Study design

In this phase I study (NCT01373047, RWH 11-335-99) we treated two cohorts of three patients with anti-CEA CAR-T HAIs without or with systemic IL2 support (Figure 1). Cohort 1 was treated with CAR-T HAIs in intrapatient dose escalation fashion (10^8, 10^9, and 10^{10} cells) without IL2. Those in the cohort 2 received 3 HAI of 10^{10} CAR-Ts in addition to continuous systemic IL2 infusion at 75,000 U/kg/day via an ambulatory infusion pump.

Eligible patients had measurable unresectable CEA-positive LM or detectable serum CEA levels and failed one or more lines of conventional systemic therapy. Minimal extra-hepatic disease in the lungs or abdomen was permitted. No commercial sponsor was involved in the study. Clinical assessments were scheduled on infusion days, and on days 1, 2, 4, and 7 post-infusion. Liver MRI and PET examinations were performed within one month prior to the first infusion and then within one month following the third CAR-T HAI. The study radiologist (BS) graded responses according to modified RECIST (mRECIST) and immune related response criteria (26). Safety evaluation was performed per protocol. Severity of adverse events was graded using the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0.

Human CAR-T cell production

The 2nd generation anti-CEA scfv-CD28/CD3ζ (Tandem) chimeric antigen receptor was cloned into the MFG retroviral backbone as previously described (FDA BB IND 10791) (18). Briefly, the tandem molecule was generated by fusing the hMN14 sFv-CD8 hinge segment of the IgTCR (IgCEA) in the MFG retroviral backbone with a hybrid CD28/CD3ζ molecule. The construct was verified by sequencing. The clinical retroviral vector supernatant was produced using PG13 cells to generate gibbon ape leukemia virus pseudotyped viral particles as described (27). All clinical batches were prepared at Indiana University vector production facility (Indianapolis, IN) and stored at −80°C.

Rhode Island Blood Center personnel performed leukapheresis at the Roger Williams Medical Center (RWMC, Providence, RI). Anti-CEA CAR-Ts were prepared at the RWMC Cell Immunotherapy and Gene Therapy (CITGT) Good Manufacturing Practice (GMP) Facility with standard operating procedures (SOPs) for processing, manufacturing, expansion, dose harvesting, labeling, storage and distribution. Briefly, patient peripheral blood mononuclear cells (PBMCs) were isolated from leukapheresis product using Ficoll (Sigma, St; Louis, MO). We then activated PBMCs for 48–72 hours in tissue culture flasks (BD Falcon, Franklin Lakes, NJ) containing AIM V media (Life Technologies, Grand Island, NY) supplemented with 5% sterile human AB serum (Valley Biomedical, Winchester, VA), 50 ng/mL of anti-CD3 monoclonal antibody (OKT3; Ortho Biotech, Horsham, PA) and 3000 U/mL of IL2 (Prometheus, San Diego, CA).

Using the spinoculation method (28), 7.2 – 14.4 × 10^8 T cells were transduced in retronectin (Takara Bio Inc, Japan) coated 6-well plates in AIM V media with 5% human AB serum, 3000 U/ mL of IL2, and protamine sulfate (MP Biomedicals) at low speed centrifugation for 1 hour at room temperature. Three transductions were carried out over 24-hrs. After

transduction, cells were washed and incubated for 48–72 hours at 37°C. CAR-Ts were further expanded in Lifecell tissue culture bags (Baxter, Deerfield, IL) for 10–14 days. CAR-T growth curves and cell viability were examined periodically and cell growth media was replaced as required. CAR-Ts were examined by flow cytometry with fluorescently labeled antibodies specific for CD3 (UCHT1, Invitrogen, Frederick, MD), CD4 (SK3, BD Biosciences, San Jose, CA), CD8 (3B5, Invitrogen), and CAR expression (WI2 antibody, Immunomedics, Norris Plains, NJ). The WI2 antibody was prepared as an APC conjugate (WI2-APC; Molecular Probes). Flow cytometry was performed on a CyAn (Beckman Coulter, Brea, CA) or LSR-II (BD Biosciences, San Jose, CA) machine. In vitro activity of patient products was measured by bioluminescence cytotoxicity assay. Luciferase-expressing CEA+ tumor cells were mixed with anti-CEA CAR-T at various ratios in 96-well round bottom plates and loss of bioluminescence from each well measured (29).

We prepared clinical doses using a Fenwal cell harvester system (Baxter, Deerfield, IL) in freezing media containing PlasmaLyte (Baxter), 20% human bovine albumin (Valley Biomedicals), 10% DMSO (Bioniche Pharma, Lake Forrest, IL) and IL2. Bacterial and fungal cultures were monitored for 14 and 28 days respectively. We performed assays for endotoxin with LAL Endotoxin assay kits (Lonza, Walkersville, MD). The clinical dose was stored in liquid nitrogen and thawed immediately prior to infusion.

Product delivery

At baseline, a mapping angiogram was performed via a common femoral artery approach. The gastroduodenal and right gastric arteries, in addition to other potential sources of extrahepatic perfusion, were embolized with microcoils. For CAR-T infusions, the same arterial access procedure was carried out and the cells were hand injected via a 60cc syringe at a rate of <2cc/second with a total volume of 100cc. Angiography with calibrated contrast rate was performed after the first 50cc and at completion of the CAR-T infusion to confirm preserved arterial flow. Infusions were delivered into the proper hepatic artery when possible. In cases of aberrant hepatic arterial anatomy, where either the right or left hepatic artery did not arise from the proper hepatic artery, the dose was split based upon lobar volume calculations. In such cases, split doses were delivered separately into the right and left hepatic arteries to ensure proportionate CAR-T delivery to both lobes.

Correlative studies

Normal liver and LM core needle (16-gauge) biopsies were obtained under sonographic guidance at baseline and at the time of the third CAR-T HAI. Three cores were obtained for normal liver and LM, with each confirmed by cytology. For each case, 4–5 μm sections were stained with H&E and additional unstained slides were stained with anti-CEA antibody (TF 3H8-1, Ventana, Tucson, AZ). All immunohistochemical stains were performed on the Ventana Medical System at Our Lady of Fatima Hospital (Providence, RI). All slides were reviewed in blinded fashion and graded for necrosis and fibrosis. Fibrosis was scored as follows: 0% = grade 0; 5–10% = grade 1; 11–50% = grade 2; >50% = grade 3. Necrosis was scored as follows: 0% = grade 0; 0–10% = grade 1; 11–50% = grade 2; >50% = grade 3. Flow cytometry was performed on fresh biopsy tissue for CAR-T cells and peripheral blood as described above.

We measured serum IFNγ levels in all patients by ELISA (EBioscience, San Diego, CA). Samples were purified with the Purelink DNA Isolation Kit (Life Technologies, Grand Island, NY) according to the manufacturer's instructions. Patient serum was screened for anti-CAR antibodies one month after treatment by flow cytometry. We mixed CAR+ or CAR- Jurkat cells with 100ul of 1:1 diluted patient serum and then stained with secondary goat anti-human immunoglobulin.

CAR DNA was measured from patient whole blood genomic DNA by qPCR performed at the Boston University Analytical Core Facility. SYBR Green technology was used and CAR positive samples were identified using 100uM 28F2 forward (5′-GCAAGCATTACCAGCCCTAT-3′) and zr2 reverse (5′-GTTCTGGCCCTGCTGGTA-3′) primers (custom, Sigma Aldrich, St. Louis, MO). Plasmid DNA containing the CAR gene was used as a positive control qPCR. Additional primers were used to amplify CD3, GAPDH, and RPL13A (Bio-Rad, Hercules, CA). Raw cycle threshold (Ct) values were normalized to the average of the two reference genes (RPL13A and GAPDH) and we used the DeltaDelta Ct method to analyze the results. Wet-lab validated and MIQE-compliant primers were purchased from BioRad (Hercules, CA).

RESULTS

Study design and patient characteristics

We enrolled eight patients with unresectable CEA+ adenocarcinoma LM who progressed on an average of 2.5 (range 2–4) lines of conventional systemic therapy (Table 1). Six patients completed the protocol (Figure 1A), one patient withdrew due to an unrelated infection prior to treatment, and another patient withdrew due to extrahepatic disease progression prior to his third CAR-T HAI. Of the patients that completed the protocol, 4 were male and 2 were female. Five patients had stage IV colorectal carcinoma and one patient had pancreatobiliary ampullary carcinoma. The average age was 57 (range, 51–66). Patients presented with substantial disease burdens, with the average size of the largest LM being 8.4 cm (range, 1.7–14.4) and five patients having more than 10 LM. The mean CEA level upon enrollment was 807 ng/ml (range, 2–3265). Five of eight patients had synchronous colorectal LM and the mean disease-free interval was 27.3 months (range, 9 to 37) for patients with metachronous LM. All further analyses include only the six patients who completed the study.

CAR-T cell product assessment

The leukapheresis product from each patient was analyzed by flow cytometry prior to and following transduction with anti-CEA CAR construct. For all patients, the mean percentage of CD3+ cells following leukapheresis was 55% (range, 12.0–82.0) and increased to 91% (range, 72–97) following activation and transduction (Figure 1B). The mean CD4:CD8 ratio was 2.4 (range, 1.4–4.7) in the leukapheresis samples and 0.8 (0.2–2.2) in the final products (not shown). The transduction efficiency (CAR+) ranged from 10% to 64%, with a mean of 45% (Figure 1B). Negligible FoxP3 staining was detected among CAR+ T cells prior to infusion (not shown). Cells in the final products were 85% viable prior to infusion (range,

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