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Metastatic gastric cancer target lesion complete response with Claudin18.2-CAR T cells

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SUMMARY

Treatment of hematologic malignancies with patient-derived anti-CD19 chimeric antigen receptor (CAR) T-cells has demonstrated long-term remissions for patients with otherwise treatment-refractory advanced leukemia and lymphoma. Conversely, CAR T-cell treatment of solid tumors, including advanced gastric cancer (GC), has proven more challenging due to on-target off-tumor toxicities, poor tumor T-cell infiltration, inefficient CAR T-cell expansion, immunosuppressive tumor microenvironments, and demanding preconditioning regimens. We report the exceptional results of autologous Claudin18.2-targeted CAR T cells (CT041) in a patient with metastatic GC, who had progressed on four lines of combined systemic chemotherapy and immunotherapy. After two CT041 infusions, the patient had target lesion complete response and sustained an 8-month overall partial response with only minimal ascites. Moreover, tumor-informed circulating tumor DNA (ctDNA) reductions coincided with rapid CAR T-cell expansion and radiologic response. No severe toxicities occurred, and the patient's quality of life significantly improved. This experience supports targeting Claudin18.2-positive GC with CAR T-cell therapy and helps to validate ctDNA as a biomarker in CAR T-cell therapy.

Clinical Insight: Claudin18.2-targeted CAR T cells can safely provide complete objective and ctDNA response in salvage metastatic GC.

INTRODUCTION

Chimeric antigen receptor (CAR) T-cell therapy is capable of effective responses and durable remissions in patients with advanced hematologic malignancies[1 2]. Unfortunatley, CAR T-cell treatments in solid tumors must overcome obstacles including CAR T-cell in vivo expansion, difficulty infiltrating immunosuppressive stroma, off-tumor toxicity, and intense chemotherapy lymphodepleting regimens[3 4]. With deep tissue invasion and peritoneal penetration, especially with signet-ring/diffuse histologies, many gastric cancers (GCs) are unresectable and treatment options include only palliative chemotherapy, human epidermal growth factor receptor-2 (HER2)/vascular endothelial growth factor (VEGF)-targeted, or immune checkpoint inhibitor therapies. Five-year overall survival for patients with GC ranges from 5% to 20% with a median of 11 months.[5] Chemotherapy backbones coupled with immunotherapy or targeted agents offer objective tumor responses and survival benefits, but the vast majority of patients with GC remain incurable.[6–11]

Claudin18.2 (CLDN18.2) is a highly-selective cell-surface molecule with limited expression in normal gastric tissues but significantly higher expression in primary GCs and their metastases.[12] Hence anti-CLDN18.2-targeted chimeric antigen receptor (CAR)-T cells, termed CT041, were developed for treatment of patients with GC in the salvage setting.[13] A Chinese phase 1 clinical trial of Claudin18.2-specific CAR-T cells in various gastrointestinal cancers reported the interim results of the first 37 patients of which 28 had gastroesophageal junction (GEJ) or GC and 25 had at least two lines of therapy. Initial outcomes observed that 57.1% of patients achieved a partial response, 17.9% had stable disease, and 25% had progressive disease (PD), but none with a complete response.[14]

METHODS

Study treatment

We initiated a phase 1b multicenter, open-label study of CT041 (NCT04404595) actively enrolling at six sites in the USA. The protocol is available in online supplemental file 1. Results from all sites are pending treatment of additional patients and will be reported. All participants gave informed consent to participate in the study before taking part.

Table 1 Demographic and clinical characteristics for Patient 1 on the CT041 protocol

Demographic characteristics	
Age category	30–39 years
Alcohol	No
Smoking	No
Illicit drug use	No
Family history of cancer	No
Clinical characteristics	
Prior lines	Line of therapy
FOLFOX	First line
Trastuzumab	First line, second line
Pembrolizumab	First line, second line
FOLFIRI	Second line
Paclitaxel	Third line
DCF	Fourth line
Additional prior therapy	
HIPEC	Four treatments
Staging	
TNM categories	cTx/cNx/pM1 (peritoneum)
Performance status	
ECOG*	1
Location of primary tumor	
GEJ	Positive biopsy
Stomach body	Positive biopsy
Histological subtype	
Adenocarcinoma	Poorly differentiated, signet-ring/diffuse
Time from diagnosis to CT041 treatment	19 months (12/2019 to 7/2021)
HER2	
First biopsy	IHC equivocal (2+)
Second biopsy	ISH ratio 2.5
PD-L1	
First biopsy	CPS 3
Second EGD biopsy	CPS 8
Microsatellite stability	
First biopsy	Stable
Claudin18.2 IHC	Positive
++	40%
+++	60%
Mucin 17 IHC	Negative

*Eastern Cooperative Oncology Group (ECOG) performance status scores range from 0 to 5, with higher scores indicating greater disability.
CPS, combined positive score; CT041, Claudin18.2-targeted CAR T cells; DCF, docetaxel, cisplatin, 5-fluorouracil; EGD, esophagogastroduodenoscopy; FOLFIRI, 5, fluorouracil, leucovorin, irinotecan; FOLFOX, 5, fluorouracil, leucovorin, oxaliplatin; GEJ, gastroesophageal junction; HIPEC, hyperthermic intraperitoneal chemotherapy; IHC, immunohistochemistry; ISH, in situ hybridization; PD-L1, programmed death ligand-1; TNM, tumor-node-metastasis.

Patient characteristics

Patient 1 was in their late 30s with metastatic, poorly differentiated, signet-ring GC, diagnosed December 2019 (table 1, figure 1A). The HER2 immunohistochemical status was equivocal (2+) with an in situ hybridization (ISH) ratio of 2.5 indicating gene amplification. Biopsies of the GEJ (first) and gastric fundus (second) demonstrated programmed death ligand-1 combined positivity scores of 3 and 8, respectively. Their tumor was microsatellite stable. Metastatic locations at screening included two peritoneal nodules along the dome of the urinary bladder and the peritoneal wall. Non-target lesions included gastric wall thickening and ascites.

The patient consented for the trial in May 2021 after progression through four systemic lines of therapy in addition to multiple cytoreductive surgeries combined with heated intraperitoneal chemotherapy (HIPEC) (figure 1A). The patient had first-line progression on 5-fluorouracil, leucovorin, oxaliplatin combined with trastuzumab and pembrolizumab. This was complicated by immune-related colitis that was treated to resolution with prednisone, infliximab, and vedolizumab. The patient then had second-line progression on 5-fluorouracil, leucovorin, and irinotecan, third-line progression on paclitaxel (without VEGF inhibition due to gastrocutaneous fistula formation), and fourth-line progression on docetaxel, cisplatin, and 5-fluorouracil (DCF).

The patient's multiple previous cytoreductive surgeries and HIPEC reduced their peritoneal cancer index from 20 to 0 but was halted due to progressive tissue friability and development of the gastrocutaneous fistula that permitted venting of liquid oral intake in addition to refluxed bile. Due to progressive intraperitoneal bowel obstruction, the patient received most of their calories by total parenteral nutrition (TPN) for 5 months before enrollment. Just prior to CT041 infusion, the patient needed a nasogastric (NG) tube to attenuate consistent bilious vomiting due to progressive small bowel obstruction. Despite these multiple comorbidities, the patient continued to remain active throughout the day as energy permitted, completed their own activities of daily living, and assisted with their family as able. Thus the patient was scaled as 'ECOG 1' (Eastern Cooperative Oncology Group) per trial inclusion criteria.

The central immunohistochemical review of their archived tumor tissue showed 40% (++) and 60% (+++) CLDN18.2 positivity (figure 1B).

CLDN18.2 expression analysis by immunohistochemistry

The formalin-fixed, paraffin-embedded (FFPE) tumor tissues were immunostained by the central laboratory (Labcorp Drug Development) using a mouse anti-human CLDN18.2 antibody (Clone number HK19Z2 CARsgen Therapeutics) or an anti-rabbit/anti-mouse negative control (Lecia Biosystems Ref#NC499). Briefly, following deparaffinization and rehydration, FFPE tissue sections were exposed to 3% H_2O_2 in methanol to eliminate endogenous peroxidase activity. Bovine serum albumin (1%) was used to block for 30 min at room temperature (RT). The primary antibody was incubated overnight at 4°C. Sections were then rinsed with 1×PBS (phosphate-buffered saline) and 0.5% PBST (phosphate-buffered saline with Tween) and incubated with peroxidase-conjugated secondary antibodies (ChemMate DAKO

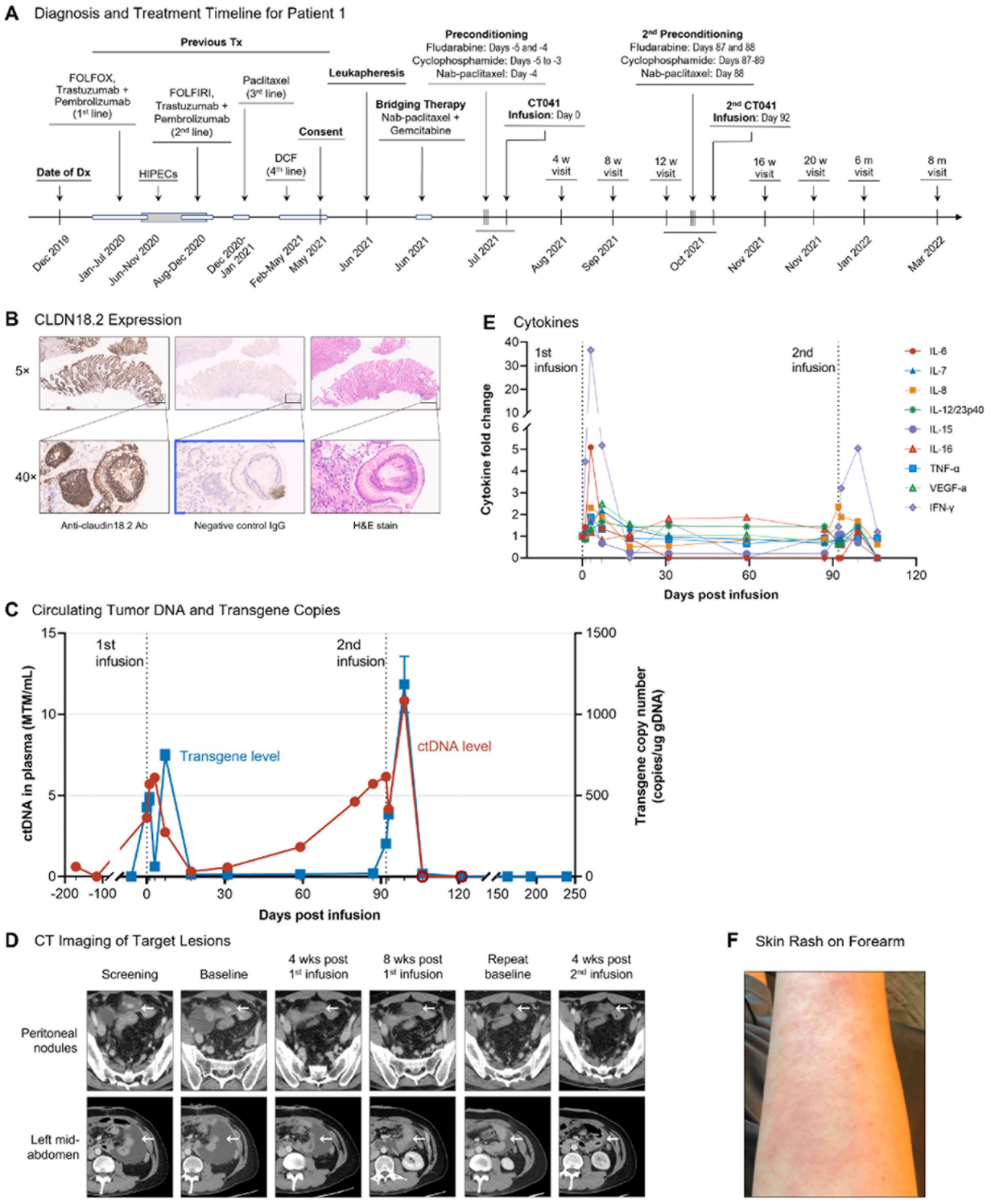

Figure 1 Clinical timeline of Patient 1 treated with CT041. (A) Initial diagnosis, treatment history, and follow-up visits for Patient 1, who received two CT041 infusions, 250×10^6 CAR-T cells each. (B) CLDN18.2 expression in gastric biopsy via immunohistochemistry, 5× and 40× magnification. (C) Tumor-informed circulating tumor DNA (ctDNA) in plasma measured in mean tumor molecules (MTM)/mL via qPCR and CAR-transgene copies/µg genomic DNA in peripheral blood via qPCR. Open spheres, ctDNA negative; closed spheres, ctDNA positive. (D) Complete response of target lesion at peritoneal nodules (arrows, top) and decreasing loculated ascites from left midabdomen (arrows, bottom) via contrast-enhanced axial CT scans. (E) Serum cytokines including interleukins, TNF-α, VEGF-A, and IFN-γ via enzyme-linked immunoassays. Fold changes normalized to Day 0 value (first infusion). (F) Skin rash following first CT041 infusion. CAR, chimeric antigen receptor; CLDN18.2, Claudin18.2; CT041, Claudin18.2-targeted CAR T cells; DCF, docetaxel, cisplatin, 5-fluorouracil; dx, diagnosis; FOLFIRI, 5, fluorouracil, leucovorin, irinotecan; FOLFOX, 5, fluorouracil, leucovorin, oxaliplatin; HIPEC, hyperthermic intraperitoneal chemotherapy; IFN, interferon; IL, interleukin; m, months qPCR, quantitative PCR; TNF, tumor necrosis factor; tx, treatment; VEGF, vascular endothelial growth factor, w/wks, weeks.

EnVision Detection Kit, Peroxidase/DAB, Rabbit/Mouse, DAKO) for 45 min at RT. Sections were visualized using a diaminobenzidine staining kit (DAKO), and then counterstained with hematoxylin, dehydrated, cleared, mounted, and photographed. A positive staining result was indicated when the cell membrane of tumor cells had yellow or brown staining without significant background staining. Different levels of CLDN18.2 expression were evaluated by two experienced pathologists using a 4-point scale. Score 0 indicates no CLDN18.2 expression; scores of 1+, 2+, and 3+, indicate weak, medium, and strong expression of CLDN18.2, respectively.

Leukapheresis at medical site

Prior to leukapheresis, the complete blood count with differential laboratory test was ordered at the medical site for Patient 1 to determine the levels of white blood cells, lymphocytes, monocytes, red blood cells, and platelets. Testing for transmittable infectious diseases (human immunodeficiency virus, HIV; hepatitis B virus, HBV; hepatitis C virus, HCV; coronavirus disease 2019, COVID-19) was performed. A standard leukapheresis procedure was conducted per institutional standard, processing about two to three times total blood volume (collection targets: $\geq 2\times10^9$ mononuclear cells apheresis product in ≥125–150 mL and ≥180 mL plasma). After collection, the fresh apheresis product and plasma were shipped at 2–8°C to the contracted cGMP manufacturing facility.

Generation of CT041 at cGMP manufacturing facility

Autologous CT041 CAR-CLDN18.2 T cells for Patient 1 were manufactured through unit operations as described previously.[13 14] Briefly, the manufacturing process included peripheral blood mononuclear cell enrichment, T-cell activation, transduction with lentiviral vector, free vector removal, magnetic bead removal, cell expansion in bioreactor, and harvest. Final CT041 drug product was formulated with a cryopreservation solution containing 4% DMSO (dimethyl sulfoxide) and 4% human serum albumin. The CT041 cell product was subsequently cryopreserved in freezing bags and stored in vapor phase liquid nitrogen. Before product release, CT041 cells were subjected to various release tests including identity, impurity, and safety. The CT041 product met all release test specifications. The frozen CT041 product was transported to the medical site for infusion.

CT041 characterization by flow cytometry

The characteristics of the CT041 drug product, including expression of the anti-CLDN18.2 CAR on transduced patient T cells, were determined using flow cytometry at the cGMP manufacturing facility. Briefly, CT041 cells were stained with a combination of the following anti-human fluorescently labeled antibodies as previously described[13]: CD4-APC (BD Biosciences Cat. No. 641398), CD8-BV510 (BD Biosciences Cat. No. 563919), CD3-BV421 (BD Biosciences Cat. No. 563798), CD45RA-FITC (BD Biosciences Cat. No. 347723), CD62L-PE (BD Biosciences Cat. No. 341012), CD56-PE (BD Biosciences Cat. No. 340363) and CAR-PE (CARsgen Therapeutics). Data was acquired using an MACSQuant Analyzer 10 (Miltenyi Biotec) and the analyses were performed using FlowJo V.10.0.

CT041 infusion at medical site

The frozen CT041 cells were transported in vapor phase nitrogen to the patient's bedside and thawed by immersing the whole vacuumed overwrap pack in a water bath at 37–38°C. The thawed cells were gently mixed then immediately infused via gravity. CT041 cells were infused within 1 hour of thawing and bags were thawed one at a time. Each infusion bag was affixed with a drug product label stating, "FOR AUTOLOGOUS USE ONLY" and containing unique patient identifiers. Prior to the infusion, two healthcare providers at the medical site independently verified the information was correctly matched to the patient.

Preconditioning regimen prior to CT041 infusions

For the first infusion, the subject received a preconditioning regimen of fludarabine 25 mg/m^2 (46 mg) on Days −5 and −4, nab-paclitaxel 100 mg on Day −4, and cyclophosphamide 250 mg/m^2 on Days −5 to −3 (455 mg). For the second infusion, the subject received the same regimen with adjustments relative to their body surface area, and nab-paclitaxel was given at 100 mg/m^2: fludarabine 25 mg/m^2 (47 mg) on Days −5 and −4, nab-paclitaxel 100 mg/m^2 (185 mg) on Day −4, and cyclophosphamide 250 mg/m^2 on Days −5 to −3 (465 mg).

Copy number analysis by quantitative real-time PCR

To monitor in vivo expansion and persistence of CT041 cells post infusion, whole blood from Patient 1 was collected at various time points in EDTA tubes and stored frozen at −80°C before analysis by the central laboratory. DNA was extracted from frozen samples and amplified in duplicate with primers and TaqMan probes specific for the CAR-transgene using the QuantStudio Flex Real-time PCR System. Amplification was performed using the following reaction conditions: 50°C for 2 min, 1 cycle of 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C for 1 min. Low, medium, and high-quality control samples were included in every run.

Circulating tumor DNA analysis by tumor-informed ctDNA assay

Circulating tumor DNA (ctDNA) in Patient 1 was measured longitudinally using a patient-informed assay as previously published.[10 15] Briefly, a tumor specimen and blood specimens were sent by the University of California San Diego GI Oncology clinical trials team for whole exome sequencing and subsequent generation of proprietary multiplex PCR primer pairs targeting 16 highly ranked tumor-specific variants. Blood samples for longitudinal surveillance were also collected.

Serum cytokine levels

Serum cytokine levels, including interferon (IFN)-γ, tumor necrosis factor (TNF)-α, VEGF-A, interleukin (IL)-16, IL-15, IL-6, IL-7, IL-8, and IL-12/23p40, were measured by the central laboratory at several time points before and after CT041 infusions using a commercially available multiplex Meso Scale Discovery (MSD) platform according to the manufacturer's instructions. Briefly, serum was loaded onto precoated 96-well MULTI-SPOT plates, along with the detection antibody solution. MSD buffer was then added, and the plate was read in the MSD instrument. The assays were performed in duplicates and values are represented as the average fold change over Day 0.

RESULTS

First infusion

Enrollment of Patient 1 after determination of CLDN18.2 positivity initiated the US Phase 1b/2 CT041 trial. The patient continued on DCF chemotherapy during trial screening procedures followed by a 2-week washout prior to leukapheresis (figure 1A). The patient's apheresis collected 10.1×10^9 cells that were sent for CT041 manufacturing, during which the patient was bridged with one cycle of nab-paclitaxel 100 mg/m^2 and gemcitabine 1000 mg/m^2. After our hospital received their CT041, the patient proceeded to chemotherapy washout over 3 weeks. Outpatient preconditioning comprising fludarabine, nab-paclitaxel (flat-dose of 100 mg), and cyclophosphamide (FNC; Days −5 to −3) was completed without complication. The patient was admitted on Day −1, and CT041 was infused (250×10^6 cells) on Day 0. The patient remained inpatient for 1 week to monitor for cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), or other adverse events. Meanwhile, the patient used the medical unit's gym daily, including ≥1 mile walks on the treadmill and free-weights. The patient experienced Grade 1 CRS (fever), managed with anti-pyrectics. Transient adverse events experienced at Grade 2 or higher included lymphopenia, leukopenia, anemia, skin rash, elevated alkaline phosphatase, and vomiting (table 2).

Tumor-informed ctDNA peaked after CT041 infusion and quickly reduced by Day+7 when the patient was discharged. The ctDNA continued to reduce through Day+17 but remained positive (figure 1C). Quantitative PCR (qPCR) indicated CT041 expansion quickly peaked by Day+7 at ~750 copies/µg genomic DNA, concurrent with reduced ctDNA (figure 1C). Two peritoneal target lesions seen at baseline were markedly diminished post CT041 infusion (figure 1D, top). Additionally, decreasing loculated ascites in the left mid-abdomen was observed in parallel to patient-reported improvements in abdominal distention (figure 1D, bottom). The patient had partial response (PR) per RECIST (Response Evaluation Criteria in Solid Tumors) V.1.1 at week 4 as target lesions

Table 2 Adverse events after first CT041 infusion and within 30 days

Adverse events*	Highest grade	Longest duration, days†	Treatment
Hematologic			
Lymphopenia	4	3	
Leukopenia	3	8	
Anemia	3	2	
Other			
Cytokine release syndrome‡ Fever	1	2	Acetaminophen
Skin rash	2	35	Clobetasol propionate; hydrocortisone
Alkaline phosphatase increase	2	13	
Vomiting	2	6	Ondansetron, lorazepam, prochlorperazine
Tachycardia	1	30	
Cough	1	8	
Gagging	1	5	
Chills	1	3	
Nausea	1	2	Lorazepam
Myalgia	1	1	

*Adverse events (AEs) within 30 days following the first dose of preconditioning and CT041 infusion, graded according to National Cancer Institute's Common Terminology Criteria for Adverse Events V.5.0.
†Duration at highest grade, inclusive of the start and end dates. If an AE had multiple instances at the highest grade, the duration was summed. If an AE occurred before CT041 infusion, the total duration of the event was included.
‡CRS graded according to American Society for Transplantation and Cellular Therapy criteria: Lee, *et al*.[29]
CAR, chimeric antigen receptor; CRS, cytokine release syndrome; CT041, Claudin18.2-targeted CAR T cells.

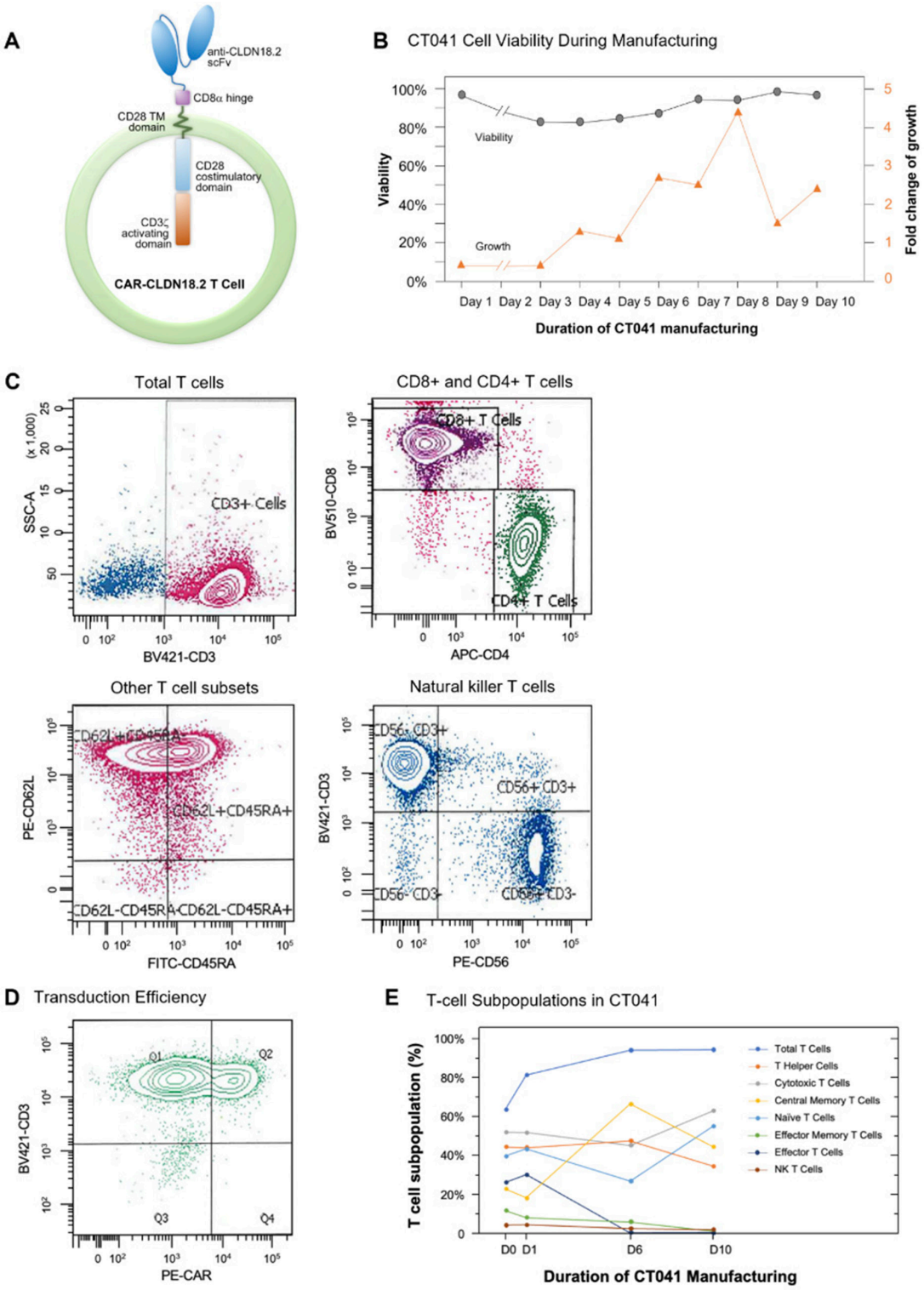

Figure 2 Characterization of CT041 CAR-T-cell product. (A) Schematic of an autologous CT041 CAR-CLDN18.2 T cell consisting of an extracellular humanized anti-CLDN18.2 scFv (blue) and a CD8α hinge (purple), connected to an intracellular CD28 costimulatory domain (cyan) and a CD3ζ activating domain (brown) by a CD28 transmembrane domain (dark green). (B) Cell viability (black) and growth rate (orange) of CT041 during the 10-day manufacturing process, determined daily using NC 200 cell counter. (C) T-cell subpopulations, characterized and quantified by flow cytometry analysis using different cell-surface markers, including total T cells (CD3+CD45+) (upper left); T helper cells (CD4+CD3+CD45+) and cytotoxic T cells (CD8+CD3+CD45+) (upper right); central memory T cells (CD62L+CD45RA–CD3+CD45+), naïve T cells (CD62L+CD45RA+CD3+CD45+), effector memory T cells (CD62L–CD45RA–CD3+CD45+), effector T cells (CD62L–CD45RA+CD3+CD45+) (bottom left); and natural killer T cells (CD56+CD3+CD45+) (bottom right). (D) Transduction efficiency of CT041 as quantified by flow cytometry with anti-CD3 antibody plus PE-CAR. (E) T-cell subpopulations as determined at different time points by flow cytometry during manufacturing.

disappeared and five non-target lesions showed non-complete response (CR)/non-PD. Serum cytokines were evaluated during and post-CT041 infusion including IL-6, IL-7, IL-8, IL-12, IL-15, IL-16, IFN-γ, TNF-a, and VEGF-A (figure 1E). Circulating IL-6 increased 5-fold increase from Day 0 to Day+3 and subsequently declined by Day+7. Circulating IFN-γ also peaked by Day+3 with a 37-fold increase indicating robust activation of T cells. Other circulating cytokines negligibly increased following CT041 infusion. On ~Day+14, the patient had developed a patchy, flat erythematous rash over their bilateral dorsal forearms that resolved without intervention (figure 1F).

Clinically, the patient had improved energy, significantly reduced bilious vomiting that permitted removal of their NG tube, and diminished gastrocutaneous fistula output. Ascites-related abdominal distention did not recur, relieving the need for serial paracenteses. The patient could eat some solid food and had a rectal bowel movement for the first time in approximately 5 months.

Second infusion

With declining circulating CT041 evident by qPCR, persistent radiographic residual disease, and measurable ctDNA post-CT041 infusion, we considered a second CT041 infusion between weeks 12 and 16 to deepen tumor response. The patient required NG tube re-insertion prior to the second infusion as bilious vomiting resumed. A repeat baseline CT scan was completed and the patient met re-infusion eligibility (figure 1D). The second FNC preconditioning regimen included 100 mg/m^2 nab-paclitaxel instead of flat dosing. The patient received the second CT041 infusion (250×10^6 cells) inpatient on Day+92 post-initial infusion. During this infusion, the patient experienced transient Grade 3 lymphopenia and Grade 2 CRS (fever, hypotension) necessitating antipyrectics and broad-spectrum antibiotics. Tocilizumab and filgrastim were considered but not administered due to subsequent fever reduction. The patient resumed using the unit's gym treadmill daily but had significantly more fatigue than the first infusion. Again, ctDNA showed a rapid peak following CT041 infusion followed by an equally rapid decrease (figure 1C). CT041 vector transgene showed similar but higher expansion, peaking Day 7 at 1185 copies/µg gDNA (figure 1C). At 4 weeks post-second CT041 infusion, the patient's PR deepened into a radiographic target-lesion CR per RECIST V.1.1. There was complete resolution of a non-target perisplenic nodule and gastric wall thickening. Only trace non-target ascites was observed. Further, the ctDNA was now negative, with no molecular residual disease (MRD). Clinically, the patient had no rash after re-infusion and again resumed oral intake. The patient's NG tube was removed again, and their energy level significantly improved, permitting them to independently care for their children. Remarkably, surveillance imaging showed ongoing CR until 8 months after the first CT041 infusion, at which time the patient experienced progression.

Table 3 CT041 product phenotype

Phenotype	Percentage (%)
Total T cells (CD3+CD45+)	94.6
Other lymphocytes (CD3−CD45+)	5.4
T-cell subtypes (CD3+CD45+)	
Cytotoxic T cells (CD8+)	62.9
T helper cells (CD4+)	34.5
NK T cells (CD56+)	1.9
T-cell differentiation: CD62L vs CD45RA (CD3+CD45+)	
Naïve T cells (CD62L+CD45RA+)	54.9
Central memory T cells (CD62L+CD45RA-)	44.3
Effector memory T cells (CD62L- CD45RA-)	0.5
Effector T cells (CD62L- CD45RA+)	0.3

CAR, chimeric antigen receptor; CT041, Claudin18.2-targeted CAR T cells; HER2, Human epidermal growth factor receptor-2; NK, natural killer.

CT041 characteristics

CAR-CLDN18.2 T cells contain a unique, humanized anti-CLDN18.2 single-chain variable fragment (scFv), a CD8α hinge region, a CD28 transmembrane region, a CD28 costimulatory domain, and a CD3ζ activating domain (figure 2A). The CT041 product maintained >80% viability throughout production with a maximum 4.4-fold daily increase in growth within 10 days (figure 2B). The CD3+ T-cell population was gated and sorted into subpopulations, with a majority of CD8+ cytotoxic T cells by Day 10. Flow cytometric data demonstrated that the final CT041 product contained 94.6% total T cells with 34.5% CD4+ helper T cells, 62.9% CD8+ T cells (figure 2C, table 3), and 26.7% viable CAR-CLDN18.2-positive T cells (figure 2D, table 3). T-cell and subpopulation characterization showed the selective expansion of CD8+ cytotoxic T cells and CD62L+CD45RA− central memory T cells during CT041 manufacturing (figure 2E).

DISCUSSION

Prior reports of solid tumor CAR-T-cell trials showed substantial toxicities including CRS, ICANS, hematologic and on-target, off-tumor toxicities.[16] For example, a HER2-directed CAR-T-cell treatment-related death occurred in a colon cancer patient with lung and liver metastasis due to cytokine storm.[15] Here, Patient 1 had manageable side effects with CT041, possibly due to the restricted expression of CLDN18.2 in normal tissues. Despite receiving two CT041 infusions, no Grade 3 or higher CRS or other dose-limiting toxicities occurred, possibly ascribed to CT041's unique anti-CLDN18.2 scFv.13. Peak CAR copies after the second infusion were 58.2% higher than the first infusion peak, suggesting CT041 could expand during re-infusion. The ctDNA reduction coincided with the rapid expansion of CT041 copy numbers during first and second infusions, suggesting CT041 reduced the total

tumor burden below MRD, similar to previous reports of immunotherapy in solid tumors.[17 18] Notably, duration of tumor response surpassed CT041 persistence in peripheral blood, suggesting CAR-T-cell integration into the solid tumor microenvironment might differ from that in hematologic malignancies. For the first time, this CAR-T study implemented tumor-informed ctDNA monitoring in addition to radiographic disease monitoring to determine when to repeat CT041 infusion, resulting in a target-lesion CR.[19] The infusion of CT041 without significant CRS, ICANS, or hematological toxicities, suggests the potential for outpatient CAR-T-cell administration in patients with solid tumors in highly-selected centers.[20]

We report the successful implementation of CT041 in last-line metastatic GC and detail a sustained target lesion complete response (CR) clinically, molecularly, and radiographically until 8 months post-initial infusion. CT041 exhibited promising antitumor activity even in a chemotherapy-refractory patient who had failed four lines of systemic treatment and multiple cytoreduction surgeries with HIPEC treatments.

Paclitaxel is clinically recommended as a GC monotherapy at 80 mg/m^2 days 1, 8, and 15 every 4 weeks and GC clinical trials of nab-paclitaxel have shown response at 260 mg/m^2 every 3 weeks.[21 22] Considering the patient previously progressed on a paclitaxel-based regimen and the nab-paclitaxel lymphodepletion dosage was initially just 100 mg flat and later 100 mg/m^2, the antitumor activity observed in this patient is likely attributed to CT041 infusions and not to the bridging or preconditioning regimens. Interestingly, another patient with signet-ring GC previously treated with anti-Claudin18.2 antibody (zolbetuximab) also had an ongoing partial response to CT041 infusion, suggesting enhanced cell-therapy anticancer activity (G.P. Botta, unpublished data).[23 24] Previous studies have demonstrated that FC-based lymphodepletion reduces immunosuppressive factors and improves cell expansion and persistence of CAR-T cells in hematologic cancers.[25] Further, nab-paclitaxel has been combined with cyclophosphamide for the preconditioning regimen in another solid tumor CAR-T-cell trial.[26] Importantly, other studies have suggested that nab-paclitaxel may selectively deplete the tumor stroma potentially through a stromal SPARC (secreted protein acidic and rich in cysteine) interaction. As stroma may proliferate in multiple solid malignancies, increased intratumoral concentration of drugs may be possible when co-administered with nab-paclitaxel.[27 28] Therefore, the low-dose nab-paclitaxel-based preconditioning regimen was introduced to improve T-cell trafficking within the tumor immunosuppressive microenvironment. Indeed, the successful expansion of CAR-T-cells after two infusions further confirms the FNC regimen's positive impact.

Patients with metastatic GC, specifically signet-ring/diffuse histologic subtypes, experience significant comorbidities due to disrupted digestive function. Most are cachectic due to disease burden and limited oral caloric intake necessitating enteric feeding or TPN. Further, few efficacious chemotherapeutic treatment options are available, and our patient became refractory to all standard of care therapies. Despite performance status and systemic therapeutic challenges, the patient had successful multidisciplinary treatment and target lesion CR in the setting of a diffuse, metastatic GC after CT041 CAR-T-cell therapy in the salvage setting.

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Collaborators CT041 Study Group.

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