Journal for ImmunoTherapy of Cancer

Efficacy and safety of autologous tumor-infiltrating lymphocytes in recurrent or refractory ovarian cancer, colorectal cancer, and pancreatic ductal adenocarcinoma

Rodabe Amaria,[1] Anne Knisely ,[2] David Vining,[3] Scott Kopetz,[4] Michael J Overman ,[4] Milind Javle,[4] Mara B Antonoff,[5] Ching-Wei D Tzeng,[6] Robert A Wolff,[4] Shubham Pant,[4] Kathryn Lito,[2] Kelly Rangel,[2] Bryan Fellman,[7] Ying Yuan,[7] Karen H Lu,[2] Donastas Sakellariou-Thompson,[1] Cara L Haymaker ,[8] Marie-Andrée Forget,[1] Patrick Hwu ,[9] Chantale Bernatchez ,[10] Amir A Jazaeri[2]

To cite: Amaria R, Knisely A, Vining D, *et al*. Efficacy and safety of autologous tumor-infiltrating lymphocytes in recurrent or refractory ovarian cancer, colorectal cancer, and pancreatic ductal adenocarcinoma. *Journal for ImmunoTherapy of Cancer* 2024;**12**:e006822. doi:10.1136/jitc-2023-006822

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/jitc-2023-006822).

RA and AK contributed equally.

Accepted 09 January 2024

For numbered affiliations see end of article.

Correspondence to
Dr Amir A Jazaeri;
AAJazaeri@mdanderson.org

ABSTRACT

Background Tumor-infiltrating lymphocyte (TIL) therapy has shown efficacy in metastatic melanoma, non-small cell lung cancer, and other solid tumors. Our preclinical work demonstrated more robust CD8 predominant TIL production when agonistic anti-4-1BB and CD3 antibodies were used in early ex vivo TIL culture.

Methods Patients with treatment-refractory metastatic colorectal (CRC), pancreatic (PDAC) and ovarian (OVCA) cancers were eligible. Lymphodepleting chemotherapy was followed by infusion of ex vivo expanded TIL, manufactured at MD Anderson Cancer Center with IL-2 and agonistic stimulation of CD3 and 4-1BB (urelumab). Patients received up to six doses of high-dose IL-2 after TIL infusion. Primary endpoint was evaluation of objective response rate at 12 weeks using Response Evaluation Criteria in Solid Tumors version 1.1 with secondary endpoints including disease control rate (DCR), duration of response, progression-free survival (PFS), overall survival (OS), and safety.

Results 17 patients underwent TIL harvest and 16 were treated on protocol (NCT03610490), including 8 CRC, 5 PDAC, and 3 OVCA patients. Median age was 57.5 (range 33–70) and 50% were females. Median number of lines of prior therapy was 2 (range 1–8). No responses were observed at 12 weeks. Ten subjects achieved at least one stable disease (SD) assessment for a DCR of 62.5% (95% CI 35.4% to 84.8%). Best response included prolonged SD in a patient with PDAC lasting 17 months. Median PFS and OS across cohorts were 2.53 months (95% CI 1.54 to 4.11) and 18.86 months (95% CI 4.86 to NR), respectively. Grade 3 or higher toxicities attributable to therapy were seen in 14 subjects (87.5%; 95% CI 61.7% to 98.4%). Infusion product analysis showed the presence of effector memory cells with high expression of CD39 irrespective of tumor type and low expression of checkpoint markers.

Conclusions TIL manufactured with assistance of 4-1BB and CD3 agonism is feasible and treatment is associated with no new safety signals. While no responses were observed, a significant portion of patients achieved SD suggesting early/partial immunological effect. Further research is required to identify factors associated with resistance and functionally enhance T cells for a more effective therapy.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Tumor-infiltrating lymphocyte (TIL) immunotherapy has shown promising results in melanoma, non-small cell lung cancer, and cervical cancer, among other solid tumors, even in immune checkpoint blockade refractory patient populations. Preclinical data demonstrated robust production of $CD8^+$ predominant TIL when a 41BB agonist was added to ex vivo culture, even in cancers thought to not have robust T-cell infiltration.

WHAT THIS STUDY ADDS

⇒ We looked at efficacy of TIL immunotherapy in several cancer populations not traditionally known to be immunotherapy-sensitive, including colorectal, pancreatic, and ovarian cancer. While no objective responses were observed, 63% of patients demonstrated stable disease, including evidence of antitumor activity in one patient with pancreatic cancer who experienced reduction in tumor burden lasting for over 1 year, with no new safety signals demonstrated.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The results of this trial are important to guide future TIL modifications and/or combination therapies to increase efficacy of TIL.

BACKGROUND

Adoptive cell therapy (ACT) using tumor-infiltrating lymphocytes (TIL) typically consists of ex vivo expansion of TIL from fresh, surgically obtained tumor sample, using interleukin (IL)-2 with subsequent administration

of the expanded TIL product to the patient after preconditioning non-myeloablative lymphodepleting chemotherapy. Despite a time-consuming (often 3–6 weeks) process, TIL therapy offers unique advantages compared with other ACT, including diverse T-cell clonality capable of recognizing an array of tumor antigens, superior tumor-homing capacity, and low off-target toxicity. Additionally, higher levels of TIL in the tumor tissue, especially CD8+T cells, are associated with improved oncological outcomes in multiple solid tumor types,[1–3] demonstrating the importance of T cells to control cancer and the promising role of TILs as biomarkers and as a therapeutic modality to improve survival in this setting.

Since the recognition of the crucial importance of lymphodepleting chemotherapy prior to TIL infusion and administration of IL-2 postinfusion, multiple clinical trials have demonstrated durable response rates with TIL treatment, largely in melanoma.[4] Trials involving metastatic melanoma patients treated with TIL prior to the immune checkpoint inhibitor era have demonstrated high response rates of approximately 40%–50%,[4–8] with durable complete response (CR) rates of up to 20%.[9] More recent studies using TIL in patients with checkpoint inhibitor refractory melanoma demonstrate response rates closer to 25%–30%[9–11]; however, one phase 3 study reported a response rate of almost 50% in this patient population.[12] Recent trials investigating the use of TIL therapy in other metastatic solid tumors have demonstrated clinical efficacy with durable responses in cervical cancer,[13 14] non-small cell lung cancer (NSCLC),[15] colorectal cancer (CRC),[16] and breast cancer,[17] although these studies all involved small patient cohorts with approximately 10 patients or fewer.

Encouraging clinical data regarding the use of TIL therapy in ovarian cancer (OVCA) were reported in the 1990s[18–20], but little is known about the efficacy of TILs in recurrent platinum-resistant OVCA patients in the current era of new and improved protocols for ACT; the same is true for patients with metastatic or relapsed CRC and pancreatic ductal adenocarcinoma (PDAC). These three cancer types in the recurrent/refractory setting represent cohorts with overall guarded prognoses and poor response rates to second-line therapies, highlighting the importance of the development of novel treatment options for these patients. Preclinical work performed at our institution to optimize T-cell growth conditions, using IL-2, anti-CD3, and anti-4-1BB, demonstrated the ability to grow TIL in these types of solid tumors and thus paved the way for this trial.[21 22]

METHODS

Patient selection

Male and female patients between the ages of 18 and 70 years old with recurrent/refractory high-grade non-mucinous OVCA, CRC, or oligometastatic PDAC with an area of tumor amenable to excisional biopsy were considered for enrollment. For the OVCA cohort, patients were required to have failed at least two prior lines of chemotherapy or have platinum-resistant disease. CRC and PDAC patients were eligible if they had metastatic disease considered incurable with currently available therapies and had derived maximal benefit from or were refractory to conventional frontline therapy. PDAC patients with ascites or carcinomatosis were not eligible. Patients who had received prior ACT were excluded. All patients were required to have a clinical performance status of Eastern Cooperative Oncology Group (ECOG) of 0 or 1, acceptable end-organ function and hematologic cell counts both at the time of enrollment and within 24 hours of starting lymphodepleting chemotherapy, and at least one target lesion on imaging (aside from the lesion used for generation of TIL) to be used for response assessment. Patients who were deemed to be immunocompromised (eg, HIV infection, transplant recipient, or clinically significant autoimmune disease) were not eligible for participation in the study. Additional inclusion and exclusion criteria can be found on the NCI website under clinical trial NCT03610490.

Study design

This was a non-randomized, multicohort, open-label phase II trial. The study planned to enroll 10 subjects per disease cohort in the first stage, with possible expansion to a second stage (for cohorts with confirmed responses), guided by a modified Simon's two-stage design. The null hypothesis of a historical response rate of 5% was tested against the estimated experimental cohort response rate of 20%. The study was stopped early and did not reach the target 30 patients in the first stage due to loss of availability of urelumab as well as lack of clinical responses.

Ex vivo TIL expansion and manufacturing was conducted at the MDACC TIL lab under conditions that included IL-2 in conjunction with anti-CD3 and 4-1BB stimulation with use of urelumab. The second phase of TIL expansion using the previously described rapid expansion protocol (REP) was conducted at the MDACC Stem Cell Transplantation and Cellular Therapy Center.[11] The treatment schema is illustrated in online supplemental figure 1. Following enrolment, subjects were scheduled for a surgical procedure for excisional biopsy of tumor for TIL manufacturing. The surgical approach was based on consideration of imaging and patient-specific factors and carried out by attending surgeons in respective surgical departments at MDACC. The standard lymphodepletion chemotherapy regimen consisted of cyclophosphamide 60 mg/kg (or 30 mg/kg in patients who had heavy prior chemotherapy use) on days −7 and −6 and fludarabine 25 mg/m^2 (dosed as per renal function) on days −5 to −1. Freshly harvested ex vivo expanded autologous TILs were pooled, concentrated, washed and infused intravenously on day 0, at least 24 hours from the last dose of fludarabine. Patients received intravenous IL-2 (600 000 IU/kg) starting the day after TIL infusion every 8–12 hours as

per institutional standard of care for up to six doses or as tolerated and remained hospitalized until adequate hematologic recovery was demonstrated.

Study assessments

Patient response to treatment was assessed via serial imaging (either CT, MRI, or PET scan) which was performed at screening, baseline, and then at 6, 12, 18, and 24 weeks post TIL infusion, or until progression. Response was evaluated using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. A confirmation scan was not necessary to define a partial response (PR) but was required for CR confirmation and optional for progressive disease (PD) designation. The primary endpoint was objective response rate (ORR as defined as CR/PR) at the 12-week time point. Secondary objectives included disease control rate (DCR as defined as CR/PR/stable disease (SD)), progression-free survival (PFS), overall survival (OS), and treatment-emergent adverse events (TEAEs).

Adverse event (AE) monitoring was performed continuously from the time of initial study intervention throughout treatment and post-treatment follow-up, using the NCI Common Terminology Criteria for Adverse Events version 4.03 for grading.

TIL expansion and reactivity assessment

TILs were expanded from 1 to 3 mm per side tumor fragments as previously described.[22] Briefly, five tumor fragments were put in culture in a G-Rex 10 flask (Wilson Wolf Manufacturing, New Brighton, Minnesota, USA) in 20 mL of TIL complete media (TIL-CM) with 30 ng/mL of anti-CD3 (OKT3 clone), 10 µg/mL of agonist anti-4-1BB antibody and 6000 IU/mL IL-2. Four to seven days after initiating the culture, a 20 mL of TIL-CM containing 6000 IU/mL IL-2 was added for a total of 40 mL. Half of the media was replaced with new TIL-CM containing 6000 IU/mL IL-2 every 3–4 days until the cells completely covered the bottom of the flask. The cell suspension was filtered with a 40 µm cell strainer (Corning, Tewksbury, Massachusetts, USA) before in-process cryopreservation. In the second phase of expansion, TILs were thawed, rested for 1–3 days and propagated by REP. Briefly, on day 0, 5×10^6 TILs were seeded with 1×10^9 irradiated allogeneic pooled peripheral blood mononuclear cell (PBMC) feeders (1 TIL:200 PBMC ratio) in a GREX-100M along with 30 ng/mL of anti-CD3 (OKT3 clone) and 3000 IU/mL of IL-2 in REP-CM (50% TIL-CM and 50% AIM V (Thermo Fisher Scientific). On day 4 or 5, REP-CM supplemented with IL-2 was added. Cell concentration was determined on day 7 and TILs were subcultured with Aim V supplemented with IL-2. IL-2 was added again on day 9/10 and day 11/12. Expanded TILs were harvested for infusion on day 14 and infused fresh.[23] All cultures were maintained under the current Good Tissue Practice and current Good Manufacturing Practice.

Flow cytometry

TILs were stained in FACS Wash Buffer (1×DPBS with 1% bovine serum albumin) for 30 min on ice using fluorochrome-conjugated monoclonal antibodies from BD Biosciences against CD3 (PerCP-Cy5.5, clone SK7), CD4 (BUV496, clone SK3), CD8 (Alexa 700, clone RPA-T8), PD1 (BV650, clone EH12), CD73 (BV711, clone AD2), CTLA-4 (BV786, clone BNI3), CD45 (BUV395, clone HI30), CD103 (BUV661, clone ber-ACT8), CD69 (BUV737, clone FN50), CD27 (FITC, clone M-T271), CD45RA (V450, clone HI100), CCR7 (PerCP-Cy5.5, clone G043H7), and CD28 (PE-Cy7, clone CD28.2). A live/dead yellow dye from Thermo Fisher was used to exclude dead cells and antibodies against TIGIT (FITC, clone MBSA43), CD25 (APC-e780, clone BC96), LAG3 (PE, clone 3DS223H) and CD39 (PE-Cy7, clone ebioA1) from this company were also used in this stain. An antibody against TIM3 (BV605, clone F38-2E2) from Biolegend was also used. Cells were fixed in 1% paraformaldehyde solution for 20 min at room temperature following surface staining. Samples were acquired using the BD Fortessa×20 and analyzed using FlowJo Software V.10 (TreeStar).

Statistical analysis

Summary statistics were used to summarize demographic and clinical characteristics of the evaluable population (those that had TIL harvested and administered). ORR was described with frequencies, percentages and 95% CIs. Similar estimates were used for DCR. PFS and OS were summarized using the Kaplan-Meier estimates. PFS was defined as date of TIL administration to earliest date of progression or death due to any cause. Patients who had not experienced PD or death at the time of data cut had survival times censored at date of last tumor assessment. OS was defined as the time from date of TIL infusion to death due to any cause. Patients not deceased at the time of data cut-off had survival times censored on the last date of known survival status. All statistical analyses were performed using Stata/MP V.17.0.

Procedure for managing conflicts of interest

Most of the authors' conflicts of interest were not relevant to this work. Iovance was a supporter of this project, however, the TIL manufacturing was performed independently of Iovance by the MD Anderson Cancer Center TIL Lab using MDACC-developed expansion methods.

RESULTS

Patient characteristics, treatment details, and overall response to TIL therapy

A total of 27 patients consented to study participation. Of these, there were 10 screen failures and 1 subject who had TIL harvested but was not treated with TIL, yielding an evaluable analysis set of 16 subjects, including 8 patients with CRC, 5 patients with PDAC, and 3 patients with OVCA. Table 1 includes the demographic and summary statistics of the study population. The median age at the

Table 1 Demographic and clinical characteristics of evaluable study population (n=16)

Characteristic	N	%
Age at consent		
N	16	
Mean (SD)	54.44 (9.95)	
Median (min-max)	57.50 (33.00–70.00)	
# of Prior Tx		
N	16	
Mean (SD)	2.63 (1.78)	
Median (min-max)	2.00 (1.00–8.00)	
# of cells		
N	16	
Mean (SD)	1.30e+11 (2.07e+11)	
Median (min-max)	7.60e+10 (2.03e+10–1.5e+11)	
# of IL-2 doses		
N	16	
Mean (SD)	5.00 (1.71)	
Median (min-max)	6.00 (1.00–6.00)	
Race		
Asian	1	6.25
White or Caucasian	15	93.75
Gender		
Female	8	50.00
Male	8	50.00
Ethnicity		
Hispanic or Latino	1	6.25
Not Hispanic or Latino	15	93.75
Cohort		
Colorectal	8	50.00
Ovarian	3	18.75
Pancreatic	5	31.25
Prior ICI Tx		
No	14	87.50
Yes	2	12.50
Best overall response		
PD	6	37.50
SD	10	62.50
Colorectal		
PD	3	37.50
SD	5	62.50
Ovarian		
PD	1	33.33
SD	2	66.67
Pancreatic		
PD	2	40.00
SD	3	60.00

PD, progressive disease; SD, stable disease.

time of enrolment was 57.5 years (range 33–70), and 8 patients (50%) were female. The majority of patients were white (n=15, 93.8%) and non-Hispanic (n=15, 93.8%). Patients had received a median of 2 prior lines of therapy (range 1–8) prior to TIL therapy. Two patients (12.5%) had received prior immune checkpoint blockade therapy (1 OVCA and 1 CRC patient, both received pembrolizumab).

The median number of TIL infused was 7.6×10^{10} (range 2.03×10^{10}–1.5×10^{11}). Patients on trial received median doses of cyclophosphamide and fludarabine of 2 (range 2–2) and 3 (range 1–5), respectively. Median doses of IL-2 administered were 6 (range 1–6).

There were no clinical responses on study yielding an ORR of 0% (97.5 one-sided CI 0% to 20.6%). A total of 10 subjects achieved at least 1 SD assessment for a DCR of 62.5% (95% CI 35.4% to 84.8%). The best responses by patient cohort (at 6 weeks) were as follows: CRC (5 SD, 3 PD), PDAC (3 SD, 2 PD), and OVCA (2 SD, 1 PD) (online supplemental figure 2). At 12 weeks, 5 patients (31.3%) had SD, including 3 CRC, 1 PDAC, and 1 OVCA patient. Figures 1–3 illustrate responses using spider, waterfall, and swimmer plots, respectively. Best response included prolonged SD in a patient with PDAC lasting 17 months. In this PDAC patient, although treatment never reduced the size of the primary lesion, several of the metastases decreased in size or disappeared, as demonstrated in figure 4.

Survival analyses

The median follow-up time for subjects was 18.9 months (range: 2.2–44.8). The median PFS across cohorts was 2.53 months (95% CI 1.54 to 4.11) (figure 5A). The median PFS for the CRC, OVCA, PDAC cohorts was 2.79, 2.52, and 2.43 months, respectively (figure 5B). The median OS was 18.86 months (95% CI 4.86 to NR) (figure 5C). The median OS for the CRC, OVCA, PDAC cohorts was 21.42, 4.86, and 14.49 months, respectively (figure 5D).

Treatment toxicities

Grade 3 and above TEAEs are summarized in table 2. Two subjects reported zero grade 3 TEAEs. Patients generally experienced transient blood immune cell count decreases due to lymphodepletion. Grade 4 platelet count decrease was observed in 12 subjects (75%; 95% CI 48% to 93%). Four subjects (25%; 95% CI 7% to 52%) experienced grade 4 neutrophil count decreased. Grade 3 anemia was observed in 7 subjects (44%; 95% CI 20% to 70%). The maximum grade across all TEAEs was observed in 13 (81%; 95% CI 54% to 96%) subjects for grade 4 and 1 (6%; 95% CI 0% to 30%) for grade 3. A total of two grade 5 AEs occurred but were unrelated to treatment.

TIL Infusion Product Phenotype

Infusion product phenotypes were generally enriched for CD8+TIL compared with CD4+, which was expected because of the use of 4-1BB agonistic antibody in the manufacturing (figure 6A). Four effector memory

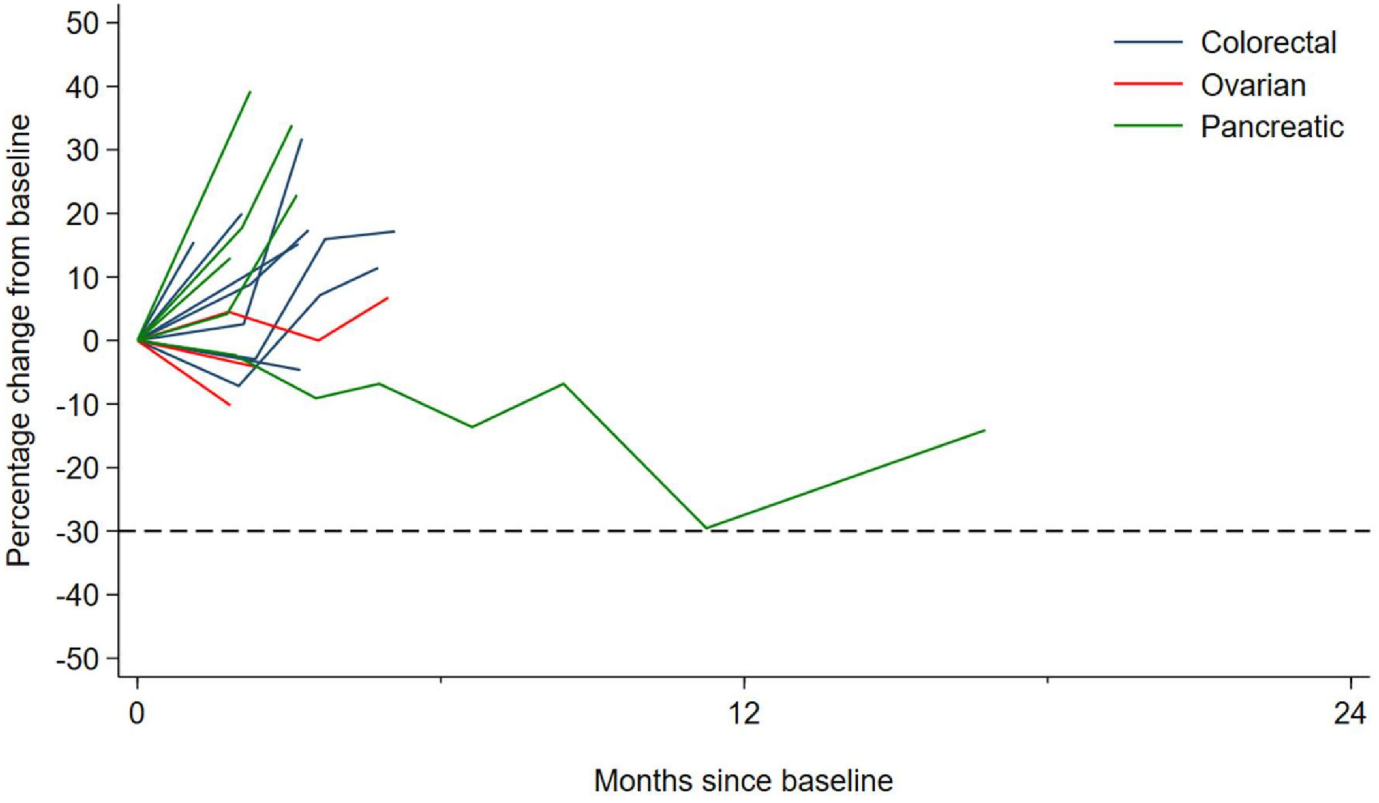

Figure 1 Spider plot. Response change in size of target lesions from baseline (% change; n=16) over time per Response Criteria in Solid Tumors version 1.1 by disease cohort.

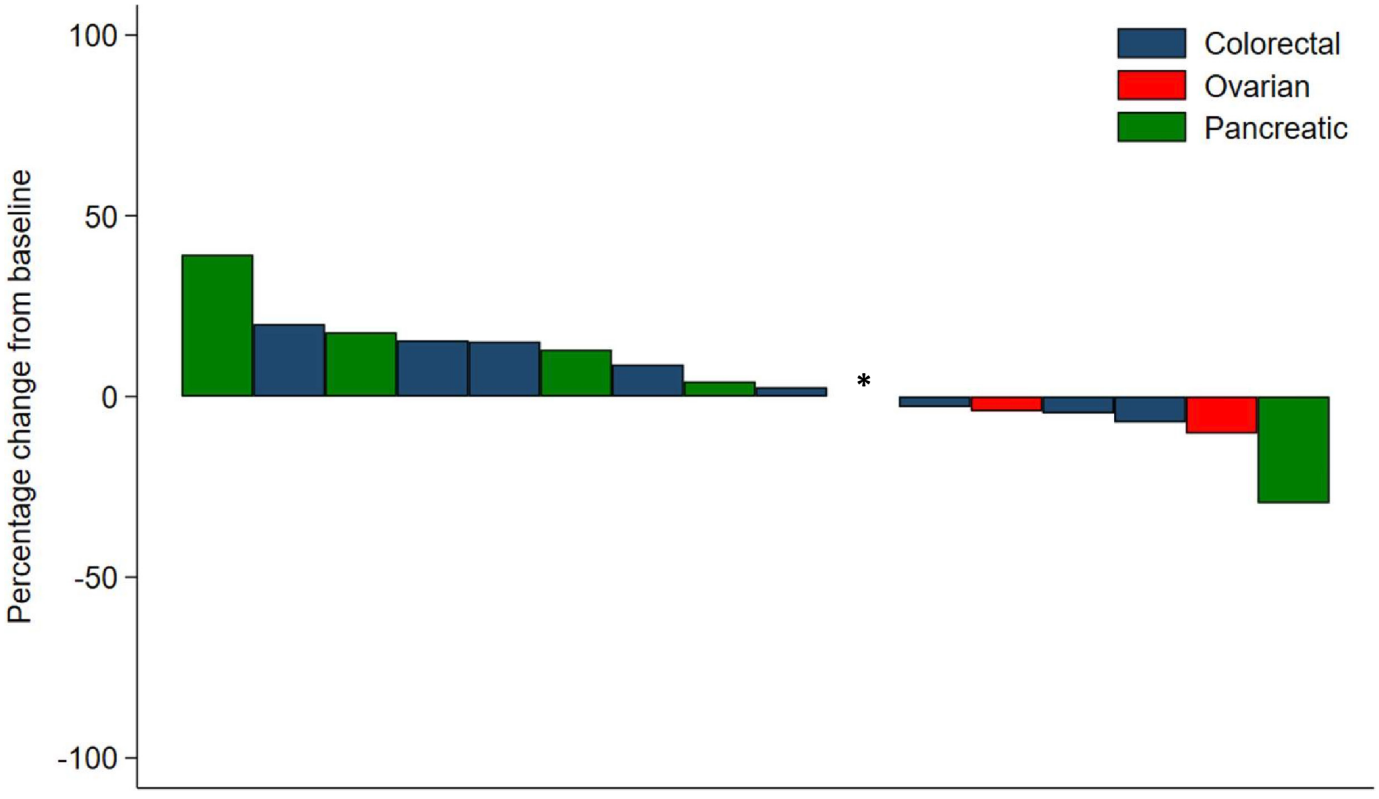

Figure 2 Waterfall plot. Best response change in size of target lesions from baseline (% change; n=16) per Response Criteria in Solid Tumors version 1.1 by disease cohort. *This patient had a 0% change in target lesion sum of diameters.

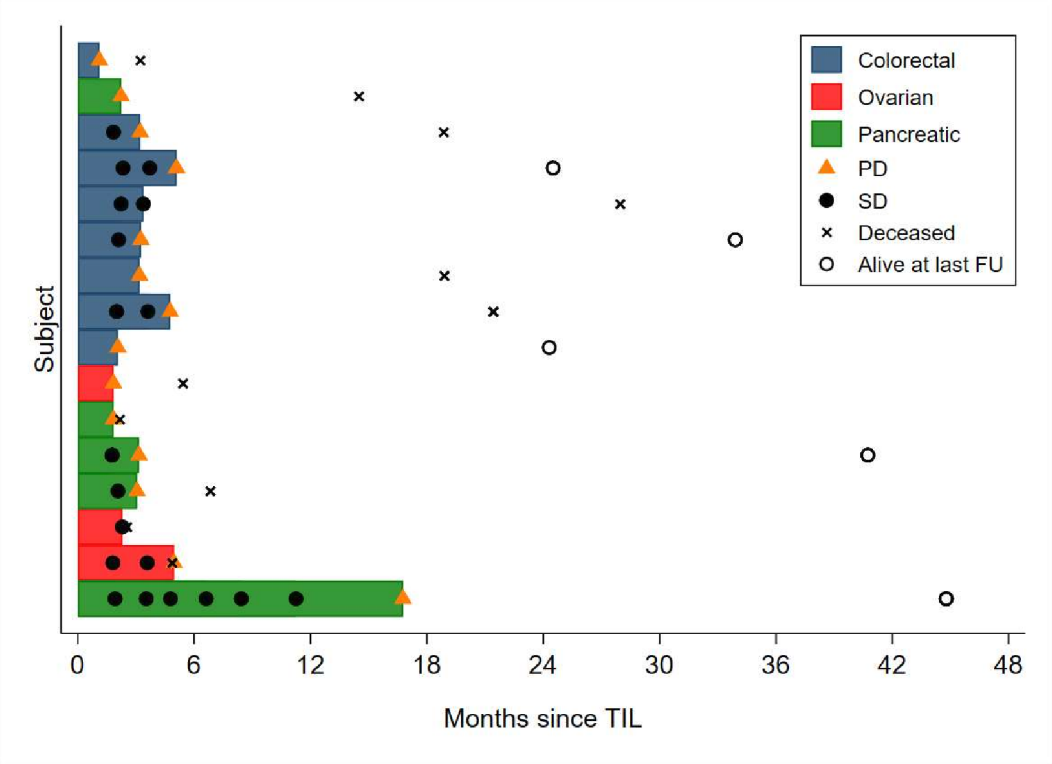

Figure 3 Swimmer plot. Disease status (stable disease (SD) or progressive disease (PD)) by disease cohort based on Response Criteria in Solid Tumors version 1.1 for the entire study cohort (n=16). Each bar represents one patient. FU, follow-up.

(EM) subsets were identified, with the majority defined as CD27-CD28- (EM3) irrespective of tumor type (figure 6B).[24 25] The TIL EM3 phenotype is associated with a differentiated cytotoxic phenotype although the low expression of checkpoint markers such as PD1, CTLA4, LAG3, TIM3 and TIGIT across the bulk CD8+ population as well as CD4+, indicates a strongly activated TIL product that does not have exhaustion attributes (figure 6C,D). This activated phenotype is supported by the high expression of CD39 across all tumor histologies as well as the expression of CD69 on CD8+TIL (figure 6C,D).

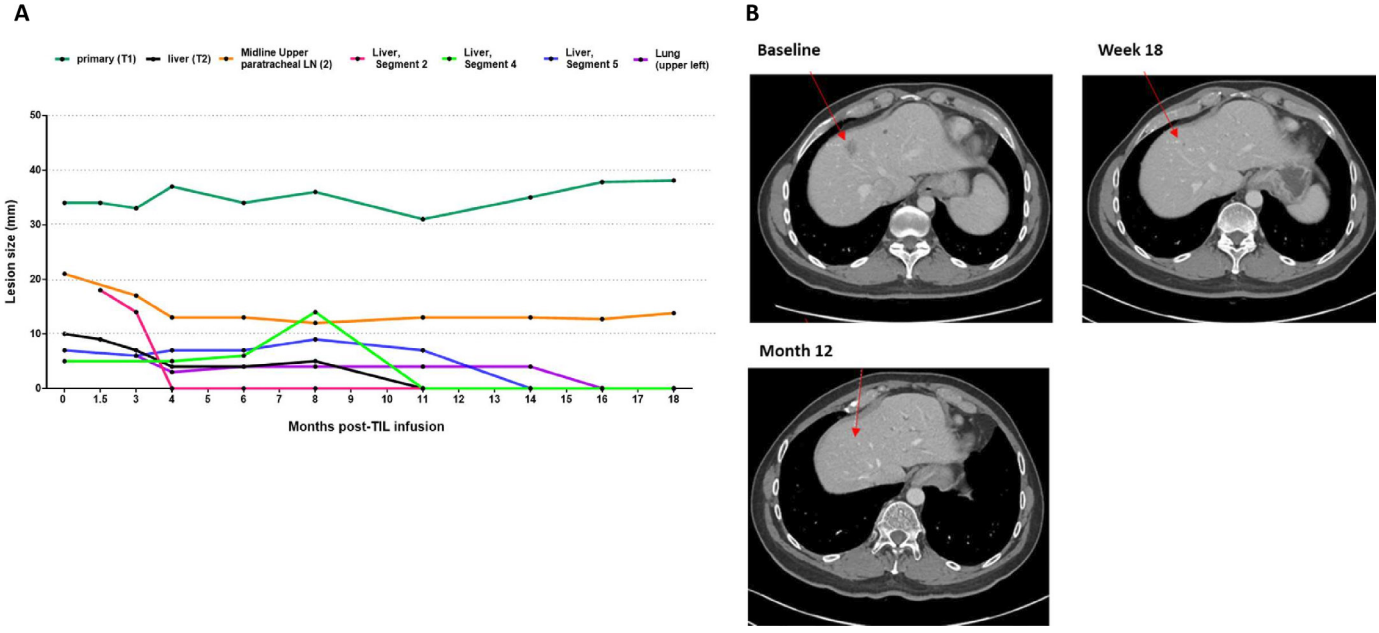

Figure 4 PDAC patient with prolonged stable disease. (A) Primary and metastatic lesion sizes in mm over time after tumor-infiltrating lymphocyte (TIL) infusion. T1 and T2 represent target lesions; the rest are non-target lesions. (B) Serial CT images demonstrating reduction in size of target liver lesion. PDAC, pancreatic ductal adenocarcinoma.

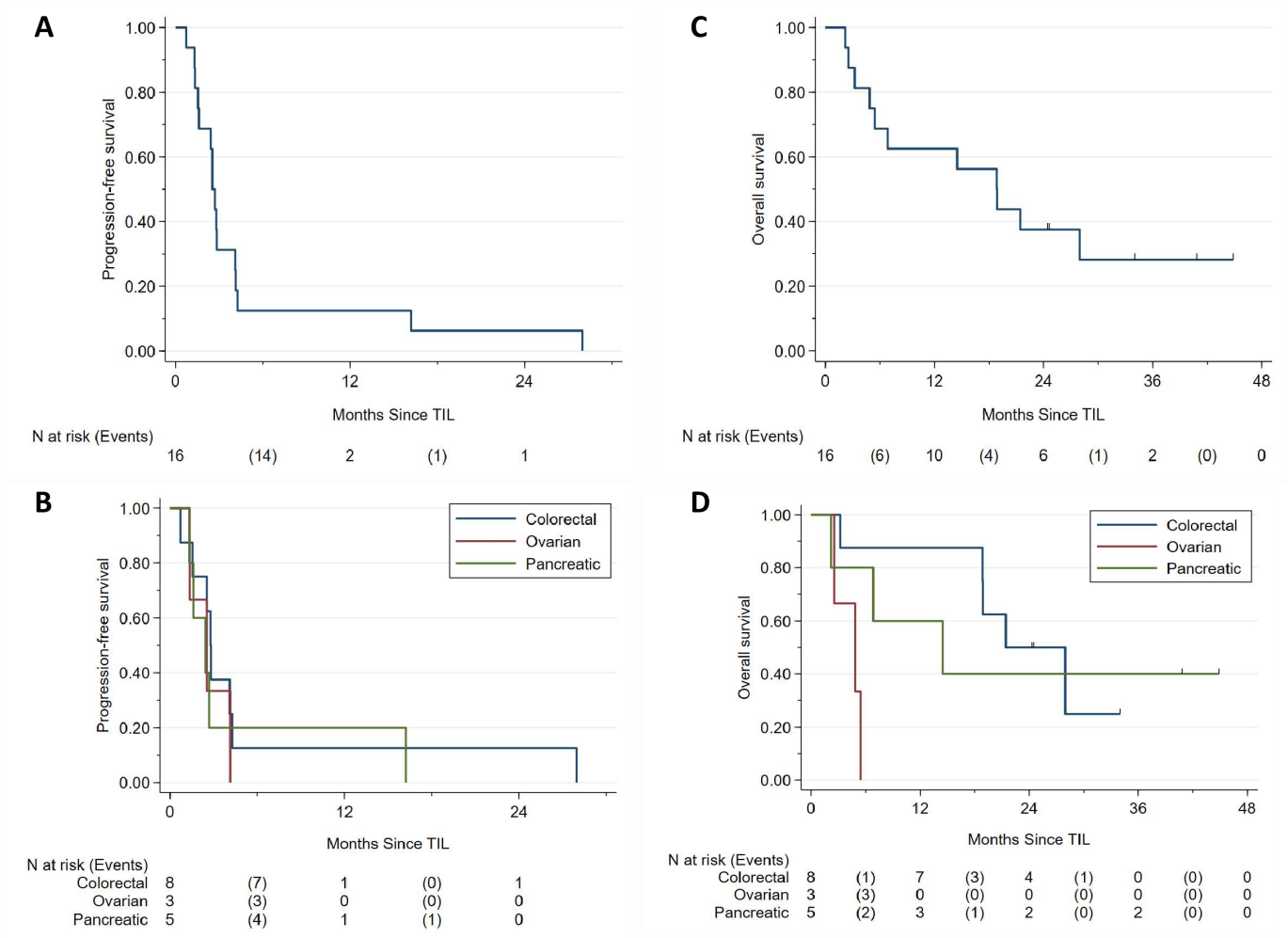

Figure 5 Kaplan-Meier curves. (A) Progression-free survival (PFS) of study population (n=16). (B) PFS of study population by disease cohort. (C) Overall survival (OS) of study population (n=16). (D) OS of study population by disease cohort. TIL, tumor-infiltrating lymphocyte.

DISCUSSION

This phase II trial of TIL therapy at a single center for the treatment of patients with CRC, PDAC, and OVCA refractory to standard therapies yielded a DCR of 62.5% but an ORR of 0%. Median PFS and OS were 2.53 months and 18.86 months, respectively. The study was feasible without any unexpected safety concerns, with the majority of toxicities representing expected cytopenias following lymphodepleting chemotherapy. Although the majority of patients progressed shortly after TIL treatment, 10 patients had SD at 6 weeks for a DCR of 63%. It should be noted, however, that only five patients continued to have SD at 12 weeks (the primary timepoint for efficacy analyses), so it is possible that the initial disease control signal is related to the lymphodepleting chemotherapy that patients received.

Importantly, there is one patient with pancreatic cancer with metastatic disease to the liver and peritoneum with prolonged SD and survival worth highlighting as proof of concept that this non-genetically modified, first-generation TIL product may have the potential to control PDAC to some extent. His tumor was *KRAS* mutated (G12D), *BRCA* wild type, and mismatch repair (MMR) proficient. As demonstrated in figure 4, although TIL treatment never reduced the size of the primary lesion, it

Table 2 Summary of grade 3 and higher TEAEs

	Grade		
Adverse event name	**3**	**4**	**Total**
Platelet count decreased	1	12	13
Neutrophil count decreased	1	4	5
Lymphocyte count decreased	1	1	2
White blood cell decreased	0	1	1
Anemia	7	0	7
Febrile neutropenia	2	0	2
Hypophosphatemia	2	0	2
Hepatobiliary disorder	1	0	1
Hypotension	1	0	1
Total	16	18	34

TEAEs, treatment-emergent adverse events.

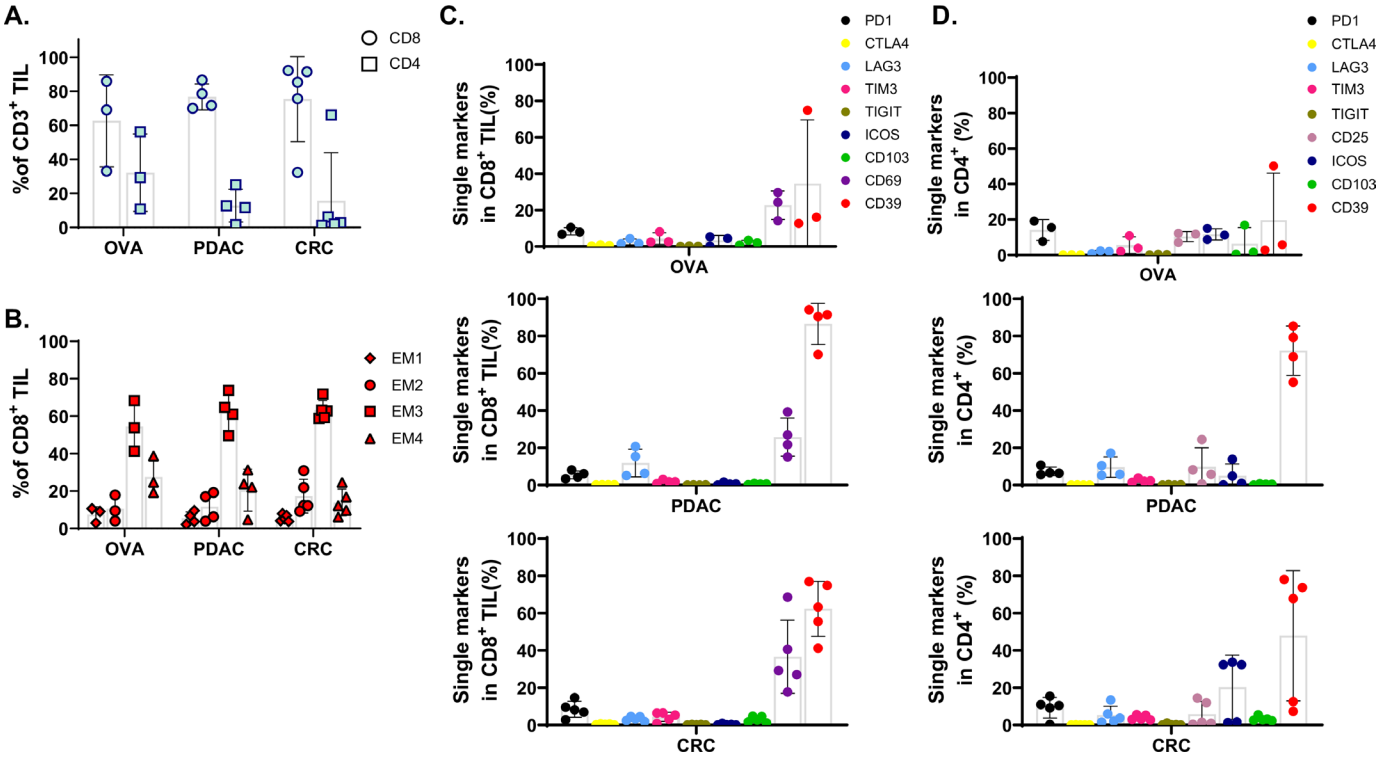

Figure 6 TIL infusion product phenotype. (A) Frequency of CD4+ and CD8+ TIL by cohort. (B) Frequency of CD8+ effector/effector memory Subsets based on CD27 and CD28 expression. (C) Single marker coexpression is shown for CD8+TIL (D) and CD4+TIL. CD25 was only assessed on the CD4+TIL. N=3 OVA, 4 PDAC, 5 CRC. CRC, colorectal cancer; PDAC, pancreatic ductal adenocarcinoma; TIL, tumor-infiltrating lymphocyte.

remained stable in size for 17 months and there was significant reduction in the size and even clearance of multiple liver metastases, without receiving any additional therapy. Although this patient never met criteria to be a responder, he did appear to have clinical benefit from therapy. Since progression (17 months post-TIL), he has received single agent pembrolizumab and two phase 1 clinical trial therapies (a RAD51 inhibitor and AZD6738, a drug targeting a somatic ARID1A mutation) and remains alive 4 years after TIL treatment. While it is not entirely clear why this patient had such notable benefit from TIL therapy, possible factors include young age (mid 40s), excellent performance status (ECOG 0), and the fact that he only received two prior lines of therapy (FOLFIRINOX and gemcitabine/abraxane) and no prior immunotherapy. Two patients on trial had received prior immune checkpoint blockade. Given the increasing use of this therapy class in cancer treatment, understanding the responses of these patients to TIL is important. These patients with stage IVB ovarian and stage IVB MMR proficient CRC achieved SD for 18 and 11 weeks, respectively, demonstrating longer responses than most patients on trial. This provides some evidence that in these tumor types, TIL therapy may be just as efficacious for patients with prior immunotherapy, although further study using g larger cohorts is warranted to validate these findings.

Other TIL immunotherapy trials have demonstrated impressive clinical response rates in metastatic melanoma[5 7 8] and advanced cervical cancer,[13 14] as well as preliminary efficacy in other tumor types including NSCLC,[26] CRC,[16] and breast cancers,[17] even in patients previously treated with immune checkpoint inhibitors.[27] However, a significant number of patients experience treatment failure via multiple potential major resistance mechanisms: lack of T-cell recognition of tumor, antigen-loss, presence of immunosuppressive cells, T-cell dysfunction/exhaustion, and lack of T-cell migration to tumor.[28] The metastatic solid tumor microenvironment is known to contain many immunosuppressive cell types including myeloid-derived suppressor cells and regulatory T cells (Tregs) that secrete anti-inflammatory cytokines that function to inhibit TIL function.[29–31]

The new manufacturing method employed in this trial, combining agonistic stimulations of CD3 and 4-1BB, was robust and yielded no manufacturing failures, with TIL reliably produced for every patient enrolled, thus providing a TIL platform process on which to build second-generation TIL products. The infusion product phenotypes in this study were generally enriched for CD8+TIL compared with CD4+TIL, which was expected based on the use of 4-1BB and our previous preclinical investigations.[21 32 33] However, the poor ORR to TIL in this trial demonstrates the need for functional improvement of TIL, with a specific focus on next-generation, genetically modified 'synthetic TILs to improve in vivo survival and functional success of TIL. There are several proposed strategies to improve the efficacy of TIL that are actively under investigation including retroviral transduction

to overexpress various cytokines as well as knock-out (KO) models of target genes (eg, PD-1 (NCT05361174), CISH (NCT04426669), SOCS1[34]) with technologies such as CRISPR or TALEN.[35–39] Although given the low expression of checkpoint molecules on the infused TIL, these molecules may not represent the major hurdle to response. The CRISPR KO approach may be utilized to render TIL non-responsive to other suppressive molecules, as highlighted in the work of Fix *et al* who recently developed a clinically scalable CRISPR-based method to delete *TGFBR2* in OVCA TIL, producing TGF-beta-resistant TIL without altering TIL expansion efficacy, immunophenotype, nor T-cell receptor clonal diversity; safety was demonstrated in this study with no evidence of CRISPR off-target effects, laying the groundwork for a novel immunotherapy for OVCA patients.[39] TGF-beta dominant negative receptor (TGF-DNRII) TIL has also been tested in metastatic melanoma patients, with a DCR of 86% and no added toxicity from gene modification.[40] This suppressive factor is expressed in the majority of solid tumors, and could be pertinent to target for all three malignancies in this case.

Alternatively, cytokines supporting TIL expansion and activation may be expressed in TIL. In a phase I trial, TILs transduced with a gene encoding IL12 driven by a nuclear factor of the activated T cells promotor (NFAT.IL12) were administered to metastatic melanoma patients (without IL-2), with a 63% ORR achieved.[37] Though the expression of IL-12 carried unwanted toxicities due to systemic exposure, strategies in development to improve targeted expression of IL-12 at the tumor site may succeed in improving the potency of TIL. In parallel, strategies involving the expression of other cytokines in TIL, such as IL-15, are currently being tested in clinical trials and could be of interest to potentiate TIL persistence and potency (NCT05470283). While next generation CAR-T approaches focus on better engineering of the antigen receptor, the challenge that lies ahead for the next generation of TIL therapy trials is to use the diverse antitumor TCR repertoire exquisite to TIL and reverse their dysfunctional state through engineering. Functionally improved TIL products are likely to require fewer cells to produce antitumor activity, making the production of the therapy less labor intensive and more cost effective.

Although this study is important in establishing the feasibility and safety of non-gene modified TIL among multiple solid tumor types, there are a number of important limitations. Importantly, it was a single-arm study so conclusions cannot be made regarding efficacy of TIL compared with standard second or third line treatment options for the various cohorts. Additionally, our conclusions are limited by a small sample size, with fewer than 10 patients in each disease cohort and only three patients in the OVCA cohort. There is a need for larger comparison trials once TIL product has been optimized for improved function.

In conclusion, generation of TIL at a single academic center for CRC, PDAC, and OVCA is feasible and treatment is associated with no new safety signals. One PDAC patient demonstrated prolonged SD lasting 17 months with decrease in size of multiple metastases. However, the majority of patients, despite initial disease control, had progression of disease within 6 months. For these tumor types, further research is required to identify host factors associated with resistance to TIL therapy, to optimize the manufacturing processes, and to use innovative genetic modification techniques to create more effective TIL cell therapy.

Author affiliations
[1]Department of Melanoma Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA
[2]Department of Gynecologic Oncology and Reproductive Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA
[3]Department of Diagnostic Radiology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA
[4]Department of Gastrointestinal Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA
[5]Department of Thoracic and Cardiovascular Surgery, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA
[6]Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA
[7]Department of Biostatistics, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA
[8]Department of Translational Molecular Pathology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA
[9]Moffitt Cancer Center, Tampa, Florida, USA
[10]SVP Discovery & Platforms, Therapeutics Discovery, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

Correction notice This article has been corrected since it was first published to reflect that RA and AK contributed equally.

Twitter Cara L Haymaker @cara_haymaker

Acknowledgements The authors wish to thank Sara Hull and Virginia Bayer for their role in helping start this clinical trial, as well as the TIL lab team members who expanded the cells to treat this cohort of patients and the Quality and Regulatory teams of the Stem Cell and CCIR groups, who provided regulatory oversight. The authors are also grateful to Iovance, BMS, and Clinigen for financial and material support of this study.

Contributors Conceptualization: RA, M-AF, PH, CB and AJ; Methodology: RA, CB and AJ; Formal analysis: BF and YY; Investigation: RA, DV, SK, MJO, MJ, MBA, C-WDT, RAW, SP, DS-T, CLH, M-AF, PH, CB and AJ; Resources: KL and KR; Data curation: BF and YY; Writing–original draft: AK and RA; Writing–reviewing and editing: RA, AK, DV, SK, MJO, MJ, MBA, C-WDT, RAW, SP, KL, KR, BF, YY, KHL, DS-T, CLH, M-AF, PH, CB and AJ; Visualization: AK and BF; Supervision: AJ; Project administration: KL, KR and AJ; Funding acquisition: PH, RA and AJ. Guarantor: AJ.

Funding This work was supported by Iovance Biotherapeutics, Bristol Myers-Squibb (provision of urelumab), Clinigen (provision of aldesleukin) and MD Anderson Cancer Center. This work was supported in part by T32 training grant CA101642 (AK) and by the National Institutes of Health through MD Anderson's Cancer Center Support Grant CA016672 (Institutional Tissue Bank (ITB)) and the Translational Molecular Pathology-Immunoprofiling Lab (TMP-IL) Moonshots Platform at the Department Translational Molecular Pathology, the University of Texas MD Anderson Cancer Center.

Competing interests RA has consulting or advisory roles with Novartis, Erasca, and Iovance and receives institutional research funding from Bristol Myers-Squibb, Roche, Novartis, OnKure, Iovance and Obsidian. MJO has consulting or advisory role with Bristol Myers Squibb, Roche/Genentech, Gritstone Bio, MedImmune, Novartis, Promega, Spectrum Pharmaceuticals, Array BioPharma, Janssen, Pfizer, 3D Medicines, Merck, Eisai and research funding from Bristol Myers Squibb, Merck, Roche, MedImmune, Phanes Therapeutics. C-WDT is a consultant for PanTher Therapeutics and receives research funding from SirTex. AJ has received institutional research funding from Bristol Myers-Squibb, Iovance, Merck, Pfizer, Aravive, Macrogenics, AstraZeneca, Imunon, Avenge Bio, Greenfire Bio, and Break

Through Cancer. He has received consulting fees from Guidepoint, Alkermes, Gerson Lehrman Group, Adicet Bio, Theolytics, Avenge Bio, and Greenfire Bio. CB received research funding from Iovance Biotherapeutics and Obsidian Therapeutics, and reports consulting or advisory roles with Myst Therapeutics and Turnstone Biologics. CB, M-AF and DS-T are now currently employees of the Cell Therapy Manufacturing Center (CTMC), Houston, Texas, USA, a joint venture between MD Anderson Cancer Center and Resilience. CLH reports receiving funding to institution from Avenge Bio, Dragonfly, BTG, Sanofi, Obsidian and Iovance; stock options from Briacell as an SAB member; travel support/honoraria from the Hope Foundation and the Society for Immunotherapy of Cancer outside the scope of the submitted work.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and this study was approved by the US Food and Drug Administration and the Institutional Review Board (IRB) at the University of Texas MD Anderson Cancer Center (IRB-approved protocol #2017-0671, NCT03610490). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. The data generated in this study will be deposited into clinicaltrials.gov.

ORCID iDs
Anne Knisely http://orcid.org/0000-0003-4200-2293
Michael J Overman http://orcid.org/0000-0001-5377-135X
Cara L Haymaker http://orcid.org/0000-0002-1317-9287
Patrick Hwu http://orcid.org/0000-0002-8293-1313
Chantale Bernatchez http://orcid.org/0000-0001-5926-3460

REFERENCES

1 Gooden MJM, de Bock GH, Leffers N, *et al*. The prognostic influence of tumour-infiltrating lymphocytes in cancer: a systematic review with meta-analysis. *Br J Cancer* 2011;105:93–103.
2 Ino Y, Yamazaki-Itoh R, Shimada K, *et al*. Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer. *Br J Cancer* 2013;108:914–23.
3 Hadrup S, Donia M, Thor Straten P. Effector CD4 and CD8 T cells and their role in the tumor microenvironment. *Cancer Microenviron* 2013;6:123–33.
4 Dudley ME, Wunderlich JR, Robbins PF, *et al*. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science* 2002;298:850–4.
5 Dudley ME, Wunderlich JR, Yang JC, *et al*. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *JCO* 2005;23:2346–57.
6 Dudley ME, Yang JC, Sherry R, *et al*. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. *J Clin Oncol* 2008;26:5233–9.
7 Radvanyi LG, Bernatchez C, Zhang M, *et al*. Specific lymphocyte subsets predict response to adoptive cell therapy using expanded autologous tumor-infiltrating lymphocytes in metastatic melanoma patients. *Clin Cancer Res* 2012;18:6758–70.
8 Rosenberg SA, Yang JC, Sherry RM, *et al*. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. *Clin Cancer Res* 2011;17:4550–7.
9 Goff SL, Dudley ME, Citrin DE, *et al*. Randomized, prospective evaluation comparing intensity of lymphodepletion before adoptive transfer of tumor-infiltrating lymphocytes for patients with metastatic melanoma. *J Clin Oncol* 2016;34:2389–97.
10 Sarnaik AA, Hamid O, Khushalani NI, *et al*. Lifileucel, a tumor-infiltrating lymphocyte therapy, in metastatic melanoma. *J Clin Oncol* 2021;39:2656–66.
11 Forget M-A, Haymaker C, Hess KR, *et al*. Prospective analysis of adoptive til therapy in patients with metastatic melanoma: response, impact of Anti-CTLA4, and biomarkers to predict clinical outcome. *Clin Cancer Res* 2018;24:4416–28.
12 Rohaan MW, Borch TH, van den Berg JH, *et al*. Tumor-Infiltrating lymphocyte therapy or ipilimumab in advanced melanoma. *N Engl J Med* 2022;387:2113–25.
13 Stevanović S, Draper LM, Langhan MM, *et al*. Complete regression of metastatic cervical cancer after treatment with human papillomavirus-targeted tumor-infiltrating T cells. *J Clin Oncol* 2015;33:1543–50.
14 Huang H, Nie C-P, Liu X-F, *et al*. Phase I study of adjuvant immunotherapy with autologous tumor-infiltrating lymphocytes in locally advanced cervical cancer. *J Clin Invest* 2022;132:15.
15 Creelan BC, Wang C, Teer JK, *et al*. Tumor-infiltrating lymphocyte treatment for anti-PD-1-resistant metastatic lung cancer: a phase 1 trial. *Nat Med* 2021;27:1410–8.
16 Tran E, Robbins PF, Lu Y-C, *et al*. T-cell transfer therapy targeting mutant KRAS in cancer. *N Engl J Med* 2016;375:2255–62.
17 Zacharakis N, Chinnasamy H, Black M, *et al*. Immune recognition of somatic mutations leading to complete durable regression in metastatic breast cancer. *Nat Med* 2018;24:724–30.
18 Aoki Y, Takakuwa K, Kodama S, *et al*. Use of adoptive transfer of tumor-infiltrating lymphocytes alone or in combination with cisplatin-containing chemotherapy in patients with epithelial ovarian cancer. *Cancer Res* 1991;51:1934–9.
19 Freedman RS, Edwards CL, Kavanagh JJ, *et al*. Intraperitoneal adoptive immunotherapy of ovarian carcinoma with tumor-infiltrating lymphocytes and low-dose recombinant interleukin-2: a pilot trial. *J Immunother Emphasis Tumor Immunol* 1994;16:198–210.
20 Fujita K, Ikarashi H, Takakuwa K, *et al*. Prolonged disease-free period in patients with advanced epithelial ovarian cancer after adoptive transfer of tumor-infiltrating lymphocytes. *Clin Cancer Res* 1995;1:501–7.
21 Sakellariou-Thompson D, Forget M-A, Hinchcliff E, *et al*. Potential clinical application of tumor-infiltrating lymphocyte therapy for ovarian epithelial cancer prior or post-resistance to chemotherapy. *Cancer Immunol Immunother* 2019;68:1747–57.
22 Tavera RJ, Forget M-A, Kim YU, *et al*. Utilizing T-cell activation signals 1, 2, and 3 for tumor-infiltrating lymphocytes (til) expansion: the advantage over the sole use of interleukin-2 in cutaneous and uveal melanoma. *J Immunother* 2018;41:399–405.
23 Forget M-A, Haymaker C, Dennison JB, *et al*. The beneficial effects of a gas-permeable flask for expansion of tumor-infiltrating lymphocytes as reflected in their mitochondrial function and respiration capacity. *Oncoimmunology* 2016;5:e1057386.
24 Romero P, Zippelius A, Kurth I, *et al*. Four functionally distinct populations of human effector-memory CD8+ T lymphocytes. *J Immunol* 2007;178:4112–9.
25 Powell DJ, Dudley ME, Robbins PF, *et al*. Transition of late-stage effector T cells to CD27+ CD28+ tumor-reactive effector memory T cells in humans after adoptive cell transfer therapy. *Blood* 2005;105:241–50.
26 Creelan B, Wang C, Teer J, *et al*. Abstract CT056: durable complete responses to adoptive cell transfer using tumor infiltrating lymphocytes (TIL) in non-small cell lung cancer (NSCLC): A phase I trial. *Cancer Res* 2020;80(16_Supplement):CT056.
27 Sarnaik A, Khushalani NI, Chesney JA, *et al*. Safety and efficacy of cryopreserved autologous tumor infiltrating lymphocyte therapy (LN-144, lifileucel) in advanced metastatic melanoma patients who progressed on multiple prior therapies including anti-PD-1. *JCO* 2019;37(15_suppl):2518.
28 Qin SS, Melucci AD, Chacon AC, *et al*. Adoptive T cell therapy for solid tumors: pathway to personalized standard of care. *Cells* 2021;10:808.
29 Becker JC, Andersen MH, Schrama D, *et al*. Immune-suppressive properties of the tumor microenvironment. *Cancer Immunol Immunother* 2013;62:1137–48.
30 Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol* 2012;12:253–68.
31 Ohue Y, Nishikawa H. Regulatory T (Treg) cells in cancer: can treg cells be a new therapeutic target? *Cancer Sci* 2019;110:2080–9.

32 Sakellariou-Thompson D, Forget M-A, Creasy C, *et al*. 4-1BB agonist focuses CD8$^+$ tumor-infiltrating t-cell growth into a distinct repertoire capable of tumor recognition in pancreatic cancer. *Clin Cancer Res* 2017;23:7263–75.
33 Shah P, Forget M-A, Frank ML, *et al*. Combined IL-2, agonistic CD3 and 4-1BB stimulation preserve clonotype hierarchy in propagated non-small cell lung cancer tumor-infiltrating lymphocytes. *J Immunother Cancer* 2022;10:e003082.
34 Wong K, Wrocklage C, Lin S, *et al*. 204 KSQ-004: unbiased pair-wise discovery of SOCS1 and regnase-1 as the top CRISPR/Cas9 dual-edit combination enhancing in vivo TIL potency against solid tumors. *J Immunother Cancer* 2021;9(Suppl 2):A215.
35 Wang S, Sun J, Chen K, *et al*. Perspectives of tumor-infiltrating lymphocyte treatment in solid tumors. *BMC Med* 2021;19:140.
36 Ritthipichai K, Machin M, Juillerat A, *et al*. 1052P genetic modification of Iovance's TIL through TALEN-mediated knockout of PD-1 as a strategy to empower TIL therapy for cancer. *Annals of Oncology* 2020;31:S720.
37 Zhang L, Morgan RA, Beane JD, *et al*. Tumor-infiltrating lymphocytes genetically engineered with an inducible gene encoding interleukin-12 for the immunotherapy of metastatic melanoma. *Clin Cancer Res* 2015;21:2278–88.
38 Forget M-A, Tavera RJ, Haymaker C, *et al*. A novel method to generate and expand clinical-grade, genetically modified, tumor-infiltrating lymphocytes. *Front Immunol* 2017;8:908.
39 Fix SM, Forget M-A, Sakellariou-Thompson D, *et al*. CRISPR-mediated TGFBR2 knockout renders human ovarian cancer tumor-infiltrating lymphocytes resistant to TGF-β signaling. *J Immunother Cancer* 2022;10:e003750.
40 SMR Congress 2017 abstracts. *Pigment Cell Melanoma Res* 2018;31:125–230. 10.1111/pcmr.12656 Available: https://onlinelibrary.wiley.com/toc/1755148x/31/1