nature portfolio

Peer Review File

Autologous multiantigen-targeted T cell therapy for pancreatic cancer: a phase 1/2 trial

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This phase I/II trial aimed at treating patients with PDAC with multiple infusions of autologous unmodified peripheral blood-derived T cell product reactive to 5 shared tumor antigens, PRAME, SSX2, survivin, MAGE-A4 and NY-eso-1..
56 patients were enrolled and 37 patients received at least one infusion, and were divided over 3 treatment arms: A: stage III or IV PDAC stable or responding to SOC chemotherapy; B: stage III or IV PDAC, progressed after SOC chemotherapy; C: resectable PDAC, receiving the T cells prior to surgery, but after neoadj chemotherapy.
The study demonstrated feasibility to grow enough T cells with defined specificity from these patients and that patients were able to receive at least 2 infusions of the cells. The treatment appeared safe with at least in cohort A a signal of efficacy. Extensive and high quality correlative studies were performed.

Comments:
What was the rationale to aim for 6 infusions per patients? Based on what other data in this particular setting? In comparison, both in TIL treated patients and in a recently published study (Borgers et al. Nat Med 2025) investigating neoantigen blood derived T cells, administration of TIL is preceded by LD chemo, and in TIL with additional IL-2 infusions. Therefore, the authors should explain why they did not choose for one large dose of T cells preceded by LD-chemo vs 30 millions T cells (a rather low dose for a solid cancer) per dose, but multiple times. In addition, LD-chemo may impact on the PDAC TME and perhaps improve T cell infiltration.

In the patients with a response (PR and CR) in arm A, did they have SD on prior treatment of were they responding to prior chemotherapy? In order to fully grasp the efficacy of the cell therapy, more detailed information would be helpful.

The duration of response of the treatment in arm A, the only arm that can be assessed in terms of RECIST response, is rather short, just over 6 months (longer for the CR patient). Perhaps important to put this into context of other SOC treatments, to better emphasize that this is substantially better compared chemotherapy.

Similarly, for patients in arm C, 2 patients are alive at more than 5 years. Please put this into context.

Upon infusion, and at FU, dominant T cell clones (TCRVb seq) persisted in patients that were tested. Whether these cells contributed to immune reconstitution is difficult to say. However, in some patients it appears that the frequency increased at FU timepoints, at least suggesting that there was Ag recognition. Was there a correlation with outcome in these patients?

In addition, when looking at individual tumor-specificities, patients in arm A having a response had higher frequencies of TAA-specific cells compared to SD and PD patients, the latter groups not showing signs of epitopes spreading. In contrast, in the SD group of patients in arm B the frequency of TAA-specific T cells was much elevated compared to PD. I find it strange that this was not found in arm A, as these patients were in general in a better disease situation. Why?

PRAME-specific T cells were more frequent than any other TAA-specific population. Was this correlated with PRAME expression by these PDAC? Was there a difference in responders vs non-responders?

In some patients, prior to infusion, non-TAA antigen-specific T cells were already present in the circulation, oftentimes reactive to WT-1 and MC1, which expanded after cell therapy. I could argue that these antigens are probably more relevant

antigens that the 5 chosen TAA, and should be included in the induction protocol. Why were these not included?

In figure 4 the functionality of persisting and expanding TAA-specific T cells was shown. The results are highly encouraging and demonstrate that in these responding patients TAA and non-TAA reactive T cells can persist and remain functional over a prolonged period of time.
In the PR patients that progressed later on, what was the mode of escape to such a wide T cell repertoire?

In general, this is a very interesting study, providing early evidence of response in this complicated and dismal disease. I assume that based on a tumor biopsy, the expression of an extended amount of TAA could be assessed, and based on this a T cell product could be generated. In addition, it would be interesting to combine a single dose of T cells with LD-chemo to perhaps increase the ORR in this patient population as a comparison to this approach (outside the scope of this study), but could be discussed.

Reviewer #2

(Remarks to the Author)
A. The authors conducted, TACTOPS, a phase I/II trial involving an autologous ex-vivo expanded polyclonal , Th1-polarized T cell therapy which targets a spectrum of tumor associated antigens (TAAs) in three different patient cohorts. The antigens targeted include PRAME, SSX2, MAGEA4, NY-ESO1, and Survivin.
The trial population was unique as it involved: Arm A stage III/IV stable on 3 months of therapy; Arm B stage III/IV that had progressed; Arm C neoadjuvant with chemotherapy; and refractory patients.

B. The work described is original and when further developed may provide a novel therapeutic option for patients with pancreatic cancer.

C. Objectives: (1) feasibility of administration and safety; (2) autologous T cells would persist in vivo; (3) persistence would be associated with clinical benefit

The effort is commended. The main objectives which explored feasibility and safety were executed. Feasibility criteria only met in Arm A (the cohort with stable disease on active treatment), whereas those receiving 2nd line or neoadjuvant did not meet criteria. Due to limited numbers in each cohort, I agree that clinical outcome assessments are limited but the association between persistent T cell impact and durability of response is worth further exploration.

D. This effort assessed feasibility and safety so statistical goals were descriptive in nature.

E. The development of the T cell product and transcriptomic assessment in vivo was well conducted and methods easy to follow and appear to be reproducible. The limitations of this project include: (1) the three very different patient populations limited the ability to successfully administer the goal 6 infusions. It is unclear why the team went in this direction, as it also makes any trends regarding clinical benefit difficult to assess. (2) the lack of baseline T cell marker presence limits quantitative and qualitivative changes impressed by the T cell product, and if all 6 infusions were needed.

F. If possible, I would analyze the baseline markers and provide information regarding change over time in reference to that versus to the subsequent administration timepoints.

G. References were appropriate.

H. Clarity and presentation of context was optimal.

Reviewer #3

(Remarks to the Author)
This is an early phase 1 trial of targeted T cell therapy in patients with PDAC. As previously shown the strategy is feasible and safe and the infused T cells persist in patients.

Main issue with this trial is that it is not possible to determine if there is activity as either the cells were administered in combination with chemotherapy or in the post operative setting. In cohort 2 there was no activity. Several experiments may help to elucidate this issue:

1. One option could be to generate personalized oprganoids from enrolled patients to test ex vivo if the cells have killing capabilities both alone and in combinations.

2. Pair biopsies should be conducted to show that the cells localize to the targeted tumors. If not, strategies should be explored to overcome that issue.

3. Even if active and able to target the tumors, ICI may be needed to unleash the full potential of these cells.

4. Study should be designed to test efficacy without confounding factors such as concomitant chemotherapy.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I would like to thank the authors for their extensive answers to my questions! No further questions.

Reviewer #2

(Remarks to the Author)
The authors have addressed the concerns raised. I would support publication with the addition of the described edits.

Reviewer #3

(Remarks to the Author)

EDITORS COMMENTS:

Your Article, "Autologous multiantigen-targeted T cell therapy for pancreatic ductal adenocarcinoma (PDAC): results of a Phase I, three-arm, nonrandomized clinical trial (TACTOPS)" has now been seen by 3 referees. You will see from their comments below that while they find your work of interest, some important points are raised. We are interested in the possibility of publishing your study in Nature Medicine, but would like to consider your response to these concerns in the form of a revised manuscript before we make a final decision on publication.

In a revised manuscript, we consider of importance to;

(i) critically discuss the decision to aim for 6 infusions in line with comments by referees 1 and 2

Response: Per the editors request we have now provided a detailed explanation behind the rationale to perform 6 infusions in our response to the reviewers and have also revised the manuscript to include this information.

(ii) state clearly whether or not the primary endpoint was met in arms b and c.

Response: This has now been clearly articulated in the revised manuscript and is also included below.

The primary study endpoints were (i) safety and (ii) feasibility of completing 6 infusions (defined as a series, per the clinical protocol).

(i) Safety – this endpoint was met.
Infusions were well tolerated by patients in all three study arms. Only a single grade 3 tSAE (transient lipase elevation) was reported. The original submission (**Table 2**, **Supp. Table 4**) includes all AE information.

(ii) Feasibility of completing 6 infusions – this was not met in any of the study arms. **Supp. Table 3** provides the specific details of the # of doses infused and reasons why the series was not completed.

Per the clinical protocol, if ≥2 of the first 6 subjects in each arm were unable to receive all 6 infusions (i.e. unable to complete a series) the strategy was determined unfeasible. However, study enrollment could continue in each respective arm.

By this definition, completing the infusion series in all arms was not feasible. However, the reasons that patients did not/could not receive all 6 infusions was not due to insufficient cells or infusion-related toxicities in the vast majority of cases. In 86% of cases infusions were discontinued due to disease progression (n=14), surgical complications (n=4) or cancer-unrelated death (n=1).

Arm A: Although 2 of the first 6 Arm A patients did not receive a full series of 6 infusions (so the feasibility endpoint was not met), the median number of infusions per patient in Arm A was 6 (range 2-6).
Arm B: Median number of infusions per patient was 2.5 infusions (range 1-6)
Arm C: Median number of infusions per patient was 3 (range 1-6)

(iii) We also feel it is important to show analysis of target expression as commented on by referee 1

Response: Although target antigen profiling on tumor material was not a study pre-requisite, we now supplement the IHC data in our original submission showing expression of PRAME and Survivin in tumor material from pts #9, #25, #12, and #54 [**Fig. 4(d), (k)**; **Supp. Fig. 9(i), (m)**] with bulk RNA

sequencing data that was performed on formalin-fixed paraffin-embedded (FFPE) slides for 5 of our patients. The slide processing was performed by Novogene and our response letter now reports the detection of both targeted and non-targeted TAAs in **Response Table 1**. Furthermore, in response to reviewer 1 (questions 6 and 7), we now provide additional context regarding our original target antigen choice as well as other TAAs that we are profiling for potential inclusion in a future trial.

(iv) Data on infiltration of T cells in the tumour, if possible.

Response: Fortunately, in Arm C of our protocol we included a provision to receive tissue from resected patients to evaluate T cell infiltration. Our original manuscript submission included data for pts #25, #12 and #54 showing CD3 detection in tumor samples (by IHC + flow cytometry) and confirmation that the tumor-infiltrating T cell content was, in part, derived from the infused product (TCRvβ deep sequencing) [**Fig. 4 (k, l)**; **Supp. Fig. 9 (i, j, m, n)**].

Based on feedback from the editor and reviewers, we have now revised our manuscript (**new Extended Data Figure 1**) and provided a detailed response to reviewer 3 (Q2), in which we have included all available CD3 detection in tumors (IHC and/or flow cytometry) and provenance (TCRvβ deep sequencing).

Since neither Arm A nor Arm B included provisions for protocol-mandated tumor biopsies, we can provide no new data on these patients. However, we would highlight that during the study we were fortunate to attain excess material from a standard of care biopsy on Arm A pt#9 and were able to detect CD3+ T cells infiltrating the tumor, of which 0.19% were confirmed to be line-derived, based on deep sequencing data. These data are included in **Fig. 4d** of our original submission.

(v) We do not think it is necessary to perform patient-derived organoid experiments to analyse functionality of T cells.

Response: We appreciate this concession.

REVIEWERS COMMENTS:

Reviewer #1 (Remarks to the Author):

This phase I/II trial aimed at treating patients with PDAC with multiple infusions of autologous unmodified peripheral blood-derived T cell product reactive to 5 shared tumor antigens, PRAME, SSX2, survivin, MAGE-A4 and NY-eso-1. 56 patients were enrolled and 37 patients received at least one infusion, and were divided over 3 treatment arms: A: stage III or IV PDAC stable or responding to SOC chemotherapy; B: stage III or IV PDAC, progressed after SOC chemotherapy; C: resectable PDAC, receiving the T cells prior to surgery, but after neoadj chemotherapy.

The study demonstrated feasibility to grow enough T cells with defined specificity from these patients and that patients were able to receive at least 2 infusions of the cells. The treatment appeared safe with at least in cohort A a signal of efficacy. Extensive and high quality correlative studies were performed.

General comments: We thank reviewer 1 for their critical review and feedback of our work. Below we provide a point-by-point response to each comment raised. Overall, in addition to revising sections of the

text we have revised a Supp. Table and have added 2 new Extended Data Figures and a new Supp. Figure, which we believe have substantially improved our revised manuscript.

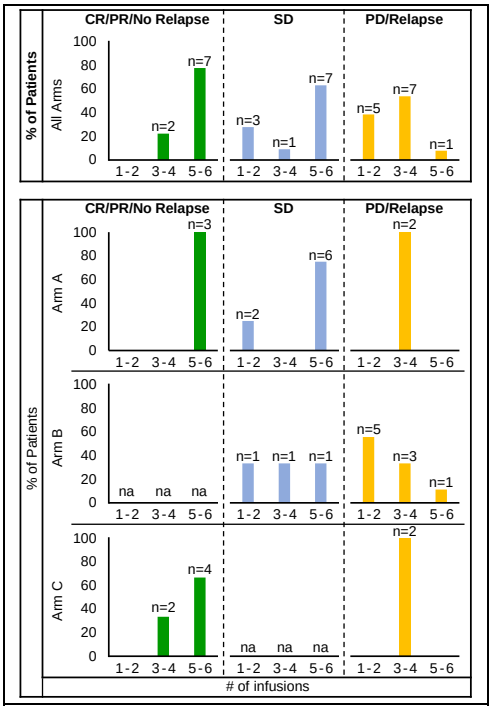
Comments:

Q1. What was the rationale to aim for 6 infusions per patients? Based on what other data in this particular setting? In comparison, both in TIL treated patients and in a recently published study (Borgers et al. Nat Med 2025) investigating neoantigen blood derived T cells, administration of TIL is preceded by LD chemo, and in TIL with additional IL-2 infusions. Therefore, the authors should explain why they did not choose for one large dose of T cells preceded by LD-chemo vs 30 millions T cells (a rather low dose for a solid cancer) per dose, but multiple times. In addition, LD-chemo may impact on the PDAC TME and perhaps improve T cell infiltration.

Response:

(a) Rationale for 6 infusions per patient (@1x10^7/m^2) - we thank the reviewer for this question. In reality there were a number of factors that influenced this decision:

1. The infused cell dose was primarily influenced by initial FDA interactions and perceived safety concerns, given that the target tumor antigens were a mix of cancer testis antigens and shared tumor associated antigens. This is a major element that distinguishes our work (and potential safety profile) from that of Borgers et al, who targeted neoantigens that are exclusively present on malignant cells. Indeed, when we first proposed a multi-antigen-targeted product, the FDA mandated an antigen escalation clinical trial in which T cells (0.5x10^7 cells/m^2) targeting a single antigen were prepared and infused to at least 2 patients. Once safety had been established within a 4 week monitoring period to evaluate treatment-related toxicities, we were allowed to escalate by targeting 2 antigens in a single product, followed by the same safety evaluation period and subsequent permission to antigen escalate to 3 antigens, and so on. Once the safety of a product targeting all 5 antigens had been established, we were allowed to initiate our conservative dose finding study across a narrow range of 0.5-2x10^7 cells/m^2, 2x infusions per patient.



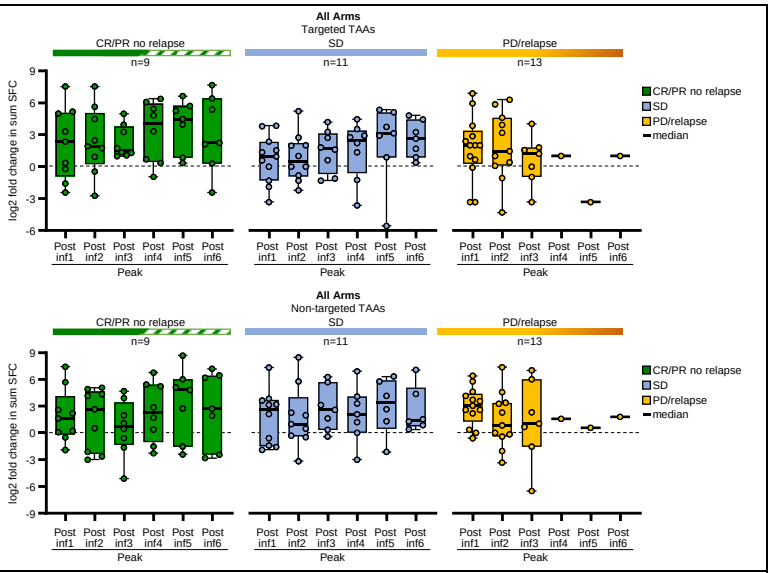
Response Figure 1. Association between # of infusions and outcome. Responders (CR/PR/No relapse), SD and Non-responders (PD/Relapse) are segregated based on the number of infusions administered. Results are reported as % of patients.

Hence, in the current study, we sought to increase the infused cell dose (cumulatively) through performing repetitive infusions (1x10^7/m^2, 6x infusions), which in a typical adult (2m^2) equates to 1.2x10^8 total cells. By arm, 9/13 arm A patients, 2/12 arm B patients, and 4/9 resected Arm C patients received all 6 doses. Overall, 15/34 patients received 6 infusions; 2/34 patients received 4 infusions; 8/34 patients received 3 infusions; 6/34 patients received 2 infusions; and 3/34 patients received 1 infusion.

In order to better understand the contribution of multiple infusions, as requested by reviewer 2, we have performed some more detailed analyses examining (i) the association between the # of infusions and outcome and (ii) the change in the frequency of functional T cells (directed against both targeted and non-targeted antigens) compared with baseline after each subsequent infusion. These data are now included as **Response Figures 1** and **2**, respectively and are also included in

the revised manuscript – **new Supp. Fig. 6** and **new Extended Data Fig.2**. It is important to note that, per the clinical protocol, patients stopped receiving infusions upon evidence of progression, which confounds the analysis. Nevertheless, when looking at (i) all patients (ii) by individual study arm (**Response Figure 1**, top and bottom panels, respectively) clinical benefit (CR/PR/No Relapse) and even disease stabilization appears to be associated with receiving more (3-4 or 5-6 infusions) vs fewer infusions. Furthermore, the change in the frequency of functional, tumor-specific T cells was most pronounced in responders and continued to increase/remained persistently high upon receiving additional infusions (**Response Figure 2**). Those with SD exhibited more modest expansion after receiving the first infusion with ongoing increase thereafter, whereas those who progressed/relapsed experienced a rapid decline in functional T cells.

Based on these data, both (a) immediate post-infusion expansion and (b) the persistence of functional, tumor-specific T cells at an elevated level appear to correlate with benefit, supporting an updated infusion strategy for a future clinical trial in which fewer infusions with larger cell doses are administered, preferably at an earlier phase of treatment and potentially in combination with LD chemo/ICI/IL2/other adjuvants.



Response Figure 2. In vivo T cell activity. In vivo expansion of tumor-reactive T cells. Expansion of T cells specific for targeted (top) and non-targeted (bottom) TAAs in patients from all 3 arms grouped in responders (CR/PR/no relapse) vs SD vs non-responders (PD/relapse). Results are reported as log-transformed fold change in SFC at the specified time points in relation to baseline. Each symbol represents an individual patient and median values are indicated.

2. Our decision to infuse patients on every 4 weeks was driven in large part by the Arm A cohort, in which patients received T cell infusions concurrently with standard chemotherapy. Since one of these regimens (nab-paclitaxel/gemcitabine) is administered in 4-week cycles (days 1, 8, and 15 of a 28 day cycle) while the other (FOLFIRINOX) is administered every 2 weeks, and given that we wanted to administer our T cells during the "off week" of chemotherapy (i.e. week 4 of a nab-paclitaxel cycle or week 2 of a FOLFIRINOX cycle) to minimize the impact of chemotherapy on the "fitness" of the infused T cells, we administered T-cells every 4 weeks.

3. We also considered Arm C patients when seeking to extend T cell coverage for at least 6 months post-surgery with "top-up" infusions to deal with any sites of minimal residual disease present following surgical resection of the primary mass.

(b) Cell production feasibility – As outlined in the manuscript, despite starting with peripheral blood draws for T cell preparation, we successfully expanded cells to >$8x10^8$ (**Supp. Table 2**). Thus, initiating cultures with apheresis material should facilitate production of cells at ≥10^9-10^{10} for future clinical trials.

(c) Consideration of prior lymphodepletion/combination with IL2: The reviewer raises an extremely important point. Whether lymphodepleting patients before T-cell infusions (to potentially alter the PDAC TME and improve T cell infiltration, as well as maximizing the *in vivo* expansion of the infused product) versus foregoing lymphodepletion (to maximize recruitment of the endogenous immune system) was

extensively discussed prior to embarking on the TACTOPS clinical trial. In TACTOPS, we ultimately chose not to pursue lymphodepletion to simplify assessment of both toxicity and response from the infused cells; however, now that we have established the feasibility and safety of our product, we will propose pre-infusion lymphodepletion in a future trial. We have revised the manuscript to include our rationale for the study design of TACTOPS, as well as options for future trials [including pre-T cell infusion lymphodepletion and combination approaches such as tumor-specific T cells + IL2 and/or checkpoints (as highlighted by reviewer 3)].

Q2. In the patients with a response (PR and CR) in arm A, did they have SD on prior treatment or were they responding to prior chemotherapy? In order to fully grasp the efficacy of the cell therapy, more detailed information would be helpful.

Response: We have now revised **Supp. Table 6** of the manuscript to include information regarding the response status (per RECIST v1.1) of all Arm A patients following three 28-day cycles of first-line chemotherapy but prior to initiating T cell infusions. To specifically address the reviewer's question: all 3 Arm A patients with objective responses on TACTOPS (#9 - CR; #4 - PR; #41 – PR) had a best response of SD before starting T cell infusions.

Q3. The duration of response of the treatment in arm A, the only arm that can be assessed in terms of RECIST response, is rather short, just over 6 months (longer for the CR patient). Perhaps important to put this into context of other SOC treatments, to better emphasize that this is substantially better compared chemotherapy. Similarly, for patients in arm C, 2 patients are alive at more than 5 years. Please put this into context.

Response: Patients enrolled in Arm A had locally advanced unresectable or metastatic PDAC and had already received three 28-day cycles of first-line chemotherapy prior to their first T cell infusion, which was administered during week 4 (the "off" week) of their fourth 28-day chemotherapy cycle. We have now revised **Supp**. **Table 6** of the manuscript to report the disease status of Arm A patients prior to initiating T cell infusions to provide some additional baseline context, and to note that the 3 Arm A responders all entered the study with SD.

Regarding those in Arm C that remain well at 5 years (#25 and #53), below is a summary of their clinical course, which may be helpful.

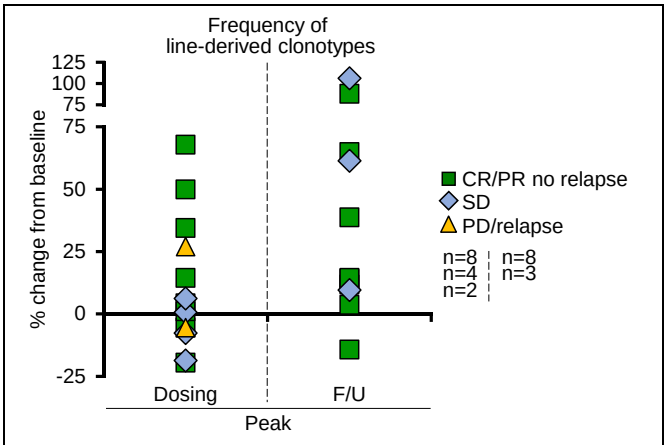
- Subject #25: This patient was diagnosed with cT2N1 PDAC in Jan 2019 and underwent neoadjuvant FOLFIRINOX (9 cycles) followed by a single pre-surgery T cell infusion and a Whipple (July 2019). Despite having received 4-5 months of neoadjuvant FOLFIRINOX, her pathologic stage was ypT2N1, putting her at high risk for relapse. Furthermore, she received no adjuvant chemo post-surgery due to ongoing neuropathy and GI symptoms. She did, however, receive 5 more T cell infusions (to complete her course of 6 total). Despite her high risk surgical pathology and inability to receive adjuvant chemotherapy, she remains cancer-free as of July 2025.
- Subject #53: This patient was diagnosed with PDAC in August 2019. His tumor was borderline resectable at diagnosis with radiographic evidence of extensive lymphatic spread (cT2N2). He underwent neoadjuvant FOLFIRINOX and one T cell infusion followed by a Whipple in Feb 2020 (ypT1cN0, TRG 2). In addition to 3 more cycles of adjuvant chemotherapy, he received 5 more T cell infusions (to complete the full course of 6). Despite his high risk stage at diagnosis (cT2N2), he remains cancer-free as of July 2025.

Only a randomized trial could prove how much infused T cells contributed independently to disease response and survival, and interpretation of any responses seen in the current trial is complex given the small size of the study, heterogeneity of the patient population, and absence of a control arm. However, we are encouraged by the robust immunological signature detected in our responders (**Figure 3** and **Supp.**

Figure 8) and the pronounced increased frequency of functional T cells that are detected early (after the first infusion) and maintained thereafter (**Response Figure 2**), which are consistent with an immunological fitness that may be associated with better outcomes.

Q4. Upon infusion, and at FU, dominant T cell clones (TCRVb seq) persisted in patients that were tested. Whether these cells contributed to immune reconstitution is difficult to say. However, in some patients it appears that the frequency increased at FU timepoints, at least suggesting that there was Ag recognition. Was there a correlation with outcome in these patients?

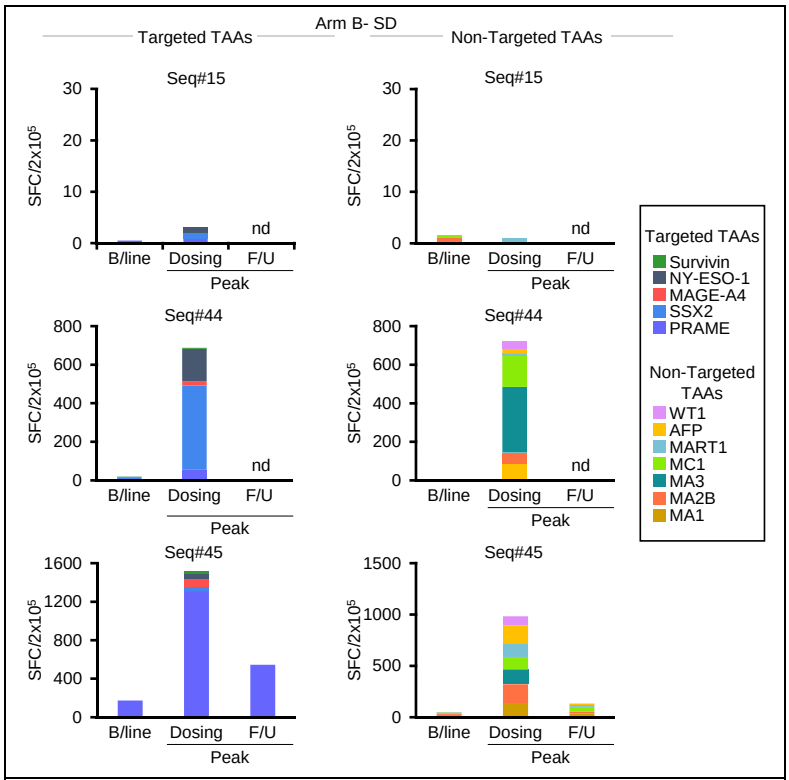
Response: As noted by the reviewer, in the current study persistence was evaluated by TCR deep sequencing analysis of peripheral blood samples collected during and after dosing to look for the presence of product-derived unique clones, while the expansion and functional capacity of tumor-reactive T cells was interrogated by performing IFNγ ELIspot analysis. As the reviewer also notes, in the original submission we comment that "In all 14 donors analyzed, product-derived clonotypes were detected at all timepoints tested, confirming that the infused autologous, non-engineered mTAA-T cells persisted *in vivo* and contributed to immune reconstitution." So the reviewer's point is well taken that perhaps the assertion that these cells contribute to immune reconstitution is not supported solely by the presentation of data demonstrating the presence of line-derived unique clonotypes over time. However, our proposition is strengthened by (i) the confirmation that our product is specific and contains T cells that are functional and reactive against the targeted TAAs, (ii) the detection of functional, antigen-specific TAA-reactive T cells in the peripheral blood of patients post-infusion, both during the dosing phase and in follow-up, and (iii) the confirmation (in a subset of individuals for whom we had deep sequencing and scRNAseq data) that line-derived clonotypes that persisted in the peripheral blood of patients during infusion and follow-up were polyfunctional [**Figure 4 (E+F, I+J); Supp. Figure 9 (I+J); Supp. Figure 10 (C+D, G+H)**]. Additionally, in **Response Figure 3** we now include data showing the change in frequency of line-derived clonotypes compared to baseline, which shows their presence at elevated levels post-infusion that remained high in follow-up. We have this data for only 14 patients in total, making it difficult to make highly robust claims regarding detection of clonotypes at a certain threshold and outcome. So, to err on the side of caution, in the revised manuscript we have removed the phrase "…contributed to immune reconstitution".



Response Figure 3. Frequency of mTAA-T cells. the change in frequency of mTAA product-derived clones as assessed by TCRvβ deep sequencing (n=14) by comparing baseline samples with those detected during dosing and in follow-up. Each symbol represents an individual patient and the patients are color-coded based on best clinical outcome.

Q5. In addition, when looking at individual tumor-specificities, patients in arm A having a response had higher frequencies of TAA-specific cells compared to SD and PD patients, the latter groups not showing signs of epitopes spreading. In contrast, in the SD group of patients in arm B the frequency of TAA-specific T cells was much elevated compared to PD. I find it strange that this was not found in arm A, as these patients were in general in a better disease situation. Why?

Response: We thank the reviewer for the opportunity to expand on the interesting SD patients in Arm B and, in particular, patients #44 and #45, who achieved disease stabilization after receiving T cells as monotherapy and subsequently had prolonged survival. As the reviewer notes, in **Figure 3** of the manuscript, the summarized data for Arm B patients who achieved disease stabilization demonstrated robust T cell reactivity to both targeted and non-targeted antigens, at least during the dosing period. When one looks at the individual patient level (**Supp. Figure 8b**; for ease of review this data is also shown below in **Response Figure 4**) of the 3 SD patients, it is the immune activity in patients #44 and #45 (who received 4 and 6 infusions, respectively) that is most notable.



Response Fig 4: **In vivo behavior of circulating tumor-reactive T cells of Arm B patients with SD**. Trends and profile of circulating T cells specific for both targeted (left side) and non-targeted (right side) TAAs in patient #15, #44 and #45. Results are reported as SFC±SEM/2x10^5 at each specified time point.

Clinically, subject #44 was initially diagnosed with PDAC metastatic to the peritoneal cavity and lung in August 2018. She received FOLFIRINOX until progression at which time she received T cells (on the TACTOPS trial) as monotherapy, with a best response of SD. She ultimately progressed, and subsequently received 2 more lines of chemotherapy, and expired in Sept 2022. Despite the presence of peritoneal metastases, which typically carries a dismal prognosis, this patient lived 30+ months after completing her T-cell infusions.

Subject #45 was enrolled in the TACTOPS trial after having undergone FOLFIRINOX chemotherapy, Whipple, and nab-paclitaxel/gemcitabine chemotherapy. She received all 6 T cell infusions (last infusion in March 2020), with a best response of SD. Of note, in the months prior to receiving T cells her CA19-9 rose sharply. However, during her 6 months of T cell infusions her CA19-9 levels stabilized, and then started to rise sharply a few months after completing on-protocol treatment. She expired in March 2021 (nearly 1 year after her final T-cell infusion) despite having already received both FOLFIRINOX and nab-paclitaxel before T cell therapy.

Both of these patients exhibited minimal tumor-directed immune reactivity prior to T cell infusions (based on baseline ELISpot results); however, the immune landscape changed upon receiving T cells, with pronounced reactivity detected against targeted and non-targeted antigens alike in both subjects, which we can speculate may have contributed to their disease status. Unfortunately we were unable to collect biopsies from these patients during disease response and at subsequent progression in order to investigate the

mechanism of immune escape. As such, we do not know whether changes in the tumor (e.g. tumor antigen modulation, MHC downregulation, upregulation of PD-L1, etc) or T cells (e.g. upregulation of inhibitory/exhaustion markers such as CTLA4 and LAG3) contributed to this phenomenon.

Q6. PRAME-specific T cells were more frequent than any other TAA-specific population. Was this correlated with PRAME expression by these PDAC? Was there a difference in responders vs non-responders?

Response: We detected PRAME in the 4 samples evaluated by IHC [original submission, **Fig. 4** (#9, #25), **Supp. Fig. 9** (#12, #54)]. Although target antigen profiling on tumor material was not a study pre-requisite, we were able to attain formalin-fixed paraffin-embedded (FFPE) slides for 5 patients: 3 Arm C patients (#10, #12, #52) whose samples were collected at the time of resection and whose best responses were relapse, no relapse, and no relapse, respectively, and 2 Arm A patients (#9, #5) whose best responses were CR and SD, respectively. These were sent to Novogene for tissue retrieval and bulk RNA sequencing. **Response Table 1** summarizes the expression profile of (i) the 5 targeted antigens, (ii) a subset of the non-targeted TAAs that we evaluated in the current study, and (iii) other TAAs of interest including mesothelin and members of the Claudin family.

Based on available data there is no clear correlation between response and detected PRAME expression, though the limited number of available samples adds complexity to interpretation.

Response Table 1

	Gene ID	Gene Name	Sequence ID					Gene Description
			#5	#9	#10	#12	#52	
Targeted TAAs	ENSG00000185686	PRAME	7.4643095	0.021379	19.43171	0.970834	2.417242	preferentially_expressed_antigen_in_melanoma
	ENSG00000241476	SSX2	2.0500881	0	0	0	0.053333	synovial_sarcoma_X_breakpoint_2
	ENSG00000147381	MAGE-A4	24.680243	0	0	0	0.127242	melanoma_antigen_family_A_4
	ENSG00000184033	NY-ESO-1	0.0998765	0	0	0	0	cancer/testis_antigen_1B
	ENSG00000089685	Survivin/BIRC5	0.9927226	1.495829	0	0	0	baculoviral_IAP_repeat_containing_5
Non-targeted TAAs	ENSG00000198681	MAGE-A1	0	0.177182	0	0	0.396254	melanoma_antigen_family_A_1
	ENSG00000183305	MAGE-A2B	0.1006327	0.030553	0	0	0.037961	melanoma_antigen_family_A_2B
	ENSG00000221867	MAGE-A3	0.0478755	0	0	0	0	melanoma_antigen_family_A_3
	ENSG00000155495	MAGE-C1	3.0813406	0.609505	0	0	0	melanoma_antigen_family_C_1
	ENSG00000081051	AFP	31.861231	1.120838	0	0	4.177785	alpha-fetoprotein
	ENSG00000184937	WT1	24.23274	1.822349	69.42121	0	2.023322	Wilms_tumor_1
	ENSG00000102854	MSLN	11.954608	3.729638	0	0	0	mesothelin
	ENSG00000163347	CLDN1	14.660865	12.69274	0.105707	7.123511	0.92893	claudin_1
	ENSG00000165215	CLDN3	3.1320251	0	0	17.12821	9.510779	claudin_3

	ENSG00000189143	CLDN4	0.3112652	1.104953	0	0.410794	0.018064	claudin_4
	ENSG00000134873	CLDN10	0.2627021	0.022788	0	10.32501	0.453013	claudin_10
	ENSG00000157224	CLDN12	0.2500949	2.801002	0.025646	1.038653	0	claudin_12
	ENSG00000066405	CLDN18	4.4458073	0.051584	0	0.086756	0.021364	claudin_18
	ENSG00000253958	CLDN23	1.4246093	0	0	0	0	claudin_23

*Quantitation reported as Fragments Per Kilobase of transcript per Million mapped fragments (FPKM)

Q7. In some patients, prior to infusion, non-TAA antigen-specific T cells were already present in the circulation, oftentimes reactive to WT-1 and MC1, which expanded after cell therapy. I could argue that these antigens are probably more relevant antigens that the 5 chosen TAA, and should be included in the induction protocol. Why were these not included?

Response: We would first like to thank the reviewer for giving us the opportunity to discuss our choice of antigen targets in the current study. When initially designing this clinical trial, we first sought to identify target antigens that were (a) expressed in PDAC and (b) known to be immunogenic to native T cells in individuals of diverse HLA types. **Response Table 2** is a literature review summary of the expression profile of our target TAAs in PDAC (along with relevant references)[1-14].

TAA	Expression in PDAC
Survivin	>75%
SSX family	3-30%
MAGE-A family	20-86%
PRAME	>30%
NY-ESO-1	2-10%

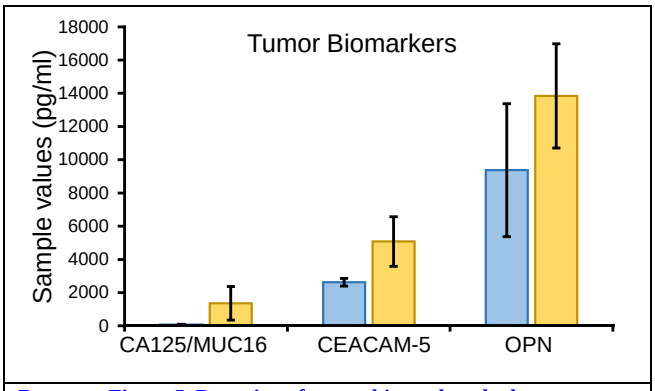
Response Table 2

To minimize immune escape we sought to target multiple antigens simultaneously. Since tumor antigen profiling was not a study pre-requisite, we hypothesized that such an approach would assure that each product generated would target at least one, and optimally multiple, antigens. Ultimately, we hypothesized that the infusion of such a tumor-targeted product would induce direct tumor lysis and that the resultant inflammatory response might result in the recruitment and activation of endogenous immune cells, resulting in *in vivo* antigen spreading with consequent associated benefit.

To address the expression of WT1 and MC1 in our patients, we evaluated their expression in 5 samples submitted for bulk RNA sequencing. As presented in **Response Table 1**, these antigens were detected in 4 and 2 samples, respectively, rationalizing their inclusion in the stimulation protocol for a future study. Claudin family members were also frequently detected, warranting further exploration. Additionally, our lab has been preclinically evaluating the immunogenicity of other shared TAAs (e.g. mesothelin – manuscript under preparation), as well as hotspot neoantigens including TP53, KRAS, NRAS, etc[15], with the goal of potentially broadening the immunogenic repertoire of the infused product in future studies. So the reviewer's comment regarding the relevance of target choice resonates deeply with us and is an area of active investigation, as we advance and optimize this modality. We now include further discussion of antigen choice in our revised submission.

Q8. In figure 4 the functionality of persisting and expanding TAA-specific T cells was shown. The results are highly encouraging and demonstrate that in these responding patients TAA and non-TAA reactive T cells can persist and remain functional over a prolonged period of time. In the PR patients that progressed later on, what was the mode of escape to such a wide T cell repertoire?

Response: Unfortunately, since we did not have access to biopsy material collected during response and at progression, we could not formally address immune escape phenomena. However, we examined known pancreatic disease serum biomarkers in 7 patients, both during clinical response/disease stabilization and disease progression, and saw an increase in serum levels of CA125/MUC16, CEACAM-5 and osteopontin (**Response Figure 5**).



Response Figure 5. Detection of serum biomarkers by human tumor biomarker luminex.

In the absence of serial tumor biopsies, we can only speculate that changes in the tumor (e.g. MHC downregulation, upregulation of PD-L1, tumor antigen modulation, etc), T cells (e.g. upregulation of inhibitory/exhaustion markers such as CTLA4 and LAG3), or both may have contributed.

Q9. In general, this is a very interesting study, providing early evidence of response in this complicated and dismal disease. I assume that based on a tumor biopsy, the expression of an extended amount of TAA could be assessed, and based on this a T cell product could be generated. In addition, it would be interesting to combine a single dose of T cells with LD-chemo to perhaps increase the ORR in this patient population as a comparison to this approach (outside the scope of this study), but could be discussed.

Response: We agree that further tumor antigen profiling could be performed, particularly if pre-treatment biopsies were a pre-requisite for study enrollment. While our response to Q7 (above) provides the rationale for choosing the antigens targeted in the current study, we are actively investigating the immunogenicity of other potential targets, with the intent of incorporating additional specificities. We also concur that prior lymphodepletion or indeed the addition of cytokine support may further potentiate the therapeutic benefit of our approach. To acknowledge all of these important points, we have now revised the manuscript to incorporate discussion of the risks and benefits.

Reviewer #2 (Remarks to the Author):

General comments: We thank reviewer 2 for their careful review and identification of areas for improvement. Below is a point-by-point response to each comment raised. Overall, in addition to revising sections of the text we have revised a Supp. Table and have added 2 new Extended Data Figures and a new Supp. Figure, which we believe have substantially improved our revised manuscript.

A. The authors conducted, TACTOPS, a phase I/II trial involving an autologous ex-vivo expanded polyclonal, Th1-polarized T cell therapy which targets a spectrum of tumor associated antigens (TAAs) in three different patient cohorts. The antigens targeted include PRAME, SSX2, MAGEA4, NY-ESO1, and Survivin. The trial population was unique as it involved: Arm A stage III/IV stable on 3 months of therapy; Arm B stage III/IV that had progressed; Arm C neoadjuvant with chemotherapy; and refractory patients.

B. The work described is original and when further developed may provide a novel therapeutic option for patients with pancreatic cancer.

Response: Regarding A and B - we appreciate the reviewer's acknowledgement regarding the novelty of our study, given the inclusion of 3 different patient populations and a new type of cell therapy. We too hope that this forms the foundation of a next generation therapeutic.

C. Objectives: (1) feasibility of administration and safety; (2) autologous T cells would persist in vivo; (3) persistence would be associated with clinical benefit

The effort is commended. The main objectives which explored feasibility and safety were executed. Feasibility criteria only met in Arm A (the cohort with stable disease on active treatment), whereas those receiving 2nd line or neoadjuvant did not meet criteria. Due to limited numbers in each cohort, I agree that clinical outcome assessments are limited but the association between persistent T cell impact and durability of response is worth further exploration.

D. This effort assessed feasibility and safety so statistical goals were descriptive in nature.

Response: Regarding C and D - we appreciate the reviewer's commendation of the effort; this was a complicated trial to conduct, and we agree that the relationship between T cell persistence and profile is encouraging.

E. The development of the T cell product and transcriptomic assessment in vivo was well conducted and methods easy to follow and appear to be reproducible. The limitations of this project include:
- (1) the three very different patient populations limited the ability to successfully administer the goal 6 infusions. It is unclear why the team went in this direction, as it also makes any trends regarding clinical benefit difficult to assess.
- (2) the lack of baseline T cell marker presence limits quantitative and qualitative changes impressed by the T cell product, and if all 6 infusions were needed.

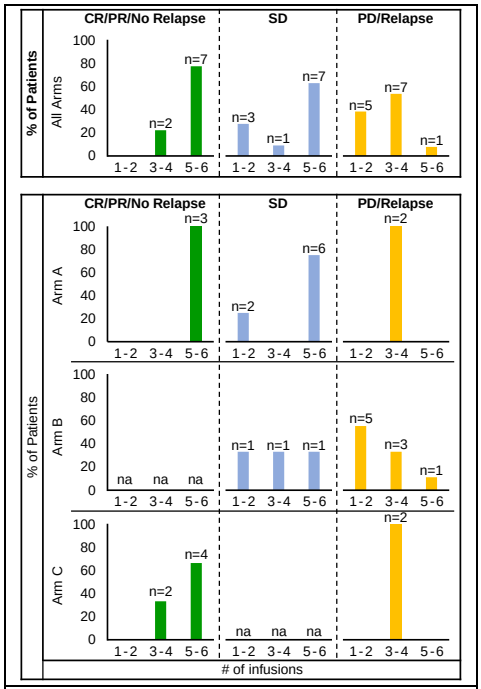
F. If possible, I would analyze the baseline markers and provide information regarding change over time in reference to that versus to the subsequent administration timepoints

Response:

E (1) - First, to address the trial design. Given the devastating nature of PDAC, we wanted to test this novel therapeutic platform and to explore signatures of safety and potential impact/benefit across a wide range of PDAC patients, from "good" (Arm C – resectable) to "intermediate" (Arm A) to "poor" (Arm B). Additionally, these patient populations had received or were receiving different concurrent/prior therapies, as outlined in **Supp. Table 1** of the manuscript. Thus, we sought to enroll patients in distinct study arms to learn about the behavior of the infused T-cells in different settings and to potentially identify the optimal patient population for T cell therapy so that we could advance our technology to a next generation trial.

E (2) + **F** – Per the reviewers request, we have now sought to better address whether all 6 infusions were needed and the contribution that each infusion made to the frequency of circulating, tumor-reactive T cells. Additionally, since reviewer 1 had similar queries, we also provide these data for their review but we would highlight that, per the clinical protocol, patients stopped receiving infusions upon evidence of progression.

To better understand the contribution of multiple infusions to clinical benefit, we examined (i) the association between the number of infusions received and outcome and (ii) the change in the frequency of functional T cells (directed against both targeted and non-targeted antigens) compared with baseline and after each subsequent infusion. These data are now included as **Response Figures 1** and **2**, respectively, and are also included in the revised manuscript – **new Supp. Fig. 6** and **new Extended Data Fig. 2**.



Response Figure 1. Association between # of infusions and outcome. Responders (CR/PR/No relapse), SD and Non-responders (PD/Relapse) are segregated based on the number of infusions administered. Results are reported as % of patients.

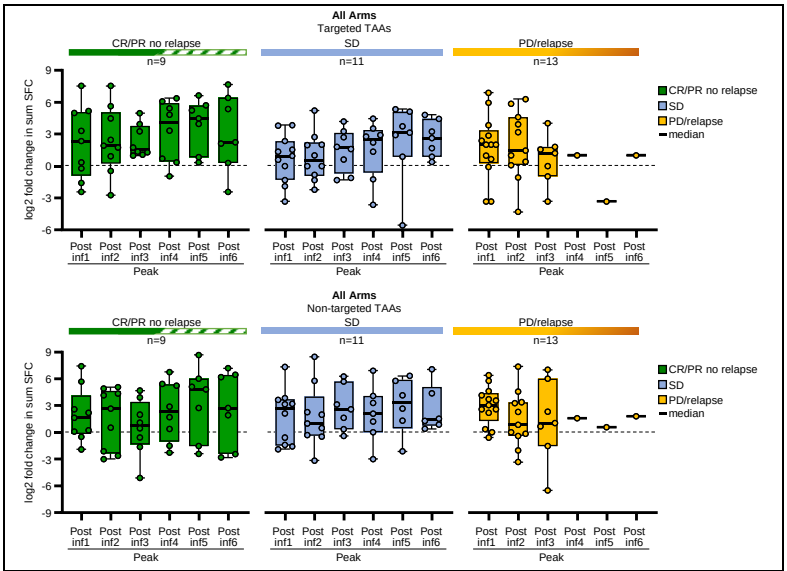
When looking at all patients together or divided into individual study arms (**Response Figure 1**, top and bottom panels, respectively), clinical benefit (CR/PR/No Relapse) and even disease stabilization appear to be associated with receiving more (3-4 or 5-6 infusions) vs fewer infusions. Furthermore, the change in the frequency of functional, tumor-specific T cells was most pronounced in responders and continued to increase or remained persistently high upon receiving additional infusions (**Response Figure 2**). Those with SD exhibited more modest expansion upon receiving the first infusion but did continue to increase thereafter, whereas those who progressed/relapsed experienced a rapid decline in functional T cells.

Based on these data, (a) immediate post-infusion expansion and (b) the persistence of functional, tumor-specific T cells at an elevated level appear to correlate with benefit.

G. References were appropriate.

H. Clarity and presentation of context was optimal.

Response: Regarding G and H - we appreciate the reviewer's comments on references, clarity and presentation.



Response Figure 2. In vivo T cell activity. In vivo expansion of tumor-reactive T cells. Expansion of T cells specific for targeted (top) and non-targeted (bottom) TAAs in patients from all 3 arms grouped in responders (CR/PR/no relapse) vs SD vs non-responders (PD/relapse). Results are reported as log-transformed fold change in SFC at the specified time points in relation to baseline. Each symbol represents an individual patient and median values are indicated.

Reviewer #3 (Remarks to the Author):

This is an early phase 1 trial of targeted T cell therapy in patients with PDAC. As previously shown the strategy is feasible and safe and the infused T cells persist in patients.

General comments: We thank reviewer 3 for their comprehensive review and below is a point-by-point response to each comment raised. Overall, in addition to revising sections of the text we have revised a Supp. Table and have added 2 new Extended Data Figures and a new Supp. Figure, which we believe have substantially improved our revised manuscript.

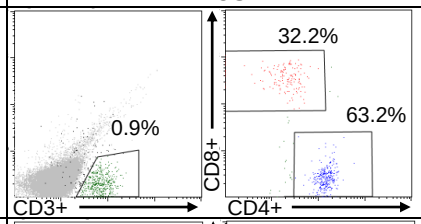
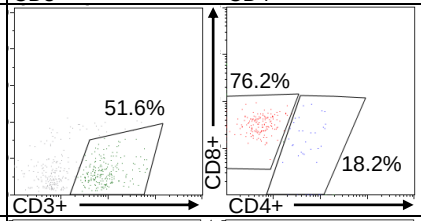
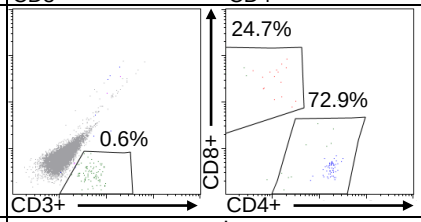
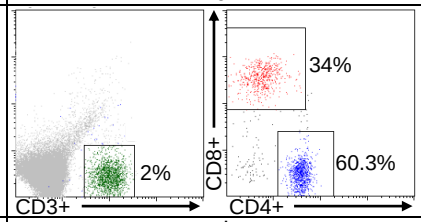
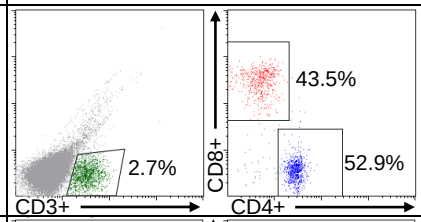
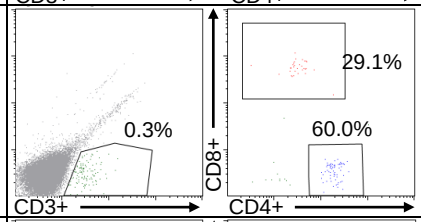
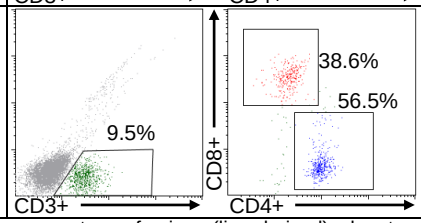
Main issue with this trial is that it is not possible to determine if there is activity as either the cells were administered in combination with chemotherapy or in the post-operative setting. In cohort 2 there was no activity. Several experiments may help to elucidate this issue:

1. One option could be to generate personalized organoids from enrolled patients to test ex vivo if the cells have killing capabilities both alone and in combinations.

Response: We thank the reviewer for this suggestion for future studies. Given the potential to develop human organoids from tumor material, we will certainly consider integrating this approach as a potency metric for future clinical trials.

2. Pair biopsies should be conducted to show that the cells localize to the targeted tumors. If not, strategies should be explored to overcome that issue.

Response: Fortunately, when designing our clinical protocol, we included tissue collection from resected patients (Arm C) to evaluate T cell infiltration into the tumor. Our original submission included data for pts #25, #12 and

Sequence ID	Detection of infiltrating T cells within tumor material	
	FACS	Deep sequencing *
#25		0.34%
#53		1.24%
#54		0.13%
#20§	nd	nd
#12		0.27%
#8§	nd	nd
#10		0.26%
#52		0.79%
#29		0.10%

*Reported as percentage of unique (line-derived) clonotypes within the total TCR sequences analyzed per tumor
§ No resected tumor material sent to the laboratory for analysis

Response Table 3

#54, which showed CD3 detection in tumor samples (IHC + flow cytometry) and confirmation that the tumor-infiltrating T cell content was, in part, derived from the infused product (TCRvβ deep sequencing) [original submission - **Fig. 4 (k, l)**; **Supp. Fig. 9 (i, j, m, n)**].

In response to this query, we have now revised our manuscript (**new Extended Data Fig. 1**) to include all available Arm C patient data. In addition, for ease of review we also include these data in the response letter (**Response Table 3**).

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