# Phase IB Study of GITR Agonist Antibody TRX518 Singly and in Combination with Gemcitabine, Pembrolizumab, or Nivolumab in Patients with Advanced Solid Tumors

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## ABSTRACT

**Purpose:** TRX518 is a mAb engaging the glucocorticoid-induced TNF receptor–related protein (GITR). This open-label, phase I study (TRX518-003) evaluated the safety and efficacy of repeated dose TRX518 monotherapy and in combination with gemcitabine, pembrolizumab, or nivolumab in advanced solid tumors.

**Patients and Methods:** TRX518 monotherapy was dose escalated (Part A) and expanded (Part B) up to 4 mg/kg loading, 1 mg/kg every 3 weeks. Parts C–E included dose-escalation (2 and 4 mg/kg loading followed by 1 mg/kg) and dose-expansion (4 mg/kg loading) phases with gemcitabine (Part C), pembrolizumab (Part D), or nivolumab (Part E). Primary endpoints included incidence of dose-limiting toxicities (DLT), serious adverse events (SAE), and pharmacokinetics. Secondary endpoints were efficacy and pharmacodynamics.

**Results:** A total of 109 patients received TRX518: 43 (Parts A+B), 30 (Part C), 26 (Part D), and 10 (Part E), respectively. A total of 67% of patients in Parts D+E had received prior anti–PD(L)1 or anti–CTLA-4. No DLTs, treatment-related SAEs, and/or grade 4 or 5 AEs were observed with TRX518 monotherapy. In Parts C–E, no DLTs were observed, although TRX518-related SAEs were reported in 3.3% (Part C) and 10.0% (Part E), respectively. Objective response rate was 3.2%, 3.8%, 4%, and 12.5% in Parts A+B, C, D, and E, respectively. TRX518 affected peripheral and intratumoral regulatory T cells (Treg) with different kinetics depending on the combination regimen. Responses with TRX518 monotherapy+anti–PD1 combination were associated with intratumoral Treg reductions and CD8 increases and activation after treatment.

**Conclusions:** TRX518 showed an acceptable safety profile with pharmacodynamic activity. Repeated dose TRX518 monotherapy and in combination resulted in limited clinical responses associated with immune activation.

*See related commentary by Hernandez-Guerrero and Moreno, p. 3905*

## Introduction

Glucocorticoid-induced TNF receptor (TNFR)-related protein (GITR; TNFRSF18/CD357/AITR) is a cell surface immune coreceptor belonging to the TNFR gene superfamily, which includes other immune costimulatory receptors, such as 4-1BB/CD137 and OX40/CD134 (1–3). GITR is constitutively expressed on Foxp3+ regulatory T cells (Treg) at high levels and at lower levels on $CD56^+$ natural killer cells. GITR is upregulated in naïve and memory T cells upon activation (1–3). The ligand for GITR is GITRL (TNFSF18), which is predominantly expressed by activated antigen-presenting cells, including dendritic cells, macrophages, and activated B cells (4, 5). GITR lacks intrinsic enzymatic activity, and upon ligation through GITRL, GITR signaling is mediated by NFκB and MAPK pathways (6, 7).

While GITR costimulation results in TRAF2/5-dependent NFκB induction and Bcl-xL upregulation in $CD8^+$ T cells, sustaining their expansion (8), its effects on Tregs are more complex. GITR ligation dampens Treg suppressive function (9–11) and attenuates the susceptibility of effector T cells to Treg-mediated suppression (12–16). In preclinical models, GITR engagement with agonist monoclonal antibody (mAb) DTA-1 resulted in Treg loss and tumor

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## Translational Relevance

Glucocorticoid-induced TNF receptor–related protein (GITR) is an immune receptor constitutively expressed on Foxp3+ regulatory T cells (Treg) and upregulated on activated T cells. TRX518 is a GITR-specific agonistic mAb. We undertook a phase I trial to evaluate safety, pharmacokinetics, pharmacodynamics, and antitumor activity of repeated dose TRX518 singly and in combination with gemcitabine or programmed death-1 inhibitors across cancer types. TRX518 was safe and well tolerated. While responses with TRX518 singly or in combination were rare, we consistently observed reductions in peripheral and, in some cases, intratumoral Tregs along with activation of intratumoral $CD8^+$ T cells. This study suggests that while TRX518 is capable of decreasing Tregs, the clinical efficacy of GITR-specific mAbs may require additional agents to effect greater Treg modulation and/or simultaneous targeting of alternative T-cell exhaustion pathways for clinical benefit.

regression (12, 17–19), although in some models, concurrent blockade of immune inhibitory coreceptors, or Fc-mediated Treg depletion was required for tumor shrinkage (13, 20).

TRX518 is a first-in-class fully humanized aglycosylated IgG1κ agonistic mAb specific for human GITR (21). The first-in-human phase I TRX518-001 study (NCT01239134) evaluated single TRX518 doses ranging from 0.0001 to 8 mg/kg, where the starting dose was based on cynomolgus monkey nonclinical safety studies, mechanistic *in vitro* experiments, pharmacokinetic, and pharmacodynamic modeling (19). We previously reported that single-dose TRX518 was safe with no reports of either dose-limiting toxicities (DLT) or related treatment-emergent adverse events (TEAE) and promising pharmacodynamic signals (19).

TRX518-003 (NCT02628574) was a phase I study wherein repeated dose TRX518 was evaluated as monotherapy, and in combination with gemcitabine, pembrolizumab, or nivolumab in adults with relapsed or refractory metastatic cancers who did not demonstrate response to standard therapies. The rationale for these combinations was based on evidence for complementary immune effects in preclinical models. Gemcitabine is an antimetabolite with direct inhibitory effects upon myeloid-derived suppressor cells (MDSC; ref. 22). Pembrolizumab and nivolumab are immune checkpoint inhibitors (ICI) that block programmed death-1 (PD-1) and reinvigorate exhausted antitumor $CD8^+$ T cells within the tumor microenvironment (23–25). Pembrolizumab and nivolumab produce durable clinical responses and are approved in multiple cancers (26–32). Here, we present final safety and efficacy data of TRX518 monotherapy and in combination with these agents.

## Patients and Methods

### Study population

Adult patients ages ≥18 years with treatment-refractory, advanced solid tumors, Eastern Cooperative Oncology Group performance status ≤1, life expectancy ≥3 months, adequate organ function, and measurable disease by RECIST v1.1 were eligible to enroll (33). Patients with prior treatment with a GITR-targeting agent were excluded. Specifically in Parts D and E, patients with known or suspected autoimmune disease and/or patients with active autoimmune disease that required systemic treatment in past 2 years were excluded although replacement therapy was permitted.

Patients were enrolled in five separate cohorts: TRX518 dose escalation (Part A), TRX518 dose expansion (Part B), TRX518+gemcitabine dose escalation (Part $C^{esc}$) and dose expansion (Part $C^{exp}$), TRX518+pembrolizumab dose escalation (Part $D^{esc}$) and dose expansion (Part $D^{exp}$), and TRX518+nivolumab dose escalation (Part $E^{esc}$) and dose expansion (Part $E^{exp}$). Enrollment to Parts C, D and E were limited to malignancies in which use of gemcitabine, pembrolizumab, and nivolumab, respectively, was considered clinically appropriate; although prior progression on anti–PD(L)1 mAb was not mandated for enrollment.

### Study design and treatment

The starting dose of TRX518 in this study was derived from the TRX518-001 study (19), and pharmacokinetic modeling that predicted that 1 mg/kg weekly would maintain peripheral GITR receptor saturation at all timepoints. Preclinical data suggested that antitumor activity required serum TRX518 to reach at least 5× the concentrations required for GITR receptor saturation peripherally. Pharmacokinetic modeling suggested that an initial loading dose of at least 2 mg/kg with subsequent dosing at 1 mg/kg every 21 days would maintain peripheral GITR receptor saturation both initially and at steady state. On the basis of these data, in Part A, patients were sequentially enrolled into one of five dose levels (1, 2 and 4 mg/kg weekly; 2 mg/kg loading followed by 1 mg/kg every 3 weeks; 4 mg/kg loading followed by 1 mg/kg every 3 weeks) whereas the subjects who received weekly doses were treated with intravenous TRX518 on days 1, 8, and 15 of each 21-day cycle. The 4 mg/kg loading followed by 1 mg/kg every 3 weeks dose was further explored in Part B. In Part C, patients were enrolled into one of two dose levels of TRX518 (2 mg/kg loading followed by 1 mg/kg every 3 weeks; 4 mg/kg loading followed by 1 mg/kg every 3 weeks) on day 2 along with gemcitabine 1,000 $mg/m^2$ on days 1 and 8 of each 21-day cycle. In Part D, patients were enrolled into one of two dose levels of TRX518 (2 mg/kg loading followed by 1 mg/kg; 4 mg/kg loading followed by 1 mg/kg) with concurrent pembrolizumab 200 mg on day 1 of each 21-day cycle. In Part E, patients were enrolled into one of two dose levels of TRX518 (same as Part D) with concurrent nivolumab 240 mg on days 1 and 15 of a 28-day cycle.

DLTs were assessed during cycle 1 of each dose level in Parts A, $C^{esc}$, $D^{esc}$, and $E^{esc}$. DLTs were defined as any grade (G) 3 or greater treatment-related hematologic or non-hematologic adverse event (AE) per Common Terminology Criteria for Adverse Events, version 4.03. The MTD was defined as the highest tested dose level of TRX518 below the DLT dose level (i.e., the dose level at which a DLT is seen in 2 or more patients). AEs were recorded for all patients. Serious AEs (SAE) were defined as any AE that was life threatening, or resulted in significant functional limitation, hospitalization, or death.

Peripheral blood (PB) samples for the assessment of anti–TRX518 antibodies and pharmacodynamic assessments were collected predose throughout treatment cycles. Pretreatment and on-treatment tumor biopsies were collected for evaluation of the tumor immune landscape.

The study was performed in accordance with the Declaration of Helsinki statement on ethical biomedical research and with the International Conference on Harmonization Guidelines for Good Clinical Practice (34). The study was approved by the local

Institutional Review Boards for each study site. All patients provided written informed consent.

### Endpoints and assessments

The primary endpoints were the incidence of DLTs, SAEs, and pharmacokinetic profile of repeated dose TRX518 monotherapy and in combination. Secondary endpoints included antitumor activity as assessed using objective response rate (ORR); presence of anti-drug antibodies (ADA); and circulating and intratumoral pharmacodynamic analyses.

Tumor response was assessed by treating investigator using RECIST v1.1 primarily (33), and secondarily by immune-related response criteria (irRC; ref. 35). Disease assessments were performed during initial patient screening (within 28 days of study entry); at the end of cycle 2, and at the end of every second cycle thereafter. Definitions of response and survival endpoints are detailed in Supplementary Data.

### Pharmacokinetics

PB samples for the assessment of TRX518 pharmacokinetics were collected predose and postdose and 1, 2, 4, and 8 hours postdose on day 1 and day 15 of the 21 (or 28) days dosing interval during treatment cycles.

Serum TRX518 levels were measured using a validated ELISA.

To evaluate for ADAs, anti-TRX518 antibodies were measured in serum using an ELISA method as published previously (36).

### Immune correlates

PB mononuclear cell (PBMC) samples were collected pretreatment and posttreatment. Flow cytometry analyses were performed on longitudinal PBMC samples from 17 patients in Parts A+B (all receiving TRX518 4 mg/kg loading), 30 patients in Part C, and 4 patients in Parts D+E. Surface and intracellular staining of human PBMCs, sample acquisition and data analysis were performed as described previously (19).

Paired pretreatment and posttreatment biopsies were available for immunofluorescence (IF) staining from 14 patients in Parts A+B (all receiving TRX518 4 mg/kg loading), 22 patients treated in Part C who had been treated for a minimum of three cycles and had undergone response assessment, and 9 patients treated in Parts D+E. IF staining was performed by the Molecular Cytology Core Facility at Memorial Sloan Kettering Cancer Center (MSKCC, New York, NY) using the Discovery XT processor (Ventana Medical Systems) as described previously (19, 37) or by the Advanced Immunomorphology Platforms at MSKCC using the Vectra Polaris imaging system (AKOYA Biosciences) and HALO software (Indica Labs) for imaging processing and quantification analyses.

### Statistical methods

Data analyses were conducted using the SAS System version 9.3 or higher (SAS Institute Inc.) in a UNIX environment and validated according to SAS Programming standards or the Prism version 9 software (GraphPad). For pharmacokinetic parameters, noncompartmental analysis was used to estimate $C_{max}$, $AUC_{\tau}$, and $t_{1/2}$ as described above. *P* values for immune modulations in longitudinal PBMC samples in responding versus nonresponding patients were calculated using two-sided unpaired *t* test. *P* values for changes in intratumoral immune cell populations in paired pretreatment and posttreatment biopsies by IF staining were calculated using two-sided paired *t* test. Statistical comparison for baseline intratumoral immune populations between patients who derived clinical benefit (CB) defined as patients with RECIST v1.1 response or stable disease (SD) lasting 120 days [+CB, progression-free survival (PFS) ≥120 days] versus. those who did not (−CB, PFS <120 days) in Part C was performed using two-sided unpaired *t* test. *P* values for survival analyses were calculated with log-rank (Mantel–Cox) test.

### Data availability

The data generated in this study are available upon request from the corresponding author.

## Results

### Patients

One hundred and nine patients with advanced solid tumors were enrolled between January 25, 2016 (first patient, first visit), and June 6, 2019 (last patient, first visit) across 7 study sites. The database was locked for final analysis on September 9, 2020.

The distribution of tumor types varied by study part (**Table 1**). In Parts A and B (TRX518 monotherapy), most patients enrolled had colorectal carcinoma (28%), melanoma (12%), and breast cancer (9%); while Part C primarily enrolled patients with pancreatic adenocarcinoma (47%) or cholangiocarcinoma (13%). Parts D and E primarily enrolled patients with choroidal melanoma (39%), non–small cell lung cancer (NSCLC, 11%), renal cell carcinoma (RCC, 8%), and cutaneous melanoma (6%). Most patients were heavily pretreated with a median of 3 prior therapies (**Table 1**). A total of 53% (16/30) of patients in Part C, and 67% (24/36) of patients in Parts D+E had progression on prior gemcitabine or ICI therapy, respectively (**Table 1**).

All 109 patients received at least one dose of TRX518 and were evaluable for safety, while 82.6% (90/109) were evaluable for efficacy per RECIST v1.1. The reasons for treatment discontinuation were withdrawal of consent (10.1%, 11/109), study termination (28.4%, 31/109), and death (60.6%, 66/109), with disease progression accounting for the latter.

### Safety

Across monotherapy arms (Parts A+B; $n = 43$), the median duration on-treatment with TRX518 was 11.4 weeks (range: 0.1–97.9 weeks). At the highest dose level (4 mg/kg loading, 1 mg/kg every 3 weeks; $n = 25$), the median duration on treatment was 11.7 weeks (range: 0.1–97.9 weeks). In the combination arms, the median duration on-treatment was 7.0 weeks with TRX518+gemcitabine (Part C, $n = 30$; range: 0.1–61.1 weeks), and 6.3 weeks with TRX518+anti–PD-1 (Parts D+E, $n = 36$; range: 0.1–65.9 weeks).

No DLTs occurred during the 21 (or 28) days DLT assessment period in cycle 1 of any part. A total of 77 patients were treated with 4 mg/kg load followed by 1 mg every 3 weeks that included 25 patients who received TRX518 monotherapy (Parts A+B), 26 patients who received TRX518+gemcitabine (Part C), and 26 patients who received TRX518+pembrolizumab (Part D) or TRX518+nivolumab (Part E).

The incidence of TEAEs was 97.7% (Parts A+B), 100.0% (Part C), 96.2% (Part D), and 100.0% (Part E), although the majority were G1 or G2 in Parts A+B, D and E, except in Part C (Supplementary Tables S1–S3). The most common (occurring in ≥5% of patients) treatment-related AEs that occurred with TRX518 monotherapy were fatigue (30.2%), vomiting (14.0%), abdominal pain (7.0%), diarrhea (7.0%), and hyponatremia (7.0%; Supplementary Table S1).

**Table 1.** Baseline characteristics of patients treated with TRX518 as monotherapy or in combination with gemcitabine, pembrolizumab, or nivolumab.

| Characteristic | Total ($N = 109$) |
|---|---|
| Median age, years (range) | 60 (28–86) |
| Age group, *n* (%) | |
| • <40 years | 4 (4%) |
| • 40–60 years | 47 (43%) |
| • ≥60 years | 58 (53%) |
| Sex, *n* (%) | |
| • Male | 47 (43%) |
| • Female | 62 (57%) |
| Race, *n* (%) | |
| • Caucasian | 98 (90%) |
| • African American | 5 (5%) |
| • Other (including Asian) | 6 (5%) |
| Baseline ECOG, *n* (%) | |
| • 0 | 58 (53%) |
| • 1 | 51 (47%) |
| Prior therapies[a] | |
| • Median (range)[a] | 3 (1, 10) |
| • Receipt of prior gemcitabine (Part C only)[b] | 16 (53%) |
| • Receipt of prior anti–PD-(L)1 and/or CTLA-4 immune therapy (Parts D/E only)[c] | 24 (67%) |
| Treatment disposition by Part, *n* (%) | |
| • Part A, all cohorts | 23 (21%) |
| • Part B | 20 (18%) |
| • Part C | 30 (28%) |
| • Part D | 26 (24%) |
| • Part E | 10 (9%) |
| Primary tumor type by Part, *n* (%) | |
| Parts A (all cohorts) + B | 43 |
| • CRC (including appendiceal and peritoneal) | 12 (28%) |
| • Melanoma | 5 (12%) |
| • Breast | 4 (9%) |
| • Other | 22 (51%) |
| Part C[b] | 30 |
| • Pancreatic adenocarcinoma | 14 (47%) |
| • Cholangiocarcinoma | 4 (13%) |
| • Other | 12 (40%) |
| Parts D + E[c] | 36 |
| • Choroidal melanoma | 14 (39%) |
| • NSCLC | 4 (11%) |
| • RCC | 3 (8%) |
| • Cutaneous melanoma | 2 (6%) |

Abbreviations: CRC, colorectal cancer; ECOG, Eastern Cooperative Oncology Group; NSCLC, non–small cell lung cancer; RCC, renal cell carcinoma.

[a]Study protocol did not place any limits on the number of prior therapies, although patients had to have received all therapies with demonstrable efficacy in any given disease (per treating investigator).

[b]16/30 (53%) of enrolled patients and 16/26 (62%) of evaluable patients in Part C had previously received gemcitabine.

[c]24/36 (67%) of enrolled patients and 24/33 (73%) of evaluable patients in Parts D and E had received prior anti–PD(L)1 or anti-CTLA-4 therapy or combination.

The AE profile of TRX518 in combination was consistent with that of individual components. We observed a 26.7% incidence of cytopenias and a 3.3%–6.7% incidence of liver function abnormalities in Part C (Supplementary Table S2). In Parts D and E, the incidence of immune-related adverse events with the addition of anti–PD-1 in Parts D and E was not higher than anticipated and included hypothyroidism (7.7%) and myositis (3.8%; Supplementary Table S3). Overall, the incidence of regimen related ≥G3 TEAE was low: 0% (Part A), 5.0% (Part B), 50.0% (Part C), 0% (Part D), 10% (Part E; Supplementary Tables S1–S3).

Three patients had fatal AEs. One patient with NSCLC treated with TRX518 monotherapy in Part A 4 mg/kg loading developed acute respiratory distress syndrome (day 31). Two patients with pancreatic adenocarcinoma treated with TRX518 4 mg/kg loading and gemcitabine in Part C separately developed perforated viscus (day 98) and cardiac arrest (day 82) in the setting of G3 esophageal infection, respectively. In all instances, the G5 events were deemed related to intercurrent illness and unrelated to study therapy.

### Antitumor activity of TRX518 monotherapy and in combination with gemcitabine or anti–PD-1

Of the 109 treated patients, 90 underwent at least one restaging study and were efficacy evaluable per RECIST v1.1. Nineteen patients did not have evaluable on-treatment scans due to clinical disease progression ($n = 13$), declining performance status ($n = 5$) and AE ($n = 1$).

In Parts A+B that explored TRX518 monotherapy, the best response observed was a confirmed partial response (PR) by RECIST v1.1 in a patient with hepatocellular carcinoma (HCC) previously treated with ipilimumab/nivolumab who remained progression free for 685 days before progressing clinically. Two patients had SD >6 months (1 each with appendiceal carcinoma and prostate adenocarcinoma). Overall, ORR to TRX518 monotherapy was 3.2% (1/31 evaluable), with a disease control rate (DCR) of 71.0% (22/31 evaluable; **Fig. 1A**; **Table 2**).

In patients treated with TRX518+gemcitabine (Part C), ORR was 3.8% (1/26 evaluable) and DCR 57.7% (15/26 evaluable; **Fig. 1B**; **Table 2**). One patient with pancreatic adenocarcinoma had a confirmed PR that lasted 428 days prior to confirmed progressive disease (PD). Five patients (one each with appendiceal carcinoma, cholangiocarcinoma, and mesothelioma, and 2 patients with pancreatic adenocarcinoma) had SD that lasted >6 months.

TRX518+anti–PD-1 combination (Parts D+E) produced modest responses: ORRs were 4.0% (1/25 evaluable) and 12.5% (1/8 evaluable), with DCRs of 32.0% (8/25 evaluable) and 50.0% (4/8 evaluable) in Parts D and E, respectively (**Fig. 1C** and **D**; **Table 2**). Across both cohorts, two confirmed responses were observed: 1 patient with anti–PD-1 naïve squamous cell cancer of the esophagus [confirmed complete response (CR)] in Part D (PFS, 446 days), and 1 patient with anti–PD-1 refractory urothelial carcinoma (confirmed PR) in Part E (PFS, 162 days), with the former ongoing at the time of data cutoff. A separate anti-CTLA-4 and tebentafusp refractory choroidal melanoma patient in Part D derived CB with durable SD (PFS, 461 days) and remains on therapy following study closure receiving therapy on a single-patient IND.

The median PFS by cohort was: 2.6 months [95% confidence interval (CI), 1.4–2.8] in Parts A+B; 1.6 months (95% CI, 1.3–3.7) in Part C; and 1.4 months (95% CI, 1.4–1.8) in Parts D+E. Median overall survival (OS) by cohort was: 10.5 months (95% CI, 5.7–15.4) in Parts A+B; 6.1 months (95% CI, 3.4–3.8.8) in Part C; and 12.5 months (95% CI, 6.0–not estimable) in Parts D+E (**Fig. 2A** and **B**).

### Pharmacokinetic analyses

Detailed pharmacokinetic data were available on 99 patients. Patients were analyzed by TRX518 dose level regardless of Part.

TRX518 exposure as estimated using mean $C_{max}$ increased in a dose proportional fashion (Supplementary Fig. S1). Noncompartmental analyses were limited to 2 ($n = 18$) and 4 mg/kg ($n = 66$) loading dose levels after excluding every 1 week patients and additional

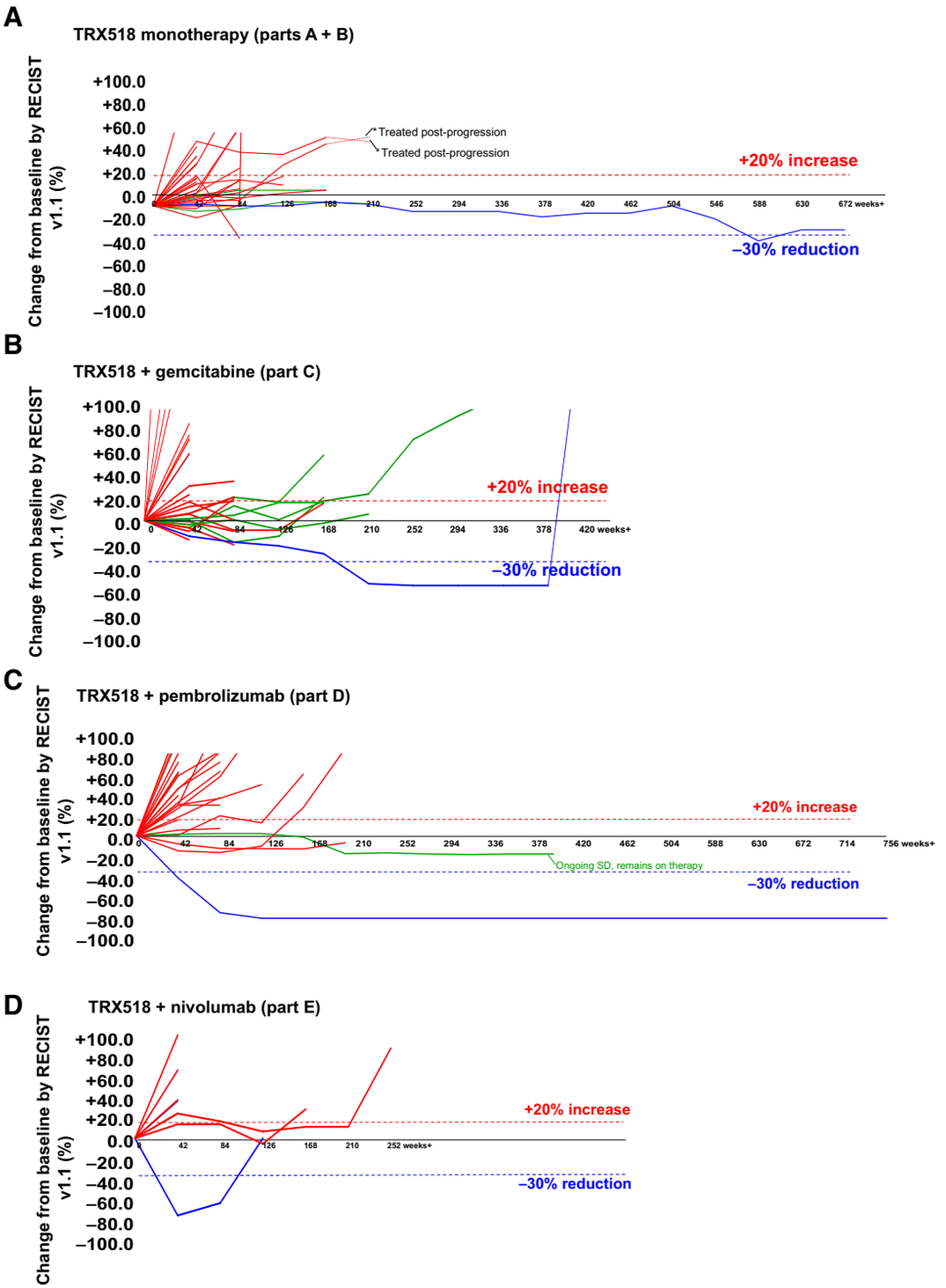


**Figure 1.**

Radiographic change of tumor burden from baseline for TRX518 as monotherapy or in combination with gemcitabine, pembrolizumab, and nivolumab—efficacy analysis set. Evaluation of response to therapy with repeated dose TRX518 monotherapy or TRX518 combinations with gemcitabine or anti–PD-1 is shown. Objective radiographic response was evaluated every 6–8 weeks using RECIST v1.1 (33), and progression was defined on the basis of radiographic or clinical progression at each treatment visit (every 2 or 3 weeks depending on cohort). Data shown are from all evaluable patients ($n = 90$) separated by study Part. TRX518 monotherapy (Parts A+B, $n = 31$ of 43 evaluable; **A**), TRX518 + gemcitabine (Part C, $n = 26$ of 30 evaluable; **B**), TRX518 + pembrolizumab (Part D, $n = 25$ of 26 evaluable; **C**), and TRX518 + nivolumab (Part E, $n = 8$ of 10 evaluable; **D**). Line color indicates response status at the time of data cutoff (blue, CR/PR; red, PD; and green, SD).

**Table 2.** ORR by RECIST v1.1 and irRECIST and DCR to TRX518 as monotherapy or in combination with gemcitabine, pembrolizumab, or nivolumab—efficacy analysis set.

| | TRX518 monotherapy | TRX518 combination therapy | | |
|---|---|---|---|---|
| | Parts A + B | Part C (TRX518 + gemcitabine) | Part D (TRX518 + pembrolizumab) | Part E (TRX518 + nivolumab) |
| No. of evaluable patients | 31 | 26 | 25 | 8 |
| RECIST v1.1 | | | | |
| CR/PR | 1 | 1 | 1 | 1 |
| SD | 21 | 14 | 7 | 3 |
| PD | 9 | 11 | 17 | 4 |
| irRC | | | | |
| irPR/iRCR | 0 | 1 | 1 | 1 |
| irSD | 20 | 10 | 7 | 3 |
| irPD | 11 | 7 | 17 | 4 |
| ORR, best (RECIST v1.1) | 3.2% | 3.8% | 4.0% | 12.5% |
| DCR (RECIST v1.1) | 71.0% | 57.7% | 32.0% | 50.0% |
| ORR (irRC) | 0.0% | 3.8% | 8.0% | 12.5% |
| Prior gemcitabine exposure in evaluable patients, *n* (%) | N/A | 16 (61.5%) | N/A | N/A |
| • ORR, best (RECIST v1.1) | | 1 (6.3%) | | |
| • DCR (RECIST v1.1) | | 8 (50.0%) | | |
| Prior ICI exposure in evaluable patients, *n* (%) | N/A | N/A | 24 (72.7%) | |
| • ORR, best (RECIST v1.1) | | | 1 (4.2%) | |
| • DCR (RECIST v1.1) | | | 6 (29.2%) | |
| • ORR (irRC) | | | 1 (4.2%) | |

patients (3) who had few post-$T_{max}$ data points, which rendered parameter estimates unreliable. TRX518 pharmacokinetic profile exhibited a pattern consistent with target-mediated drug disposition with significantly greater $C_{max}$ (88,039.4 ± 21,712.6 mg/mL vs. 43,780.1 ± 8,658.1 mg/mL, $P < 0.0001$) and $t_{1/2}$ (29.9 vs. 41.7 days, $P = 0.7404$) at 4 mg/kg loading compared with 2 mg/kg loading dose levels. On the basis of a comparison of AUCτ and $C_{max}$ and linear regression analysis of dose-normalized log-transformed AUCτ/$C_{max}$ values, we observed that TRX518 exposure increased in a dose-proportional manner over the entire dose range with slightly greater exposure with weekly than with loading schedules. Comparing 4 mg/kg loading with 2 mg/kg loading, we observed that $C_{max}$, $t_{1/2}$, and AUC were greater with the former, suggesting that this was the optimal dose and schedule (Supplementary Fig. S1; Supplementary Table S4).

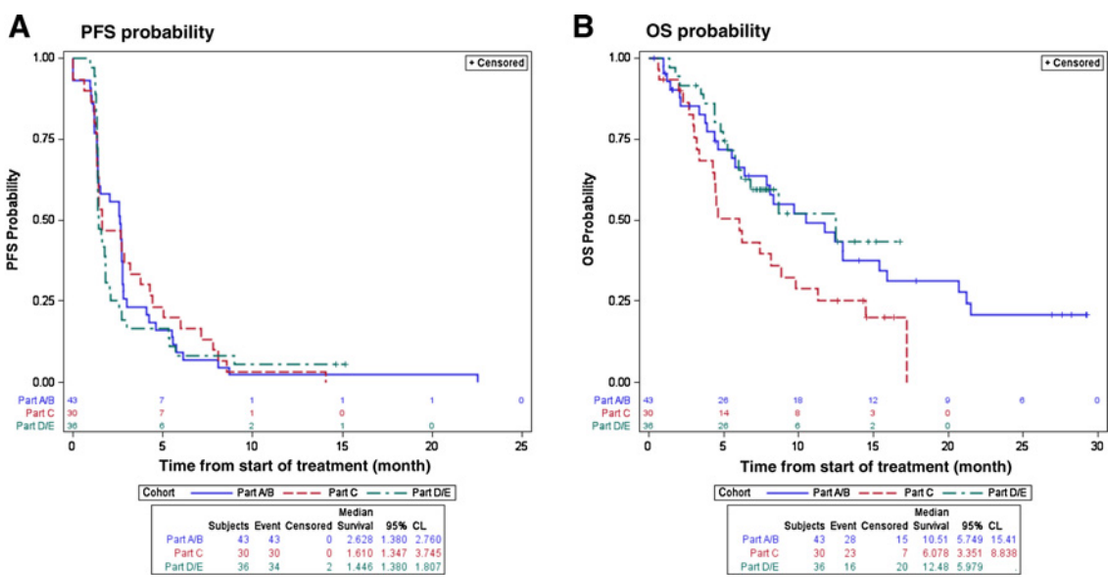


**Figure 2.**
PFS and OS for TRX518 as monotherapy or in combination with gemcitabine, pembrolizumab, or nivolumab—efficacy analysis set. PFS (**A**) and overall survival (OS; **B**) of patients treated in TRX518-003 are shown. PFS was defined as time from treatment initiation (C1D1 date) to clinical/radiographic progression, while OS was defined as time from treatment initiation (C1D1 date) to date of death. Percentages are the proportion of patients with survival at that timepoint. Numbers of patients at risk at each timepoint are shown above the *x*-axis. Tick marks indicate censored patients who had not experienced a PFS or OS event at the time of last follow-up.

Following the initial infusion of TRX518 on cycle 1 day 1 (C1D1; Parts A+B), mean clearance estimates ranged from 0.170 to 0.291 mL/hour/kg across the dose range of 1 to 4 mg/kg, and there was no consistent trend with respect to dose. Mean volume of distribution (*V*ss) estimates ranged from 47.1 to 73.9 mL/kg and were generally within approximately 1.7-fold of human serum volume (~43 mL/kg). For patients in cohorts 1–3 on a every 1 week dosing schedule, the resultant mean $t_{1/2}$ estimates ranged from 147 to 195 hours or up to approximately 8 days (Part A). For the every 3 weeks patients in cohorts 4–5 after an initial loading dose of TRX518, the resultant mean $t_{1/2}$ estimates ranged from 244 to 281 hours or up to approximately 12 days (Parts A and B).

ADA responses to TRX518 were observed in 173 samples from a total of 47 subjects (total 813 samples) with titers ranging from 1:23 to 1:5,888. The pharmacokinetic exposure of TRX518 was similar between patients with and without ADA, although given the limited number of patients upon which this was assessed, we could not reliably impute the effects of ADA upon TRX518 exposure. There was no relationship between ADA positivity and the occurrence of infusion-related reactions.

TRX518 at 0.0001 to 8 mg/kg resulted in dose-proportional modulation of GITR on human PBMCs (19). TRX518 doses > 0.5 mg/kg (mean $C_{max}$ 10.0 ± 2.04 μg/mL) resulted in serum trough coverage needed for 95% *in vitro* receptor occupancy on human PBMC and was confirmed in subsequent dose-escalation cohorts at 1.0, 2.0, 4.0, and 8.0 mg/kg (data not shown).

### Immune correlates of TRX518 monotherapy and in combination with gemcitabine or anti–PD-1 in PB

We previously reported that single-dose TRX518 monotherapy caused dose-dependent reductions in $GITR^+$ Tregs peripherally, with less consistent effects upon $GITR^+CD8^+$ T cells and $GITR^+CD4^+Foxp3^-$ T effector cells (19). In addition, we found that GITR marks $Foxp3^{hi}CD45RA^-CD4^+$ effector Tregs (eTreg) and accordingly, eTregs were more profoundly downregulated than $Foxp3^{low}CD45RA^+CD4^+$ naïve Tregs by TRX518 (19). Here, we tested similar pharmacodynamic effects in association with the clinical outcome in patients treated with repeated dose TRX518 monotherapy (Parts A+B) and in combination with gemcitabine (Part C) or anti–PD-1 (Parts D+E).

Similar to our prior study, patients in the highest-dose monotherapy cohorts (Parts A+B, 4 mg/kg loading followed by 1 mg/kg every 3 weeks; $n = 17$; Supplementary Table S5) showed general reductions in peripheral Tregs, eTregs, and $GITR^+$ Tregs, with few exceptions (Supplementary Fig. S2A). Notably, at the 4 mg/kg load followed by 1 mg every 3 weeks dose level, patients achieving SD experienced more substantial decreases in peripheral Tregs and eTregs than patients with PD (**Fig. 3A**). However, the fraction of $GITR^+$ Tregs, which ranged from 8.32% to 41.80% at baseline in these patients, was consistently reduced in all patients, and to an even greater extent in PD (compared with SD) patients (**Fig. 3A**). This suggests that Tregs (and not just the fraction of $GITR^+$ Tregs) may need to be further depleted to induce clinical responses and/or that additional biologic changes are needed to impact tumor control.

We observed similar reductions in total Tregs, eTregs, and $GITR^+$ Tregs in patients treated in Part C ($n = 30$; Supplementary Table S5) with the TRX518+gemcitabine combination (**Fig. 3B**). However, these effects followed different kinetics, where gemcitabine on days 1 and 8 led to Treg and eTreg reductions, and TRX518 on day 2 induced Treg and eTreg expansion (**Fig. 3B**). While gemcitabine-induced eTreg reduction and TRX518-induced $GITR^+$ Tregs reduction appeared more pronounced in SD/PR versus PD patients, these effects were not sustained (**Fig. 3B**). This could not be attributed to differences in baseline GITR expression on Tregs, as the frequency of GITR+ Tregs was similarly elevated (average, 41.82%, range: 9.11%–100.00%). In contrast, we observed that TRX518 administered after gemcitabine induced proliferation bursts in T cells, including Tregs (Supplementary Fig. S2B), which may explain the different kinetics of Treg modulations in these patients.

The combination of TRX518+anti–PD-1 had less consistent effects on these Treg subsets (**Fig. 3C**), with a CR patient showing increases in Tregs and eTregs but stable $GITR^+$ Tregs on day 8 (the only timepoint available for analyses) and a PD patient showing decreases in Tregs and eTregs but continuous increases in $GITR^+$ Tregs over time. In these patients, GITR was expressed in a substantial fraction of Tregs at baseline (average, 36.63%, range: 12.25%–54.95%). Previous studies have reported the ability of PD-1 blockade to induce proliferation and expansion of Tregs in addition to $CD8^+$ T cells (38–40), which could explain the inconsistent Treg reductions upon TRX518 in combination with PD-1 blockade in these patients. Accordingly, the TRX518+anti–PD-1 combination induced Ki67 upregulation in both Treg and $CD8^+$ T cells in the CR patient while the PD patients showed maximal and continuous Ki67 increases in Tregs (Supplementary Fig. S2C). In addition, we noted that the CR patient started treatment with the greatest number of circulating $CD4^+$ and $CD8^+$ T cells with an effector memory phenotype (Supplementary Fig. S2D). However, given the small numbers of patients with available samples in Parts D+E ($n = 4$; Supplementary Table S5), these results must be interpreted cautiously.

### Immune correlates of TRX518 monotherapy and in combination with gemcitabine or anti–PD-1 at the tumor site

In our prior study, we found that TRX518 decreased intratumoral Tregs, and that in preclinical melanoma models refractory to GITR agonist mAb monotherapy, GITR costimulation combined with PD-1 blockade resulted in antitumor activity that was linked to concomitant Treg reduction and intratumoral $CD8^+$ T-cell activation (19). Here, using multiplex IF, we sought to characterize the immune infiltrate from paired pretreatment and on-treatment biopsies in patients treated with TRX518 monotherapy (Parts A+B) and in combination with gemcitabine (Part C) or PD-1 blockade (Parts D+E) in relation to the clinical outcome.

In 7 of 13 evaluable patients treated at 4 mg/kg loading followed by 1 mg/kg every 3 weeks in Parts A+B (Supplementary Table S5), intratumoral Treg decreased posttreatment (**Fig. 4A**; Supplementary Fig. S3A). Notably, among these patients, the patient with PR showed the most substantial increase in $CD8^+$ tumor-infiltrating lymphocytes (TIL), which displayed stronger cytolytic profiles after treatment, as indicated by granzyme B upregulation (**Fig. 4A**; Supplementary Fig. S3A).

Of the patients treated with TRX518+anti–PD-1 in Parts D+E, paired biopsies were available from 9 patients (TRX518+pembrolizumab, 5; TRX518+nivolumab, 4; Supplementary Table S5), including 3 patients who achieved response or +CB as defined above (#30000092, CR; #71220094, PR; and #93490091, +CB). Treg staining was successfully performed in all nine cases, while $CD8^+$ staining was available in seven cases. While all but one nonresponder had increased or stable intratumoral Tregs, both responding patients had profound intratumoral Treg reductions, and the +CB patient had stably low Treg levels (**Fig. 4B** and **C**). Notably, all 3 patients with response/

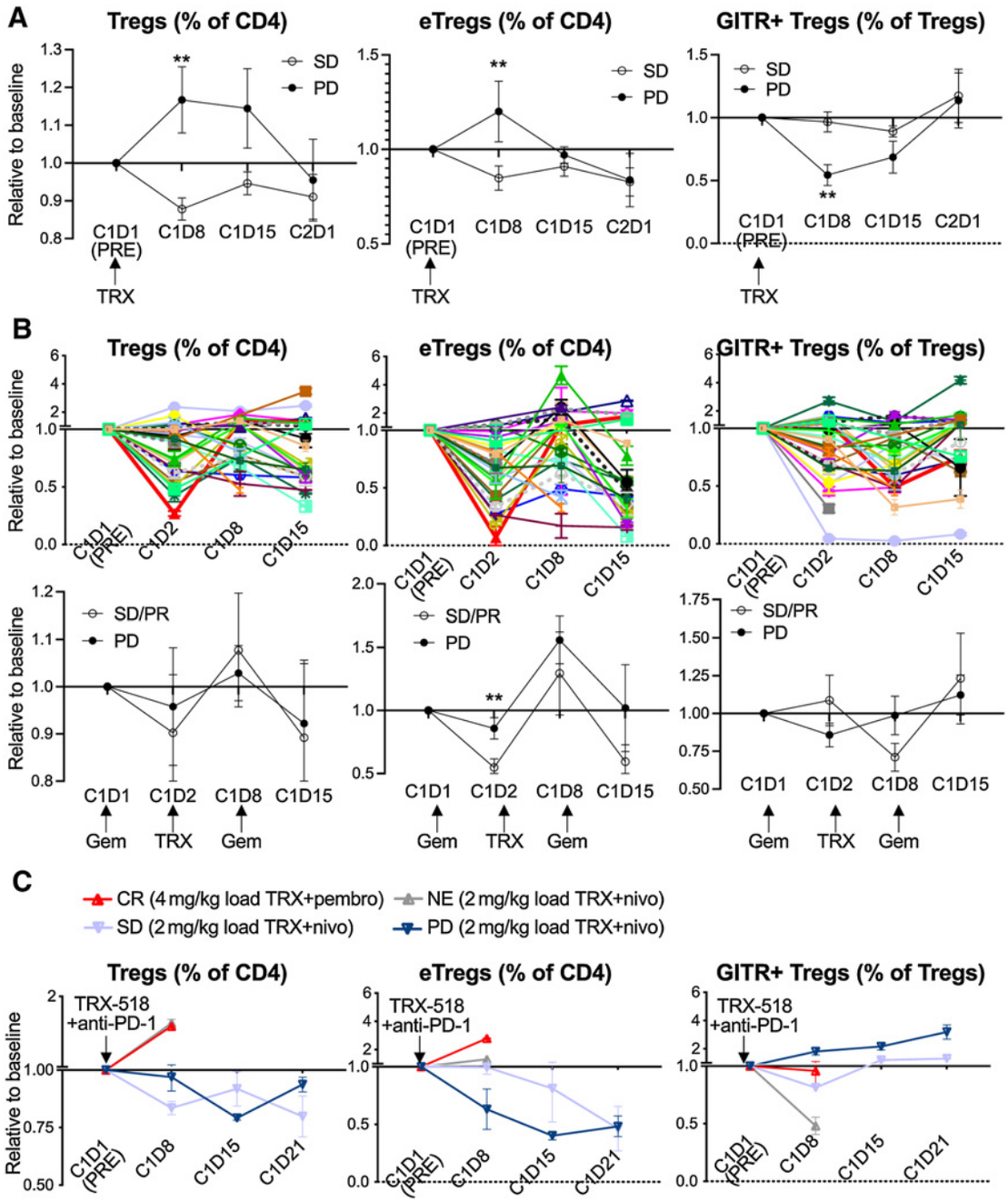


**Figure 3.**

Peripheral Treg modulations during TRX518 treatment as monotherapy and in combination with gemcitabine or PD-1 blockade. **A,** Fold changes in the indicated circulating Treg subsets by flow cytometry (left, Foxp3$^+$CD4$^+$CD3$^+$ Tregs, percentage of live single CD3$^+$CD4$^+$ T cells; middle, CD45RA$^{low}$Foxp3$^{hi}$CD4$^+$CD3$^+$ eTregs, percentage of live single CD4$^+$CD3$^+$ T cells; right, GITR$^+$ Tregs, percentage of live single Foxp3$^+$CD4$^+$CD3$^+$ Tregs) at the indicated timepoints during C1 of treatment with TRX518 monotherapy at 4 mg/kg loading dose relative to baseline (C1D1, predose) in patients experiencing SD ($n = 11$) or PD ($n = 5$) in Parts A+B. **B,** Fold changes in the same Treg subsets as in **A** in PB from patients treated in Part C with TRX518+gemcitabine at the indicated timepoints relative to baseline (top, individual patients, $n = 30$; bottom, patients evaluable for response and grouped by SD/PR, $n = 15$ and PD, $n = 11$). **C,** Fold changes at the indicated timepoints after treatment relative to baseline of the same Treg subsets as in **A** in PB from patients treated in Parts D+E with TRX518/anti–PD-1. Data are mean ± SEM in patients grouped by response (**A**; **B**, bottom) or mean ± SD of technical replicates per patient (**B**, top; **C**). Two-sided unpaired *t* test: *, $P < 0.05$; **, $P < 0.01$. NE, nonevaluable for response; Gem, gemcitabine; TRX, TRX518; pembro, pembrolizumab; nivo, nivolumab.

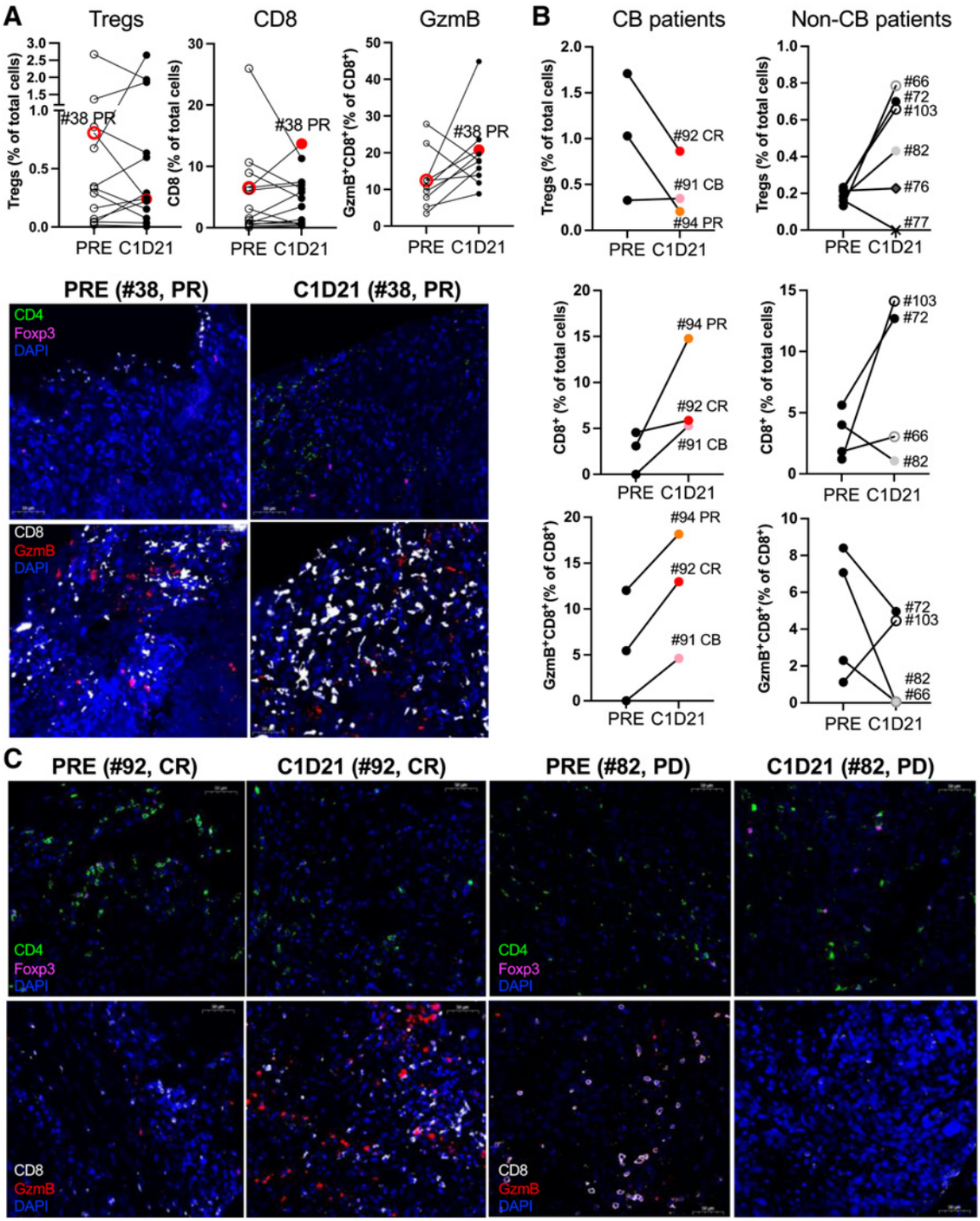


**Figure 4.**

Intratumoral immune correlates in TRX518-treated patients. **A,** Quantification (top) and representative immunofluorescent staining images (bottom) of Foxp3$^+$CD4$^+$ Tregs ($n$ = 13 evaluable cases), CD8$^+$ TILs ($n$ = 14 evaluable cases), and granzyme B (GzmB)$^+$CD8$^+$ TILs ($n$ = 10 evaluable cases) in paired pretreatment and posttreatment [cycle 1 day 21 (C1D21)] biopsies from patients treated with TRX518 monotherapy at 4 mg/kg loading dose in Parts A+B. Red, PR patient. Quantification (**B**) and representative immunofluorescent staining images (**C**) of Foxp3$^+$CD4$^+$ Tregs ($n$ = 9 evaluable cases), CD8$^+$ TILs, and GzmB$^+$CD8$^+$ TILs ($n$ = 7 evaluable cases) in paired pretreatment and posttreatment (C1D21) biopsies from patients with versus without CB treated with TRX518+anti–PD-1 in Parts D+E. Scale bar, 50 μm; 20× original magnification.

+CB had increases in both total $CD8^+$ TILs and in the proportion of cytolytic granzyme B+ $CD8^+$ TILs, indicating concomitant Treg stabilization and increased $CD8^+$ TIL numbers and activation in these patients (**Fig. 4B** and **C**). In contrast, these effects were never concordant in the nonresponder patients that were also evaluable for $CD8^+$ TIL staining (**Fig. 4B** and **C**). These results together with the findings with TRX518 monotherapy indicate that concomitant decreases in intratumoral Tregs and increases in $CD8^+$ TILs and/or their activation status were associated with response or CB.

In Part C ($n = 21$; Supplementary Table S5), we found that patients with +CB upon TRX518+gemcitabine tended to have greater infiltration with $CD4^+$ and $CD8^+$ TILs and lower proportions of Foxp3-expressing cells in $CD4^+$ TILs at baseline (Supplementary Fig. S3B). However, in Part C, we did not find consistent changes in these subsets after treatment even when patients were stratified either based on 120-day PFS +CB (Supplementary Fig. S3B) or response (data not shown). We then evaluated intratumoral GITR expression in these patients, and interestingly, we found increases in $GITR^+$ Treg infiltration upon treatment with TRX518+gemcitabine selectively in +CB patients (Supplementary Fig. S3B). This may be in line with the effects of TRX518 administered after gemcitabine inducing Treg proliferation as detected peripherally (Supplementary Fig. S2B).

## Discussion

GITR agonists have demonstrated antitumor activity singly and in combination with anti–PD-1 in a variety of tumor models preclinically (13, 20, 41–45). Several GITR agonists have been evaluated in the clinic although antitumor activity observed thus far has been limited (46–49). In this phase I study, the GITR agonist TRX518 was well tolerated at doses up to 4 mg/kg loading followed by 1 mg every 3 weeks. No DLTs were observed with TRX518 monotherapy (Parts A+B) and/or in combination with gemcitabine, pembrolizumab, or nivolumab (Parts C–E). TRX518-related AEs were generally mild or moderate in severity, as expected based on early clinical studies of this and other GITR agonists (19, 41–43, 46–50). There were no treatment-related G4 or G5 AEs and the immunogenicity profile was acceptable.

Interestingly, TRX518 monotherapy resulted in one confirmed PR in a heavily pretreated patient with HCC that lasted 685 days, in contrast to prior studies of GITR agonists wherein no confirmed responses were reported with single-agent therapy (46–49). While some patients who derived prolonged SD with TRX518 monotherapy had cancers with typically indolent courses including appendiceal carcinoma, the DCR rate in Parts A+B was 75.0% and included prolonged SD in otherwise aggressive histologies including colorectal carcinoma, prostate adenocarcinoma, and ovarian adenocarcinoma, all of whom had progressed rapidly on previous treatment. Overall, these data suggest that the immunomodulatory effects of TRX518 monotherapy may induce some tumor control, which is not sufficient to produce robust antitumor responses in heavily pretreated patients.

The TRX518+gemcitabine combination resulted in one confirmed objective response and prolonged SD in 5 patients that included patients with aggressive histologies and prior progression on gemcitabine (**Table 2**). The combination with anti–PD-1 produced two objective responses (one CR and one PR), and prolonged SD in 4 patients including some that had progressed on prior ICI therapy (**Table 2**). The objective responses were observed in both anti–PD-1 naïve [esophageal squamous cell carcinoma (#30000092, CR)] and anti–PD-1 refractory patients [urothelial carcinoma (#71220094, PR)], and durable SD was noted in a choroidal melanoma patient (#93040151) who had progressed on ipilimumab and tebentafusp. GITR engagement with TRX518-induced robust pharmacodynamic effects peripherally, decreasing Tregs, eTregs, and $GITR^+$ Tregs in most cases. While decreases in GITR+ Tregs occurred independently of therapeutic outcome, reductions in total and eTregs were more pronounced in patients achieving at least SD in Parts A+B. In Parts C–E, these effects were less consistent, possibly attributed to induction of Treg proliferation upon gemcitabine or anti–PD-1 (38–40). In general, Treg modulation peripherally occurred early, and reflected TRX518 pharmacodynamic effects; while posttreatment changes in tumor biopsies were better associated with therapeutic outcome, with concomitant Treg reductions and $CD8^+$ T-cell activation occurring in patients achieving CB.

The half-life of TRX518 is intermediate between other GITR agonists including MEDI1873, MK-4166, MK-1248, BMS-986156, and AMG-228 (46–50). At the doses tested, we consistently observed ≥95% GITR occupancy on circulating T cells despite low to moderate ADA titers. Given the activity of TRX518 singly and in combination, one potential explanation is that ADA affecting pharmacokinetics may in turn affect the pharmacodynamic response. This might occur by simply reducing the amount of drug exposure, resulting in a lower pharmacodynamic response. However, not all subjects with ADA affecting pharmacokinetics were found to also have unusual pharmacodynamics, suggesting that TRX518 ADA could have separate effects on pharmacokinetics and GITR pharmacodynamics. Whether ADA leading to increased drug clearance can also bind to (or cover) TRX518-targeted GITR epitopes remains unknown. The effect of ADA on GITR-binding warrants precise investigation for further clinical development of TRX518.

The overall AE profile and limited activity of TRX518 are consistent with data on GITR agonists including MEDI1873, MK-4166, MK-1248, BMS-986156, and AMG-228 studied singly or in combination with PD-1 inhibitors in patients with advanced solid tumors (46–50). Of these, TRX518 is most similar to other bivalent and multivalent GITR agonists such as MK-1248, BMS-986156, and AMG-228; and is structurally distinct from MEDI1873 (a hexameric GITRL molecule with human IgG1 Fc), and MK-4166 (humanized Fc intact IgG1 surrogate of the anti-mouse GITR Ab DTA-1). While no single-agent activity was noted with MK-4166, MK-1248, BMS-986156, and AMG-228, both TRX518 and MEDI1873 were notable for inducing objective responses (albeit unconfirmed with MEDI1873) in heavily pretreated patients as monotherapy.

Recent data underscore the importance of intact fragment crystallizable (Fc) region that permits Fc gamma receptor (FcγR) coengagement and in turn, FcγR-mediated clustering and cross-linking which mediates the therapeutic activity of mAb-targeting TNFR superfamily members including CD40 (51–53), in contrast to the FcγRIIIA coengagement which appears critical to the function of CTLA-4 and TIGIT-directed mAbs (54). While we observed consistent reductions in peripheral and in some cases intratumoral Tregs with TRX518, it remains unclear whether mAbs designed to effect greater Treg depletion would have produced more substantial antitumor effects. Conversely, an approach engaging both PD-1 and GITR-L has demonstrated PD-1–dependent and FcγR-independent GITR clustering (55), suggesting that a bispecific approach may overcome suboptimal TNFR clustering with FcγR-engaging mAbs.

Deeper mechanistic understanding of GITR-targeting agents has clarified the distinct dimeric nature of murine GITR-L (56, 57),

compared with trimeric human GITR-L (58)—underscoring the importance of GITR oligomerization for downstream costimulatory signaling. TRX518 achieves similar levels of human GITR oligomerization and signaling as functional anti-mouse GITR Abs (59), which cannot be achieved with their respective Fab versions, supporting the need of full anti-GITR IgG molecules to induce GITR signaling and downstream immune effects.

In summary, TRX518 has an acceptable safety profile with encouraging pharmacodynamic changes in blood and tumor-producing confirmed responses both as monotherapy and in combination with gemcitabine or PD-1 blockade. Discussion regarding further development of TRX518 is ongoing.

## Authors' Disclosures

D. Davar reports personal fees from Alpine Immune Sciences, Ascendis, Finch, Shionogi, and Vedanta Biosciences; grants and personal fees from Checkmate Pharmaceuticals; and grants from Arcus, CellSight Technologies, Immunocore, Merck, and GSK outside the submitted work; in addition, D. Davar has US patents 63/124,231 and 63/208,719 pending. R. Zappasodi reports personal fees from Leap Therapeutics during the conduct of the study as well as personal fees from iTeos outside the submitted work; in addition, R. Zappasodi has patent US20180244793A1 issued and receives grant support from AstraZeneca and Bristol Myers Squibb. G.S. Naik reports other support from Leap Therapeutics during the conduct of the study as well as other support from Merck, Zomedica, Vaxart, and Tonix Pharmaceuticals outside the submitted work; in addition, G.S. Naik has a patent for Leap Therapeutics pending. T. 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Merghoub has patent WO-2017096276 issued to Incyte; is a cofounder and holds equity in IMVAQ Therapeutics; is a consultant of Immunos Therapeutics, ImmunoGenesis, and Pfizer; has research support from Bristol-Myers Squibb, Surface Oncology, Kyn Therapeutics, Infinity Pharmaceuticals Inc., Peregrine Pharmaceuticals Inc., Adaptive Biotechnologies, Leap Therapeutics Inc., and Aprea; and has patents on applications related to work on oncolytic viral therapy, alpha virus–based vaccine, neoantigen modeling, CD40, GITR, OX40, PD-1, and CTLA-4. J.D. Wolchok reports grants and personal fees from Bristol Myers Squibb during the conduct of the study as well as personal fees from AstraZeneca, Bicara Therapeutics, Boehringer Ingelheim, Dragonfly, Georgiamune, Imvaq, Larkspur, Maverick Therapeutics, Psioxus, Recepta, and Sellas outside the submitted work. In addition, J.D. Wolchok has a patent for alphavirus replicon particles expressing TRP2 issued; a patent for Newcastle disease viruses for cancer therapy with royalties paid; a patent for anti-PD1 antibody licensed to Agenus; a patent for anti-CTLA4 antibodies licensed to Agenus; a patent for anti-GITR antibodies and methods of use thereof licensed to Agenus/Incyte; a patent for xenogeneic DNA vaccines with royalties paid from Meriel; a patent for MDSC assay with royalties paid from CellCarta; a patent for recombinant poxviruses for cancer immunotherapy pending; a patent for engineered vaccinia viruses for cancer immunotherapy pending; a patent for anti-CD40 agonist mAb fused to monophosphoryl lipid A (MPL) for cancer therapy pending; a patent for CAR-T cells targeting differentiation antigens as means to treat cancer pending; a patent for identifying and treating subjects at risk for checkpoint blockade therapy–associated colitis pending; a patent for immunosuppressive follicular helper-like T cells modulated by immune checkpoint blockade pending; a patent for phosphatidylserine targeting agents and uses thereof for adoptive T-cell therapies pending; a patent for heteroclitic cancer vaccines pending; a patent for recombinant poxviruses for cancer immunotherapy pending; and equity in Apricity, CellCarta, Ascentage, BeiGene, Imvaq, Linneaus, Georgiamune, Maverick, Tizona Pharmaceuticals, and Trieza. J.J. Luke reports service on data and safety monitoring boards of AbbVie, Immutep, and Evaxion; is a scientific advisory board member of (no stock) 7 Hills, Bright Peak, Exo, Fstar, Inzen, RefleXion, Xilio (stock), Actym, Alphamab Oncology, Arch Oncology, Kanaph, Mavu, NeoTx, Onc.AI, OncoNano, Pyxis, STipe, and Tempest; reports consultancy with (compensation) AbbVie, Alnylam, Bayer, Bristol-Myers Squibb, Castle, Checkmate, Codiak, Crown, Day One, Duke St, EMD Serono, Endeavor, Flame, Genentech, Gilead, HotSpot, Kadmon, Janssen, Ikena, Immunocore, Incyte, Macrogenics, Merck, Mersana, Nektar, Novartis, Partner, Pfizer, Regeneron, Servier, STINGthera, Synlogic, and Synthekine; receives research support from (all to institution for clinical trials unless noted) AbbVie, Astellas, AstraZeneca, Bristol-Myers Squibb [investigator-initiated (IIT) and industry trials], Corvus, Day One, EMD Serono, Fstar, Genmab, Ikena, Immatics, Incyte, Kadmon, KAHR, Macrogenics, Merck, Moderna, Nektar, Next Cure, Numab, Palleon, Pfizer (IIT and industry), Replimmune, Rubius, Servier (IIT), Scholar Rock, Synlogic, Takeda, Trishula, Tizona, and Xencor; and reports patents (both provisional) 15/612,657 (cancer immunotherapy) and PCT/US18/36052 (microbiome biomarkers for anti–PD-1/PD-L1 responsiveness: diagnostic, prognostic and therapeutic uses thereof). No disclosures were reported by the other authors.

## Authors' Contributions

**D. Davar:** Data curation, formal analysis, supervision, investigation, visualization, writing–original draft, writing–review and editing. **R. Zappasodi:** Formal analysis, investigation, visualization, writing–original draft, writing–review and editing. **H. Wang:** Formal analysis, visualization, writing–original draft, writing–review and editing. **G.S. Naik:** Conceptualization, resources, funding acquisition, methodology, writing–original draft, project administration, writing–review and editing. **T. Sato:** Supervision, investigation, writing–review and editing. **T. Bauer:** Supervision, investigation, writing–review and editing. **D. Bajor:** Supervision, investigation, writing–review and editing. **O. Rixe:** Supervision, investigation, writing–review and editing. **W. Newman:** Conceptualization, resources, funding acquisition, methodology, writing–original draft, project administration, writing–review and editing. **J. Qi:** Formal analysis, supervision, investigation. **A. Holland:** Formal analysis, supervision, investigation. **P. Wong:** Formal analysis, supervision, investigation. **L. Sifferlen:** Conceptualization, resources, funding acquisition, methodology, writing–original draft, project administration, writing–review and editing. **D. Piper:** Conceptualization, resources, funding acquisition, methodology, writing–original draft, project administration, writing–review and editing. **C.A. Sirard:** Conceptualization, resources, funding acquisition, methodology, writing–original draft, project administration, writing–review and editing. **T. Merghoub:** Supervision, investigation, writing–original draft, writing–review and editing. **J.D. Wolchok:** Supervision, investigation, writing–original draft, writing–review and editing. **J.J. Luke:** Supervision, investigation, writing–original draft, writing–review and editing.

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## Note

Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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