Biomarkers associated with blockade of prostaglandin E2 signaling by TPST-1495: a novel, dual-antagonist of E-Prostanoid receptors 2 and 4

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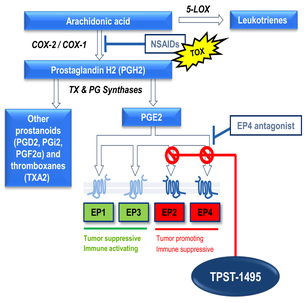
BACKGROUND

Figure 1. Prostaglandin E2 (PGE2) signaling



Prostaglandin E2 (PGE2) Signaling

- COX-2/PGE2 implicated in development and progression of multiple cancer types[1,2]
- Limitations of therapeutic COX inhibition include blocking of beneficial receptors and association with cardiovascular toxicity due to alterations in prostanoids, thromboxane, and leukotriene levels
- PGE2 elevated in tumor microenvironment
  - Mediates tumor proliferation and angiogenesis
  - Facilitates immune evasion via inhibiting myeloid and lymphoid cell effector functions and inducing immune suppressive populations
- PGE2 immune suppressive function associated with binding to E-prostanoid receptors 2 and 4 (**EP2** & **EP4**) and downstream signaling characterized by cAMP production

TPST-1495: A Novel, Specific, Potent, Dual-Antagonist of EP2 and EP4

- Anti-tumor activity, reduced immune suppression, and activated immune effector responses in syngeneic tumor models
- Dual EP2/EP4 inhibition is more active than single EP2 or EP4 antagonists or COX-2 inhibition
- A first-in-human Phase I trial of TPST-1495 as a single agent and in combination with pembrolizumab in 74 patients with treatment-refractory advanced solid tumors showed[3]:
  - Disease control rates of 37.5% for monotherapy and 40.9% for combination, with tumor shrinkage and prolonged stable disease (SD), including a confirmed partial response in a combination therapy patient with microsatellite-stable (MSS) colorectal carcinoma (CRC), an indication not normally responsive to immuno-oncology therapy
  - Manageable safety profile with once daily (QD) dosing; related AEs were predominantly grade 1-2 (no grade 4-5), gastrointestinal in nature, and on-target for the prostaglandin pathway

OBJECTIVE

To characterize TPST-1495 pharmacodynamic activities by quantifying biomarkers associated with EP2 and EP4 blockade in pre- and post-treatment specimens, testing the hypotheses that TPST-1495 can:

1) Reverse PGE2-mediated immune suppression, measured by lipopolysaccharide (LPS)-stimulated production of TNF-α in whole blood samples of patients
2) Increase systemic levels of PGE2, quantified with urinary tetranor PGE2 metabolite (PGEM) as a surrogate marker
3) Increase intra-tumoral CD8⁺ and granzyme B⁺ T cells

METHODS

Study Design (NCT04344795)

- TPST-1495 was dosed at 15, 25 or 50 mg BID or QD (continuous or days 1-5 every 7 days) as monotherapy or QD continuously in combination with pembrolizumab 200 mg IV q3 weeks (**Figure 2**)
- Key Eligibility Criteria
  - Metastatic or unresectable cancer w/ no remaining standard therapy known to confer clinical benefit
  - ECOG PS 0 or 1
  - Measurable disease (RECIST v1.1)
  - Excluded if intolerant to NSAIDs (including bleeding, ulcer), on anticoagulation therapy or at increased risk for bleeding, or experienced intolerable or unresolved immune-related adverse events (AEs) with prior checkpoint inhibitor therapy
- Study Objectives

  1°: Safety & tolerability, MTD, RP2D & schedule

  2°: Anti-tumor activity, pharmacokinetics (PK)

  Exploratory: Pharmacodynamics

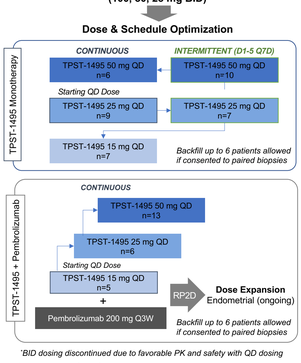


Figure 2. Study schema

Pharmacokinetics

- An HPLC-MS/MS method was developed and validated to determine concentrations of TPST-1495 in human K_2-EDTA plasma (Alturas Analytics, Inc.)
- The peak area of the product ion of TPST-1495 was measured against the peak area of the product ion of an internal standard

Inhibition of PGE2-mediated immune suppression

- Whole blood samples collected in K_2-EDTA tubes prior to and 2- or 4-hours following the first dose of TPST-1495 were cultured with 150 nM LPS in the presence or absence of PGE2 overnight at 37°C
- Cell-free supernatants were collected, and TNF-α was quantified by ELISA (Invitrogen, Inc.)
- Data were expressed as percentage of TNF-α increase in the presence of PGE2, calculated as baseline-subtracted, LPS-induced, post-dose TNF-α concentration divided by baseline LPS-induced, pre-dose TNF-α concentration

PGEM assay

- An HPLC-MS/MS assay was developed and validated to determine concentrations of tetranor PGEM in urine samples stored at -80°C (Alturas Analytics, Inc.)
- PGEM levels were expressed as a function of creatinine levels in the same samples

Immunohistochemistry (IHC)

- Biopsies collected from primary or metastatic lesions were formalin-fixed and paraffin embedded prior to performing IHC for specific markers for T cells, granzyme B (GrB), macrophages, and COX-2-expressing cells, using validated assays (CellCarta, Inc.)
- For available paired biopsies, on-treatment specimens were collected between study days 43 and 64

Data analysis

- Wilcoxon's pair-wise analysis was used to compare TNF-α or PGEM levels among patients at different dose levels (α=0.05)

RESULTS

Pharmacokinetics

- Linear PK (slope = 0.99 [95% CI: 0.57, 1.09]), with dose-dependent increase in exposure
- No changes in PK parameters with addition of pembrolizumab

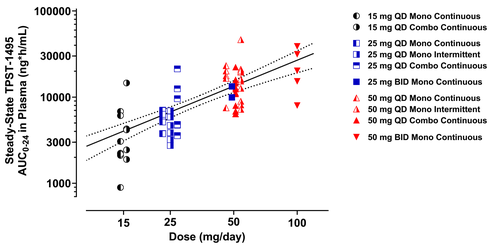


Figure 3. Relationship between dose and AUC at steady state.

Pharmacodynamics

Reversal of PGE2-Induced Immune Suppression with TPST-1495

- Whole blood samples collected prior to and 2- or 4-hours following TPST-1495 were incubated with LPS + PGE2 overnight before measuring TNF-α secretion
- Patients receiving TPST-1495 at 25 or 50 mg exhibited statistically higher median TNF-α recovery from PGE2-mediated suppression than those receiving 15 mg ($P < 0.05$ by Wilcoxon's pair-wise analysis) (**Figure 4**)

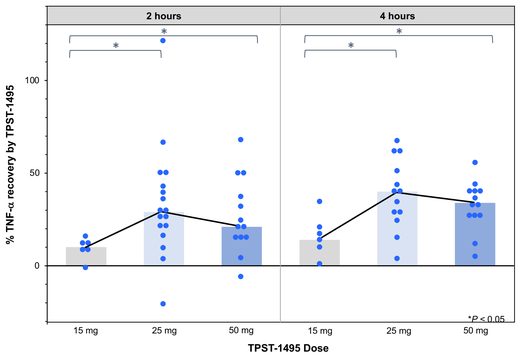


Figure 4. Reversal of PGE2-induced immune suppression with TPST-1495
Bars represent median % TNF-α recovery. Data from patients treated with TPST-1495 as monotherapy

Elevated PGEM Following TPST-1495 Dosing

- Patients treated with TPST-1495 at the 25 or 50 mg doses demonstrated significant elevations in baseline-normalized urinary PGEM (ng/mg creatinine) above those receiving 15 mg on day 2 ($P < 0.05$ by Wilcoxon's paired test), with trends of dose-dependent increases on days 8 and 22 (**Figure 5**)

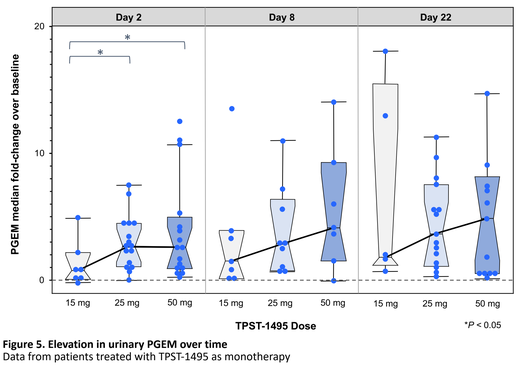


Figure 5. Elevation in urinary PGEM over time
Data from patients treated with TPST-1495 as monotherapy

Biomarkers

Baseline COX-2 Expression

- COX-2 is a key driver of PGE2 biosynthesis and associated with poor cancer prognosis
- IHC analysis revealed COX-2 expression levels >10% in 3 of 11 patients with baseline biopsies (**Table 1**), including two patients with best objectives responses of stable disease

Table 1. Percent COX-2 positive expression at baseline by patient

Patient	Cancer Type	% COX-2-positive
26[a]	**Oropharyngeal SCC**	**100**
02	**Endometrial**	**40**
63[a]	**Endometrial**	**15**
18	Pancreatic	5
03	Anal SCC	3
44	MSS CRC	3
19	CRC	0
53	CRC	0
40	CRC	0
11	Prostate	0
12	Rectal	0

CRC, colorectal carcinoma; SCC, squamous cell carcinoma
[a]Best objective response = stable disease

Case Study: Endometrial Cancer Patient 63

- One of the high COX-2-expressing patients was a 78-year-old patient with Stage IV metastatic MSS endometrial cancer and lung metastasis, with 5 prior therapies in the metastatic setting[3]
- Treatment with TPST-1495 + pembrolizumab:
  - Tumor shrinkage of -22% (**Figure 6**)
  - 2-fold increases in tumor-infiltrating CD8+ T cells in an on-treatment biopsy (**Figure 7**)
  - Increase in granzyme B⁺ CD8⁺ T cells from 1% to 4% after treatment (data not shown)

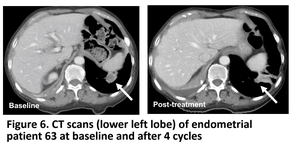


Figure 6. CT scans (lower left lobe) of endometrial patient 63 at baseline and after 4 cycles

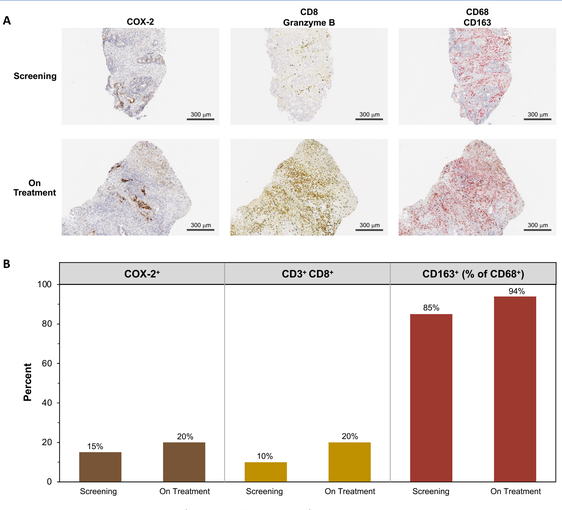


Figure 7. IHC staining of COX-2⁺, CD8⁺/granzyme B⁺, and CD68⁺/CD163⁺ cells in paired biopsies from an endometrial cancer patient at screening and on treatment. (A) Images from paired biopsies stained for COX2 (DAB: brown)/hematoxylin (blue); CD3 (HIGHDEF® yellow chromogen)/CD8 (HIGHDEF® blue chromogen)/granzyme B (Vulcan Fast red chromogen); CD163 (red)/CD68 (DAB: brown)/ hematoxylin (blue). (B) Quantitative image analysis

CONCLUSIONS & FUTURE DIRECTIONS

- TPST-1495 is a novel inhibitor of PGE2 signaling that specifically antagonizes the tumor-promoting and immune-suppressing EP2 and EP4 receptors
- In this first-in-human Phase 1 study conducted in patients with treatment-refractory solid tumors, TPST-1495 demonstrated linear PK and both immune-specific and PGE2-specific pharmacodynamic activity consistent with blockade of PGE2 signaling through EP2 and EP4 receptors
- COX-2 expression was >10% in baseline biopsies of 2 patients who experienced best responses of SD, suggesting that baseline COX-2 levels may be a predictive biomarker of response in patients
- Currently, the combination of TPST-1495 and pembrolizumab is being assessed in endometrial cancer patients, a malignancy reported to express comparatively high levels of EP2, EP4, and COX-2
- Opportunities are being explored in familial adenomatous polyposis, a high-risk pre-cancer condition
- Future analyses will include paired biopsies collected from a subset of patients providing consent for biomarker evaluation

REFERENCES: 1. Pelly et al. *Cancer Discov*. 2021; 11(10):2602-2619; 2. Tury et al. *Oncotarget* 2016;7(51):85124-85141; 3. Ulahannan et al. 2023 ASCO Annual Meeting. June 2-6, 2023. Chicago, IL. Abstract #3107

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