Dual Blockade of the EP2 and EP4 PGE2 Receptors with TPST-1495 is an Optimal Approach for Drugging the Prostaglandin Pathway

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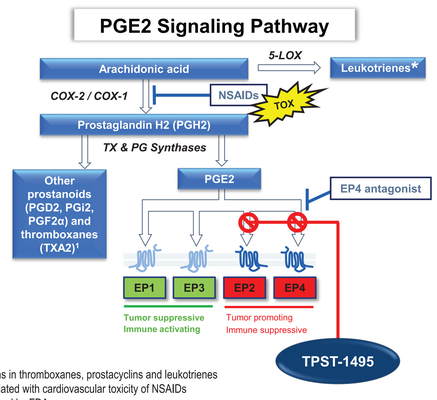
ABSTRACT

Prostaglandin E2 (PGE2) is a bioactive lipid produced by tumor cells that drives disease progression through stimulating tumor proliferation, enhancing angiogenesis and suppressing immune function in the tumor microenvironment (TME)[1, 2]. PGE2 is also a mediator of adaptive resistance to immune checkpoint inhibitor therapy via the upregulation of cyclooxygenase-2 (COX-2). While the role of PGE2 signaling in cancer is clear, how best to inhibit PGE2 for cancer treatment remains under investigation. Inhibition of COX-1 and/or COX-2 has shown promising results in observational studies and meta-analyses, but inconsistent results in prospective studies. PGE2 signals through four receptors, EP1-4, that are variably expressed on tumor and immune cells and have distinct biological activities. The EP2 and EP4 receptors signal through cAMP and drive pro-tumor activities, while the EP1 and EP3 receptors signal through calcium flux and IP3 and drive immune activation and inflammation. While COX-2 and single EP antagonists continue to be developed, the nature of PGE2 signaling supports our rationale to inhibit PGE2 by dual antagonism of the pro-tumor EP2/EP4 receptors, while sparing the pro-immune EP1/EP3 receptors. To our knowledge, TPST-1495 is the first clinical-stage dual inhibitor of both the EP2 and EP4 receptors and is distinguished from other methods of PGE2 inhibition being tested in patients.

In mouse and human whole blood assays, dual blockade of EP2 and EP4 receptors with TPST-1495 reversed PGE2-mediated suppression of LPS induced TNF-α, while single EP4 receptor antagonists were unable to block suppression at higher PGE2 concentrations. Similarly, in murine and human T cells in vitro, TPST-1495 inhibited PGE2-mediated suppression, resulting in a significant increase of TNFα production in response to stimulation with cognate peptide antigen. Importantly, EP1/3 antagonism reduced the activity of TPST-1495, providing a possible mechanism for the superiority of selective EP2/4 antagonism over COX2 inhibition. In vivo, TPST-1495 therapy alone also significantly reduced tumor outgrowth in CT26 tumor bearing mice, correlated with increased tumor infiltration by NK cells, $CD8^+$ T cells, AH1-specific $CD8^+$ T cells, and other anti-tumor myeloid and adaptive immune cell populations. We hypothesize that a yet unidentified immune or stromal cell compartment have an important role in TPST-1495 efficacy, as significant anti-tumor activity was observed in murine models lacking T Cells, including RAG-/- mice, NSG mice, and CD8a depleted mice. TPST-1495 monotherapy demonstrated a decrease of both the intestinal tumor size and number in Adenomatous Polyposis ($APC^{min/+}$) mice, as compared to EP2, EP4, and COX2 antagonism. TPST-1495 is currently being evaluated in an ongoing Phase 1 first-in-human study (NCT04344795) to characterize PK, PD, safety, and to identify a recommended phase 2 dose for expansion cohorts in key indications and biomarker selected patients.

INTRODUCTION

TPST-1495 is a First-in-Class[1] Dual EP2/EP4 PGE2 Receptor Antagonist



*Alterations in thromboxanes, prostacyclins and leukotrienes are associated with cardiovascular toxicity of NSAIDs
1 If approved by FDA
2 IC50s: 17 nM for EP2, 3 nM for EP4, and 51 nM in human whole blood assay

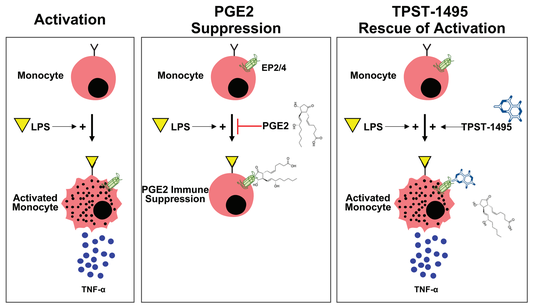
EP2/EP4 blockade effectively reverses PGE2 mediated immune suppression

- PGE2 signals through four receptors, EP1-EP4, which effect distinct tumor promoting and tumor inhibiting activities
  - NSAIDs, which block PGE2 synthesis through COX2, have been explored as IO therapeutics but have been dose-limited by toxicity due to blockade of all four EP receptors, including the immune stimulatory receptors EP1 and EP3.
- Prostaglandin (PGE2) is produced by diverse advancing malignancies and drives tumor progress through autocrine signaling and immune suppression
- Enhanced COX-2 expression and PGE2 production is a mechanism of adaptive immune resistance in response to immune checkpoint blockade therapy
- TPST-1495 features
  - First in class, highly specific antagonist inhibits only the tumor promoting EP2 and EP4 receptors
  - Oral therapy
  - Nanomolar potency
  - Targets *both* tumor cells and immune suppressive cells

RESULTS

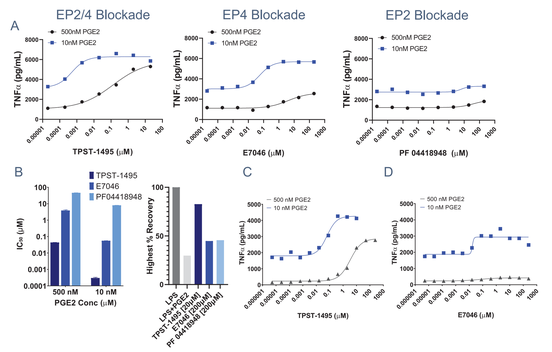
Whole Blood Assay to Measure TPST-1495 Immune Activation

TNFα readout to measure TPST-1495 mediated prevention of PGE2 suppressive activity



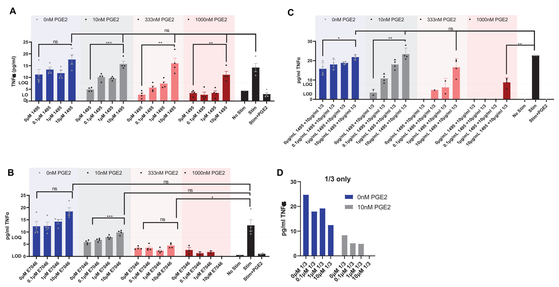
Whole blood assay is performed on fresh whole blood specimens from healthy donors. Whole blood is treated with EP inhibitors for 30 minutes @37°C, followed by 30 minute incubation with 10nM or 500nM PGE2 @37°C and finally with 0.5ug/ml LPS overnight. TNFα is then read out by ELISA.

Figure 1: Only dual antagonism of the EP2 and EP4 by TPST-1495 Restores TNFα production by monocytes at high PGE2 concentrations



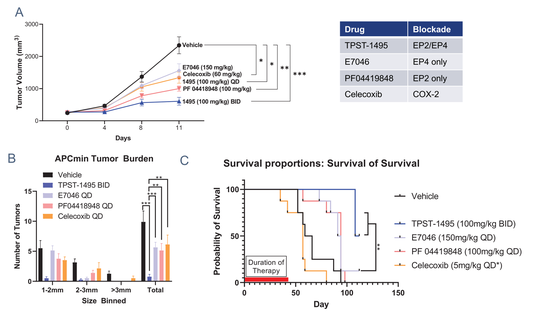
TPST-1495 is more effective than a single EP4, EP2, or Cox antagonists in mice. A,B) TNFα ELISA results from murine (A,B) and human (C,D) whole blood assays. Whole blood was procured from CROs and treated with increasing amounts of TPST-1495 (Dual EP2/4 inhibitor), E7046 (Single EP4 inhibitor), or PF 04418948 (Single EP2 inhibitor), followed by treatment with 10nM or 500nM PGE2 and 0.5ug/ml LPS ("whole blood assay"). B) IC50 and percent recovery results from whole blood assay described in (A).

Figure 2: PGE2-mediated suppression of T Cell activation is prevented by dual and specific antagonism of EP2 and EP4



TPST-1495 prevents PGE2 mediated immune suppression in human primary cells. A,B) TNFα ELISA from supernatants of $CD8^+$ enriched PBMC stimulated with CEF peptides and TPST-1495 or E7046. **C)** Same assay as in A/B, with added 10μM EP1 and EP3 inhibition. **D)** TNFα Elisa from the same assay as **A,C**, treated with increasing amounts of EP1 and EP3 inhibition. Results are representative of one donor.

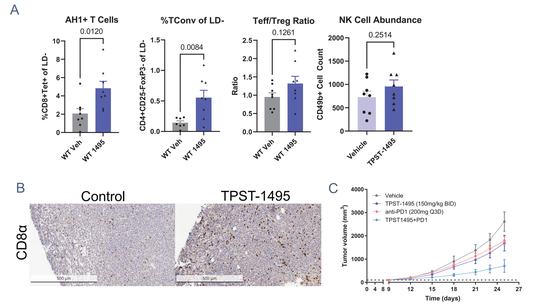
Figure 3: TPST-1495 is significantly more efficacious than either NSAIDs or single EP2 or EP4 pathway inhibitors



Drug	Blockade
TPST-1495	EP2/EP4
E7046	EP4 only
PF04419848	EP2 only
Celecoxib	COX-2

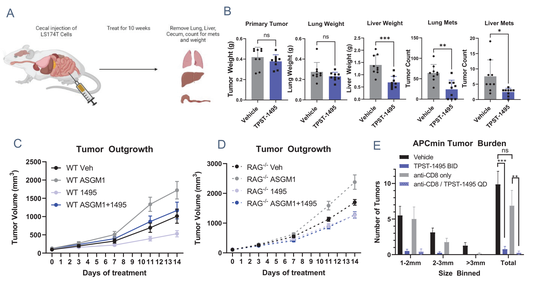
Treatment of tumors with TPST-1495 leads to greater therapeutic effect than with single antagonists.
A) Lewis Lung Carcinoma (LLC) Tumors treated with listed EP antagonists. Tumors were implanted in flanks of animals on day-11, then assorted into groups where tumor volumes averaged 200mm^3 and treated with listed EP antagonists.
B) Tumor count in small intestines of $APC^{min/+}$ mice treated for 3 weeks, starting at 13 weeks of age with orally administered TPST-1495 at 100mpk BID, E7046 at 150mpk QD, PF04418948 at 100mpk QD, or celecoxib at 60mpk QD.
C) Survival of $APC^{min/+}$ animals treated beginning at 12 weeks of age with listed EP antagonists. All antagonists were administered PO for 6 weeks, except for Celecoxib which was administered IP. Statistics were calculated by the Gehan-Breslow-Wilcoxon test.

Figure 4: TPST-1495 exhibits immunomodulatory effects and adds to checkpoint therapy



A) Results of flow cytometry from flank implanted CT26 tumors in BALB /c mice. Tumors were treated as group averages approached 100mm^3 in volume for 14 days, then sacrificed and TIL harvested. TPST-1495 was administered at 100mpk BID PO in methylcellulose.
B) CD8α Immunohistochemistry from flank implanted CT26 tumors in BABL/c mice. Tumors were treated as group averages approached 100mm^3 in volume for 14 days, then fixed in formalin and imbedded in paraffin. TPST-1495 was administered at 100mpk BID PO in methylcellulose.
C) Tumor outgrowth of CT26 Tumors treated with 150mg/kg TPST-1495 BID PO. Anti-PD-1 was administered at 10mpk in PBS IP 2x weekly.

Figure 5: T Cell-Independent effects also play a significant role during TPST-1495 therapy

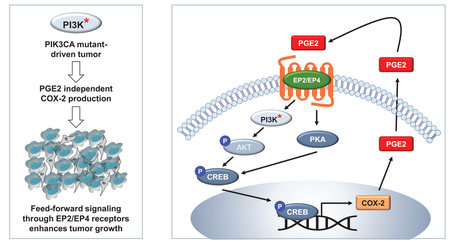


A,B) 2.5 x 10^4 of LS-174T cells were injected into the cecal wall of male NSG mice at age of 8 weeks old. After 5 days, mice were randomly divided into 2 groups treated with methylcellulose or methylcellulose containing TPST-1495 (50mg/kg, BID) by gavage for 2 weeks. Then the mice were treated with methylcellulose or methylcellulose containing TPST-1495 (25mg/kg, BID) by gavage for 7 weeks. Tumor counts and weights at the time of sacrifice, 10 weeks after the initiation of treatment.
C) Tumor outgrowth of CT26 tumors implanted in WT BALB/c Mice. 1e6 CT26 tumor cells were injected in flanks of animals and treated as averaged group tumor volumes approached 100mm^3. ASGM1 antibody was used to deplete NK cells at -1 days and time of injection of other therapeutic interventions, followed by weekly injections thereafter.
D) RAG2-/- animals were treated in the same way as BALB/c mice in (C).
E) APCmin Mice were treated at age 13 weeks for 3 weeks with vehicle or 100mpk TPST-1495 BID +/- 200ug QW aCD8 depleting antibody.

Ph1b EXPANSION DESIGN

Figure 6 PIK3CA Driver Mutation Promotes Tumor Growth and PGE2 Production

PIK3CA mutation predictive of NSAID benefit in CRC and SCCHN



- PIK3CA tumor driver mutation constitutively activates cell proliferation and production of PGE2 and may be a biomarker for TPST-1495 responsive tumors

Adopted from Yang et. Al., OncoTargets and Therapy, 2020.

TPST-1495 Near-Term Development Strategy

Maximize PTS for signal detection and potentially-broader development opportunity

* Anticipated initiation; final scope and number of arms dependent on data and finances.
[1] *Must have documented pathogenic mutation in PIK3CA.* [2] *Both wild-type and mutant PIK3CA.*

CONCLUSIONS

- Prostaglandin (PGE2) drives tumor progression in diverse malignancies through both autocrine signaling and immune suppression
- TPST-1495 is a novel, first-in-class, dual antagonist of both EP2 and EP4, two important PGE2 receptors
- TPST-1495 confers near complete restoration of immune function in cellular assays even in the presence of super-physiological PGE2 concentrations, conditions in which single EP4 or EP2 inhibitors were not effective **(Fig 1)**
- TPST-1495 therapy promotes anti-tumor activity through both T cell-independent and T-cell dependent mechanisms, as evidenced by TME infiltration of effector immune cell populations and tumor Ag-specific CD8+ T cells **(Figs 2, 4, 5)**
- TPST-1495 therapy confers a significant survival advantage compared to therapy with single EP2, EP4 antagonists or the NSAID Celecoxib in the $APC^{min/+}$ spontaneous tumor mouse model of CRC **(Fig 3)**
- TPST-1495 is currently being evaluated in a schedule and dose-finding Phase 1a clinical study in patients with advanced solid tumors (NCT04344795)
- Additional hypothesis-driven clinical studies, including in patients with PIK3CA mutant-driven cancers and in combination with pembrolizumab, are planned **(Fig 6)**

CITATIONS

1. Pelly VS, Moeini A, Roelofsen LM, et al. Anti-Inflammatory Drugs Remodel the Tumor Immune Environment to Enhance Immune Checkpoint Blockade Efficacy. Cancer Discov. 2021;11(10):2602-2619. doi: 10.1158/2159-8290.CD-20-1815.

2. Tury S, Becette V, Assayag F, et al. Combination of COX-2 expression and PIK3CA mutation as prognostic and predictive markers for celecoxib treatment in breast cancer. Oncotarget. 2016;7(51):85124-85141. doi: 10.18632/oncotarget.13200.

3. Zelenay S, van der Veen AG, Böttcher JP, et al. Cyclooxygenase-Dependent Tumor Growth through Evasion of Immunity. Cell. 2015;162(6):1257-70. doi: 10.1016/j.cell.2015.08.015.