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

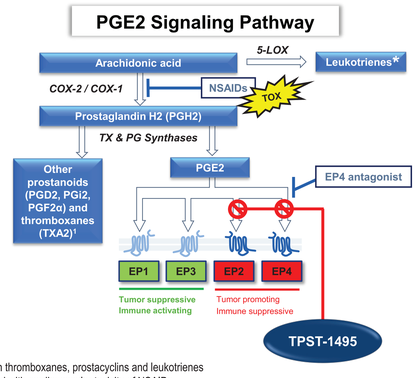
Prostaglandin E2 (PGE2) is a bioactive lipid produced by tumor cells that drives disease progression through stimulating tumor proliferation, enhancing angiogenesis and suppressing immune function in the tumor microenvironment (TME)[1, 2]. PGE2 is also a mediator of adaptive resistance to immune checkpoint 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 IFN-γ production in response to stimulation with cognate peptide antigen. 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. Immunohistochemistry analysis of resected areas of hyperplasia from the small intestines of treated $APC^{min/+}$ revealed increased infiltration of adaptive immune cells after treatment with TPST-1495. Additionally, transcriptional analysis of these samples demonstrated that TPST-1495 led to a similar transcriptional signature but greater effect on key genes downstream of prostaglandin E2 signaling as compared to single EP2 or EP4 antagonism, reinforcing that redundancy of these two receptors necessitates dual blockade. 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

Rationally designed, based on current understanding of PGE2 signaling in cancer progression



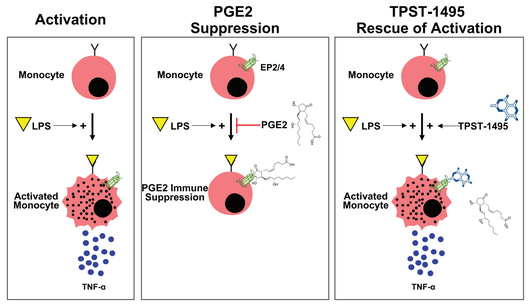
*Alterations in thromboxanes, prostacyclins and leukotrienes are associated with cardiovascular toxicity of NSAIDs
[1] If approved by FDA
[2] IC50s: 17 nM for EP2, 3 nM for EP4, and 51 nM in human whole blood assay

- Prostaglandin (PGE2) is produced by tumor cells and drives malignant progression and immune suppression
- PGE2 signals through four homologous receptors, EP1-EP4
  - EP2 and EP4 receptor signaling induces cAMP production and is "pro-tumor"
  - EP1 and EP3 receptor signaling induces calcium flux and is "pro-immune response"
- NSAIDs block PGE2 synthesis, are dose limited due to cardio- and renal toxicity and prevent signaling through the immune stimulatory receptors EP1 and EP3
- Activating tumor-driver mutations in signal transduction pathways (e.g., RAS, PIK3CA) induce COX-2 expression and PGE2 production
- Upregulated PGE2 production is a mechanism of adaptive immune resistance to immune checkpoint blockade therapy
- ***TPST-1495:***
  - First in class, orally bioavailable small molecule dual antagonist specific for EP2 (IC_{50} = 3 nM) and EP4 (IC_{50} = 17 nM) with negligible activity against EP1 and EP3 receptors (IC_{50} > 100 μM)
  - Targets both tumor cells and immune suppressive cells
  - Is the subject of an ongoing Phase 1a clinical trial (NCT04344795)

RESULTS

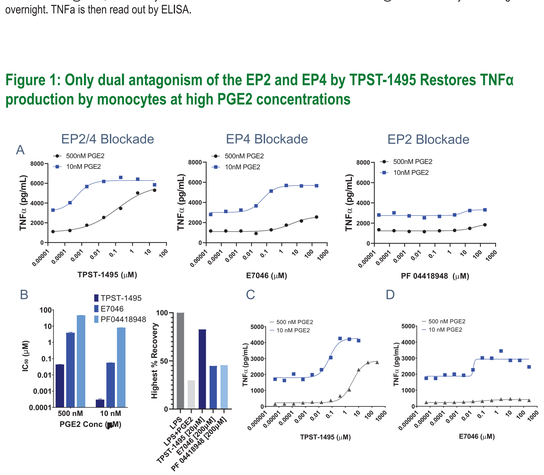
Whole Blood Assay to Measure TPST-1495 Immune Activation

TNF-α readout to measure reversal of TPST-1495 mediated PGE2 immune suppression



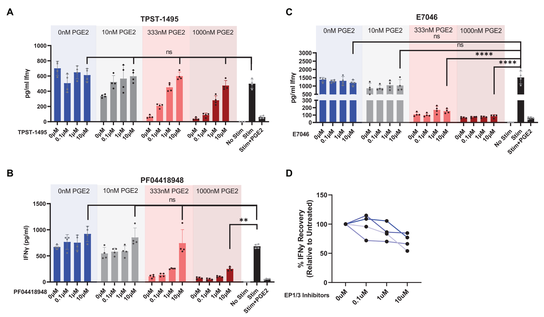
Whole blood assay is performed on fresh whole blood specimens from healthy donors. Whole blood is treated with EP inhibitors for 30 minutes @37°C, followed by 30 minute incubation with 10nM or 500nM PGE2 @37°C and finally with 0.5ug/ml LPS overnight. TNFa is then read out by ELISA.

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



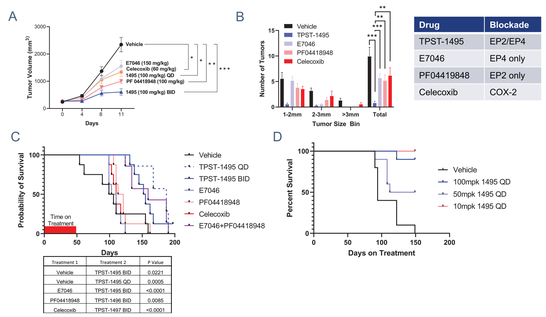
TPST-1495 is more effective than a single EP4, EP2, or Cox antagonists in mice. A,B) TNFα ELISA results from murine (A,B) and human (C,D) whole blood assays. Whole blood was procured from CROs and treated with increasing amounts of TPST-1495 (Dual EP2/4 inhibitor), E7046 (Single EP4 inhibitor), or PF 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-C) IFNγ ELISA from supernatants of CD8+ enriched PBMC stimulated with CEF peptides and TPST-1495, E7046, or PF04418948. D) IFNγ Elisa from the same assay as **A-C,** treated with increasing amounts of EP1 and EP3 inhibition. Results are representative of multiple donors.

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

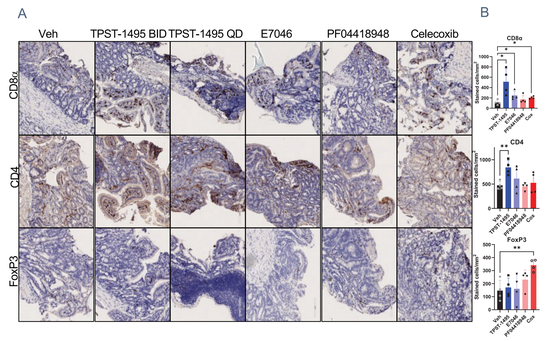


Drug	Blockade
TPST-1495	EP2/EP4
E7046	EP4 only
PF04419848	EP2 only
Celecoxib	COX-2

Treatment 1	Treatment 2	P Value
Vehicle	TPST-1495 BID	0.0221
Vehicle	TPST-1495 QD	0.0005
E7046	TPST-1495 BID	<0.0001
PF04418948	TPST-1496 BID	0.0065
Celecoxib	TPST-1497 BID	<0.0001

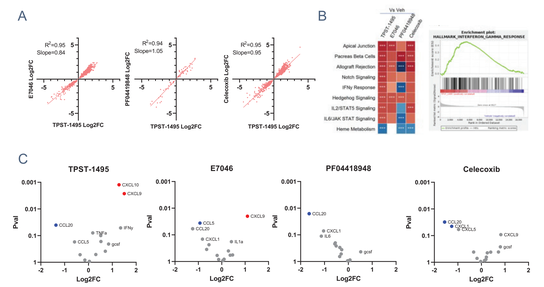
Treatment of tumors with TPST-1495 leads to greater therapeutic effect than with single antagonists.
A) Lewis Lung Carcinoma (LLC) Tumors treated with listed EP antagonists. Tumors were implanted in flanks of animals on day -11, then assorted into groups where tumor volumes averaged 200mm^3 and treated 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. Statistics were calculated by the Gehan-Breslow-Wilcoxon test.
D) Survival of $APC^{min/+}$ animals treated beginning at 6 weeks of age with orally administered QD regimens of TPST-1495. Mice were treated daily until death or humane endpoints were reached, for the full length of the experiment.

Figure 4: TPST-1495 increases $APC^{min/+}$ small intestine tumor infiltration by adaptive immune cell subsets.



A) Immunohistochemistry (IHC) of resected hyperplasias from 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.
B) Quantification of IHC in (A) by a blinded pathologist.

Figure 5: Transcriptional analysis of $APC^{min/+}$ tumors depicts redundancy of EP2 and EP4 receptors and demonstrates rationale for dual receptor blockade

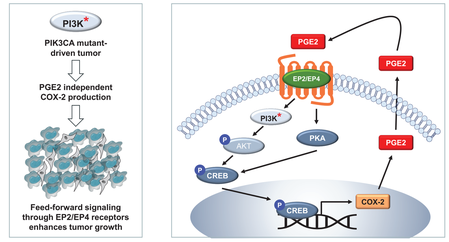


A) Significant log2 fold changes in differentially expressed genes from RNA sequencing of tumors resected from $APC^{min/+}$ mice in Figure 5B.
B) Heatmap depicting significantly upregulated gene sets from GSEA analysis performed on RNA sequencing analysis above and representative GSEA plot from IFNg signature. All results represent data from one experiment with 4 animals per group. *** indicates FDR< 0.25 for that gene set compared to Vehicle treated animals.
C) Volcano plots of selected genes from samples in (A). Blue coloration represents significantly (P_{adj} <0.05) downregulated genes while red correlation represents significantly upregulated genes. Genes in grey did not meet the significance cutoff.

Ph1b EXPANSION DESIGN

Figure 6: PIK3CA Driver Mutation Promotes Tumor Growth and PGE2 Production

PIK3CA mutation predictive of NSAID benefit in CRC and SCCHN

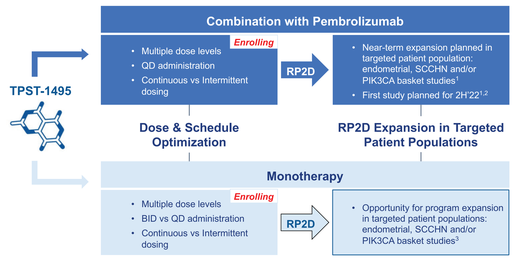


- PIK3CA tumor driver mutation constitutively activates cell proliferation and production of PGE2 and may be a biomarker for TPST-1495 responsive tumors

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



[1] Study could be either a single indication or a biomarker-based basket. [2] Timing is an estimate based on current projections. [3] With additional funding, monotherapy expansion(s) would be in select indications based on target expression and/or a PIK3CA biomarker-positive basket cohort. For either basket arm: (1) patients must have documented pathogenic mutation in PIK3CA; and (2) histologies of interest include CRC, breast, NSCLC, urothelial, gastroesophageal, anal SCC, cervical SCC

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 critical pro-tumor 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 **(Figs 1,2)**
- TPST-1495 therapy confers a significant tumor clearance and survival advantage compared to therapy with single EP2, EP4 antagonists or the NSAID Celecoxib in LLC (lung carcinoma) and $APC^{min/+}$ spontaneous tumor mouse model of CRC **(Fig 3)**
- TPST-1495 increases immune infiltrate and enhances an adaptive immune transcriptional profile in small intestine tumors from $APC^{min/+}$ mice. **(Figs 4,5)**
- 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 in targeted patient populations are under consideration **(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.

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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.