The PPAR-α Antagonist TPST-1120 Enhances Immunotherapy and Anti-Angiogenic Therapy to Inhibit Murine Renal Cancer

Michael Gillespie[1,2*], Valerie Chen[3*], Isabella Howard[1,2], Keira Smith[1,2], Dave Freund[3], Nathan Standifer[3], Dara Burdette[3], Thomas W. Dubensky[3], Sam Whiting[3], Dipak Panigrahy[1,2]

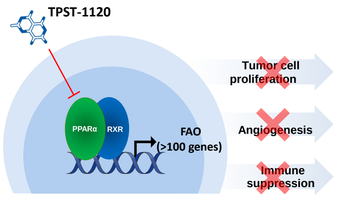
[1]Dept. of Pathology and [2]Center for Vascular Biology Research, BIDMC, Harvard Medical School, Boston, MA. [3]Tempest Therapeutics, Brisbane, CA; * MG and VC are co-equal first authors

OBJECTIVE

To evaluate effects of TPST-1120 on anti-tumor immunity in preclinical models of renal cell carcinoma (RCC)

BACKGROUND

TPST-1120: Peroxisome-Proliferator Activated Receptor-α (PPARα) Antagonist[1,2]



- **PPARα** is a transcription factor and master regulator of fatty acid oxidation (FAO), controlling >100 genes (**Figure 1**)
- **FAO** is a key cancer metabolic adaptation that supports tumor growth and metastasis
- **Genetic data** reveal that PPARα and FAO are required to sustain tumor growth
- **Inhibiting PPARα** to reduce FAO is a promising strategy to inhibit tumor growth and relieve immunosuppression

Figure 1. TPST-1120 is a first-in-class PPARα antagonist that targets both tumor cells and immune suppressive cells

- PPARα ligands modulate the switch between co-activator and co-repressor transcription complexes at PPARα-controlled target genes (**Figure 2**)
- In addition, PPARα has been shown to transrepress NF-κB signaling and inhibit angiogenesis
- RCC expresses high levels of PPARα and is a highly angiogenic cancer; current frontline treatments include chemotherapy, anti-angiogenics, and immunotherapy, but are limited by the immune suppressive state of the tumor microenvironment

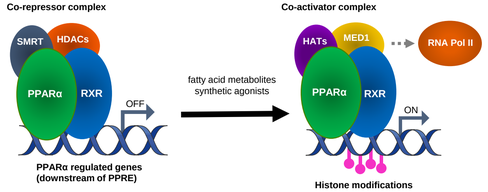


Figure 2. PPARα ligands modulate the switch between co-activator and co-repressor transcription complexes at PPARα-controlled target genes
SMRT, silencing mediator of retinoic acid and thyroid hormone receptor; HDAC, histone deacetylase; HAT, histone acetyltransferases; MED1, mediator complex subunit 1; RXR, retinoid X receptor

METHODS

***In Vitro* Cell-Based PPARα Activity Assay**

- TPST-1120 antagonist activity against PPARα was determined in a CHO cell-based reporter cell line which expresses luciferase upon PPARα activation
- Cells were incubated with agonists (oleoylethanolamide [OEA], GW7647), with or without TPST-1120, to quantify effects on PPARα

***In Vitro* Enzyme Fragment Complementation Assay**

- Activity of PPARα ligands was tested in CHO-K1 cells stably expressing engineered PPARα and MED1 proteins, each fused to a fragment of β-galactosidase
- Binding of PPARα to MED1 results in enzyme complementation and chemiluminescence, which is used to measure the effects of agonist or antagonist binding

***In Vivo* Murine Model of Renal Cell Adenocarcinoma**

- BALB/c mice 6-8 weeks of age were subcutaneously inoculated with 1x10^6 renal cell adenocarcinoma (RENCA) tumor cells
- Once tumors reached a size of ~150 mm^3, treatment with TPST-1120 alone, in combination with cabozantinib or anti-PD-1, or vehicle only, was initiated (~1 week)
  - TPST-1120 30 mg/kg once daily (QD) was administered by oral gavage
  - Cabozantinib 15 mg/kg QD was administered by oral gavage, tumor measurements were taken once a week, and mice were sacrificed on day 15
  - Anti-PD-1 200 µL was administered every 3 days by intraperitoneal injection, tumor measurements were taken on days 5, 9, and 12, and mice were sacrificed on day 12
- For quantitative analysis of cytotoxic CD8+ T cells, mice were sacrificed on day 15, and tumors were fixed and prepared for histological analysis using ImageJ software

RESULTS

TPST-1120 is a Competitive Antagonist of PPARα Ligands

- TPST-1120 potently competed against an endogenous PPARα agonist oleoylethanolamide (OEA; IC_{50} = 15 nM at 30 µM OEA) and the PPARα-specific agonist GW7647 (IC_{50} = 36 nM at 20 nM GW7647) (**Figure 3**)

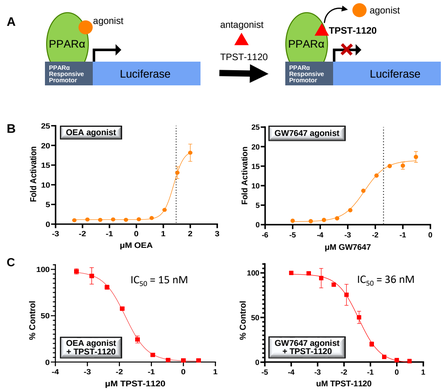


Figure 3. TPST-1120 is a Competitive Antagonist of PPARα Ligands
(A) CHO cell-based reporter assay. (B) PPARα fold-activation by OEA (left panel) or GW7647 (right panel). (C) Percent control of PPARα activation following addition of TPST-1120 to cells treated with OEA (left panel) or GW7647 (right panel). Vertical dotted lines correspond to agonist concentration used in lower panels.

TPST-1120 Destabilizes the PPARα Co-activator Transcription Complex

- MED1 is a key member of the PPARα co-activator transcription complex (**Figure 4**)
- TPST-1120 inhibited agonist-induced binding of PPARα to MED1 by destabilizing the co-activator complex and stabilizing the inactive conformation of PPARα

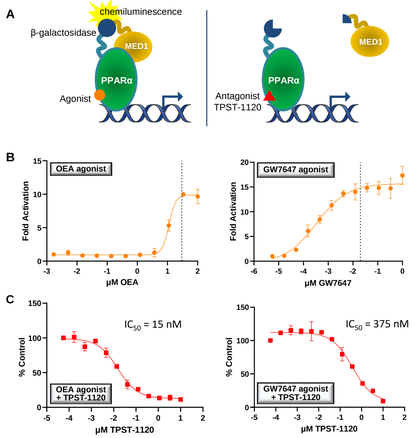


Figure 4. TPST-1120 Antagonizes Agonist-induced PPARα-MED1 Binding
(A) Enzyme fragment complementation assay in CHO-K1 cells expressing PPARα and MED1. (B) PPARα fold-activation by oleoylethanolamide (OEA; left panel) or GW7647 (right panel). (C) Percent control of PPARα activation following addition of TPST-1120 to cells treated with OEA (left panel) or GW7647 (right panel). Vertical dotted lines correspond to agonist concentration used in lower panels.

Binding of TPST-1120 to the PPARα Ligand-Binding Domain Stabilizes an Inactive Conformation

- X-ray co-crystal shows binding of TPST-1120 in the ligand-binding domain, positioning the AF-2 activation helix in an inactive conformation, which has a lower affinity for co-activator motifs and reduces activation of PPARα-regulated genes (**Figure 5**)

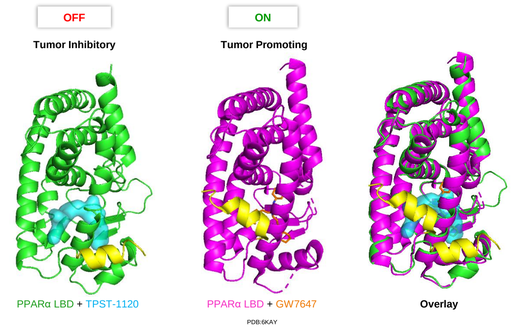


Figure 5. TPST-1120 Stabilizes the Inactive Conformation of PPARα
Comparison between the GW7647 agonist-bound PPARα ligand-binding domain (LBD) and the TPST-1120 (cyan) antagonist-bound structure shows that TPST-1120 confers the AF-2 activation helix (yellow) in an inactive conformation

Tumor Growth Inhibition by TPST-1120 Alone and in Combination With Cabozantinib or Anti-PD-1 in a Murine Model of RCC

- In a murine model of renal cell adenocarcinoma (RENCA), TPST-1120 treatment reduced tumor growth by 52%-56% as monotherapy (*P* < .0001) (**Figure 6**)
- Combination treatment with current frontline therapeutics resulted in synergistic tumor inhibition of 81% with cabozantinib and 74% with anti-PD1 (*P* < .0001)

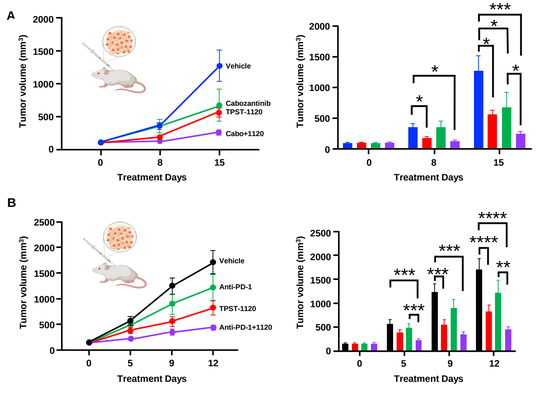


Figure 6. TPST-1120 Enhances Inhibitory Tumor Growth Effects of Chemotherapy or Immunotherapy
BALB/c mice bearing RENCA tumors were treated with TPST-1120 alone, in combination with (A) cabozantinib (N=8) or (B) anti-PD-1, (N=10) or vehicle only, and assessed for tumor growth on select treatment days post-dose. Effect of each treatment group on tumor volume is presented by treatment day. Statistical differences between treatment groups are shown in the right panels: *P < .05, ***P < .001, ****P < .0001

Increase in Tumor-Infiltrating Cytotoxic CD8+ T Cells by TPST-1120 in Murine Model of RCC

- Quantitative analysis showed TPST-1120 increases infiltrating cytotoxic CD8+ T cells in the tumor microenvironment (**Figure 7**)
- This observation is consistent with other results showing that TPST-1120 modulates the tumor microenvironment by shifting to a more immune responsive environment that allows for the influx of tumor specific CD8+ T cells

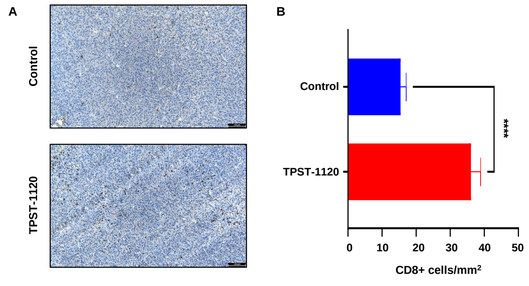


Figure 7. TPST-1120 Increases Tumor-Infiltrating Cytotoxic T-Cells
A) IHC chromogen staining of CD8+ cells in FFPE sections of RENCA implanted tumors in BALB/c mice treated with either TPST-1120 (30mg/kg) QD or vehicle. N=10. (B) Quantitative analysis using ImageJ software of representative areas of chromogen staining in tumor tissue. 8-10 representative images were taken for each of 5 samples from both groups

CONCLUSIONS

- TPST-1120 is a competitive antagonist of PPARα
- TPST-1120 inhibits agonist-induced co-activator recruitment by stabilizing the repressive conformation of PPARα
- There is a reduction in proliferating tumor cells in the tumor microenviroment in mice treated with TPST-1120
- TPST-1120 modulates the tumor microenvironment to increase the amount of infiltrating CD8+ T cells
- TPST-1120 had no notable toxicity in any treatment groups
- TPST-1120 reduces kidney cancer growth as a monotherapy while showing increased inhibition when combined with frontline chemotherapy and immunotherapy
- There is evidence for the translation of TPST-1120 into a frontline treatment for RCC
- Collectively, we demonstrate that TPST-1120 can reverse an immunosuppressive tumor microenvironment to promote anti-tumor immunity in kidney cancer in the absence of overt toxicity

REFERENCES:

1. Luo Y, Chen L, Wang G, et al. PPARα gene is a diagnostic and prognostic biomarker in clear cell renal cell carcinoma by integrated bioinformatics analysis. J Cancer. 2019;10(10):2319-2331. Published 2019 May 22. doi:10.7150/jca.29178
2. Kaipainen A, Kieran MW, Huang S, et al. PPARalpha deficiency in inflammatory cells suppresses tumor growth. PLoS One. 2007;2(2):e260. Published 2007 Feb 28. doi:10.1371/journal.pone.0000260

ACKNOWLEDGMENTS: Ingrid Koo, PhD, provided editorial support for the poster. Mouse image created with Biorender.com

