Blockade of the PPARα metabolic checkpoint with TPST-1120 suppresses tumor growth and stimulates anti-tumor immunity

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ABSTRACT

Tumors evolve to modulate metabolism to promote their own survival and to suppress tumor-specific immunity. Hypoxic conditions in the tumor microenvironment (TME) induce fatty acid oxidation (FAO), and diverse malignancies are reliant on this metabolic pathway. Additionally, suppressive immune cell populations including M2 macrophages, myeloid-derived suppressor cells and regulatory T cells preferentially utilize FAO. Peroxisome proliferator-activated receptor alpha (PPARα) is the principal transcription factor that regulates the expression of FAO genes, and this metabolic checkpoint is critical for tumor proliferation. TPST-1120 is a first-in-class selective competitive antagonist of the human PPARα. To test the hypothesis that blocking FAO with TPST-1120 confers anti-tumor efficacy, we assessed TPST-1120 in multiple syngeneic and xenograft mouse models. Blockade of PPARα with TPST-1120 mediated potent anti-tumor immune responses and significant tumor regression in syngeneic models of breast, lung, colon, pancreatic and melanoma in addition to xenograft models of CLL, AML, pancreatic and melanoma cancers as a monotherapy or in combination with chemotherapy. In pancreatic and breast cancer models, TPST-1120 augmented regression of tumor growth in combination with chemotherapy. In combination with anti-PD1, TPST-1120 treatment resulted in significant reduction of tumor growth in ovarian orthotopic (ID8) and colon (MC38) models; cured mice were completely protected against autologous tumor challenge, strongly suggesting immunological T cell memory against the primary tumor. Studies in genetic knock-out mice indicated that macrophages and antigen cross-presenting dendritic cells are required for TPST-1120 activity, mediated through thrombospondin-1(TSP-1) and stimulator of interferon genes (STING). Consistent with prior reports, inhibition of PPARa with TPST-1120 skewed macrophages *in vivo* toward an M1 effector phenotype. These results provide the rationale for evaluating TPST-1120 in patients with advanced malignancies. A Phase 1/1b open-label, dose-escalation and dose-expansion study of TPST-1120 as a single agent or in combination with systemic anti-cancer therapies is planned in early 2019.

INTRODUCTION

- **TPST-1120 is a first-in-class, orally administered, potent, small molecule selective antagonist of the human peroxisome proliferator-activated receptor alpha (PPARα)**
- PPARα is a transcription factor which induces the expression of genes that regulate fatty acid oxidation (FAO) and inflammation (Figure 1).
- PPARα and FAO gene signatures are enriched in metastatic tumors (Figure2).[1-4]
- Tumor growth is suppressed in PPARα-deficient mice.[5-6]
- TPST-1120 has significant anti-tumor activity as a monotherapy and in combination with chemotherapy or anti-PD1 Abs.
- Anti-tumor activity of TPST-1120 is mediated through: 1) direct killing of tumor cells dependent on FAO; 2) Shift FAO to glycolysis supports immune effector cells and inhibits suppressor cells; and 3) restore TSP-1 to homeostatic levels.

FIGURE 1: PPARα Induces Fatty Acid Oxidation (FAO)

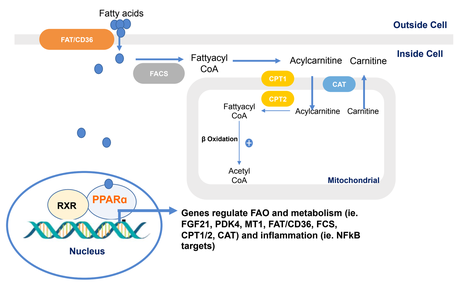


Figure 1: PPARα regulates key genes involved in FAO, metabolism and inflammation. Upon ligand binding, PPARα heterodimerizes with RXR, stimulates gene transcription of target genes involved in FAO and lipid metabolism. PPARα also negatively modulate gene transcription by inhibiting DNA-binding of several other transcription factors, such as NF-κB.

FIGURE 2: Upregulation of PPARα and FAO Genes in Diverse Tumors

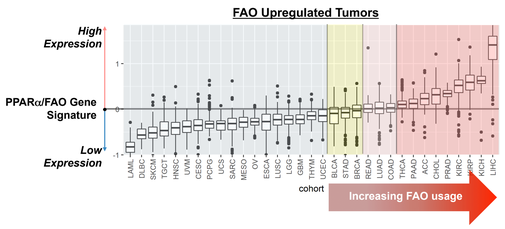


Figure 2: Enrichment of FAO associated genes including PPARα was evaluated in 10,000 unique datasets representing 33 tumor types in TCGA (cancergenome.nih.gov).

METHODS

- TPST-1120 in vitro killing was conducted on primary CLL tumor cells
- Efficacy as a monotherapy or in combination with chemotherapy or anti-PD1 was evaluated in multiple syngeneic mouse models
- To characterize its mechanism of anti-tumor immunity, TPST-1120 was evaluated in knock-out models of TSP-1, STING and BatF3.
- ELISA was used to measure plasma and tumor matrix protein thrombospondin-1 (TSP-1), and FGF21
- Gene expression analysis was performed by quantitative RT-PCR
- Flow cytometry was used to evaluate immune cell infiltrate in the tumor

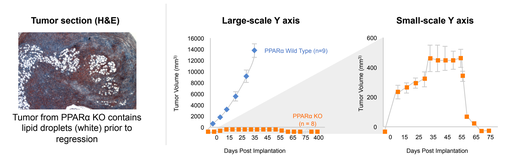
RESULTS

TPST-1120: PPARα Target Validation and Target Engagement

FIGURE 3: Spontaneous Tumor Regression in the Absence of PPARα

Tumor Establishment but Not Progression

Sarcoma (SV40 & H-ras transformed mouse embryo fibroblast tumor cell line)



Deletion of PPARα in host and tumor cells prevents usage of fatty acid oxidation (FAO)

Observation repeated in melanoma and lung tumor models

Source: Kaipainen et al., PLoS ONE 2007, 2(2): e260 and unpublished results

Figure 3: Tumor growth is inhibited in PPARα knock-out (KO) mice. Tumors establish but spontaneously regress in KO mice.

FIGURE 4: PPARα Antagonist TPST-1120 Inhibits PPARα-Dependent Targets

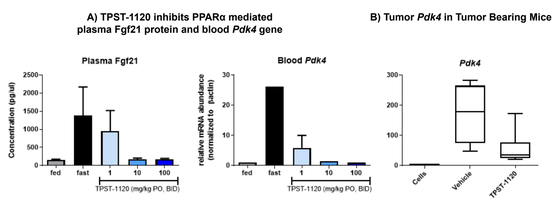


Figure 4: A.) PPARα induced Fgf21 protein and target gene *Pdk4* increases under fasting is inhibited with TPST-1120 at 1, 10, and 100mg/kg PO BID in C57BL6/N mice (non-tumor bearing). B.) *Pdk4* gene expression is inhibited in B16-F10 melanoma tumors in mice treated with TPST-1120 30 mg/kg PO BID compared to vehicle treated.

FIGURE 5: TPST-1120 Induces Direct Tumor Cell Cytotoxicity in Primary CLL Cells

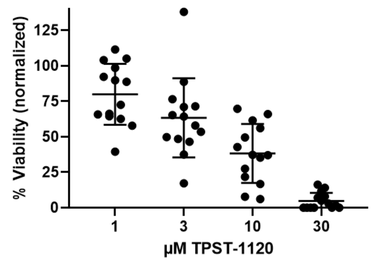


Figure 5: TPST-1120 directly inhibits proliferation of human primary CLL tumor cells in a dose dependent manner after 48 hours of incubation (n=14).

FIGURE 6: TPST-1120 Has Monotherapy Effect and Significantly Enhances Benefit of Chemotherapy

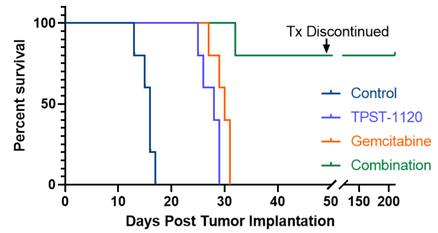


Figure 6: TPST-1120 (30 mg/kg PO BID) response against PancOH7 tumors was improved by combination with gemcitabine 50 mg/kg IP, q4d (N=5/group) in PancOH7. 4/5 mice in the combo group were alive at day 210 (treatment discontinued at day 48).

The anti-tumor effects of PPARα antagonism is dependent upon immune modulation

FIGURE 7: TPST-1120 Anti-Tumor Activity Requires Innate and Adaptive Immunity

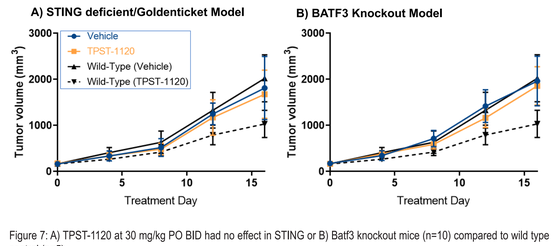


Figure 7: A) TPST-1120 at 30 mg/kg PO BID had no effect in STING or B) Batf3 knockout mice (n=10) compared to wild type control (n=5).

FIGURE 8: TPST-1120 Combination with anti-PD1 mAb Induces Significant Anti-Tumor Response and Tumor CD8 T cell Infiltration

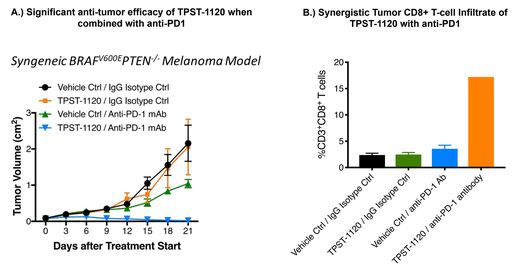


Figure 8: A) TPST-1120 has potent anti-tumor efficacy when combined with anti-PD1 in syngeneic BRAF^{V600E}PTEN$^{-/-}$ model. B.) Combination TPST-1120 plus anti-PD1 Ab shows anti-tumor efficacy is associated with synergistic increase in T cell infiltration into the TME.

FIGURE 9: TPST-1120 Anti-Tumor Efficacy is Dependent on TSP-1

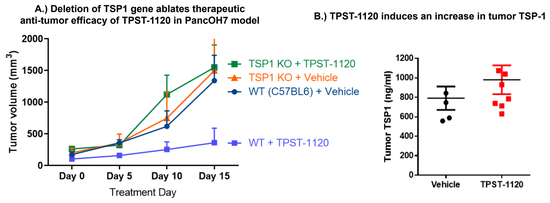
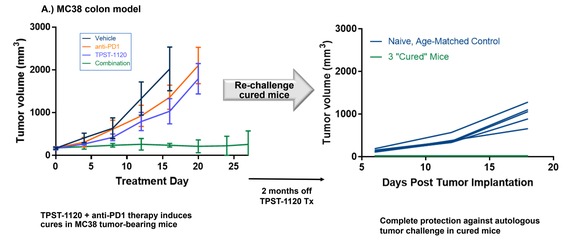


Figure 9: A) TPST-1120 has potent anti-tumor monotherapy in WT mice but this activity was lost when PancOH7 tumors implanted in TSP-1 knock-out mice. B.) Anti-tumor efficacy is associated with increase in TSP-1 levels in the TME

FIGURE 10: TPST-1120 Combination with Anti-PD1 Induces Memory Response in MC38 Colon Model



B.) Transferrable anti-tumor immunity from cured mice treated with TPST-1120 and α-PD1

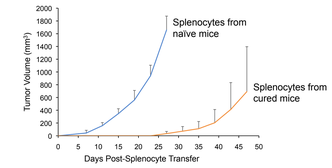


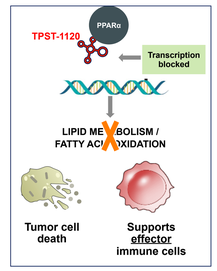
Figure 10: A.)Established MC38 tumors (100-150mm3) treated with vehicle (n=10), TPST-1120 (n=7), anti-PD1 alone (n=10), or TPST-1120 + anti-PD1 (n=15) were followed for tumor growth. By day 24 only animals in the combination group remained alive. Three were tumor free at day 60 post removal of therapy and were tumor-free upon autologous tumor re-challenge while aggressive tumor growth occurred in naïve mice. B.) Adoptive transfer of splenocytes from naïve C57BL/6 mice or MC38 tumor bearing mice cured with TPST + αPD-1 into naïve recipient C57BL/6 mice, followed by challenge with 1 x 10^6 MC38 tumor cells. Mice were then monitored for tumor growth. Tumor growth was significantly delayed in mice receiving splenocytes from cured mice compared to those from naïve mice.

CONCLUSIONS

TPST-1120 Blocks PPARα Metabolic Checkpoint

First-in-class oral PPARα antagonist

- Blocks transcription of PPARα target genes
- Shifts metabolism from FAO to glycolysis
- Multi-pronged mechanism:
  1. Direct killing of tumor cells dependent on FAO
  2. Shifting FAO to glycolysis supports immune effector cells and inhibits suppressor cells
  3. Restoration of homeostatic TSP-1 levels
- Significant anti-tumor efficacy as single agent and in combination therapies
- A Phase 1/1b open-label, dose-escalation and dose-expansion study of TPST-1120 as a single agent or in combination with systemic anti-cancer therapies in subjects with advanced solid tumors initiated in Q12019 (NCT03829436)



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