# Lipid and Immune-Based Biomarkers Associated with Clinical Response to TPST-1120: a Small Molecule Antagonist of Peroxisome-Proliferator Activated Receptor-Alpha

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Abstract #2130

## ABSTRACT

**Background**

TPST-1120 is a small molecule antagonist of peroxisome-proliferator activated receptor-alpha (PPAR-α), a regulator of fatty acid oxidation and immune suppression. TPST-1120 was well tolerated and showed signs of activity in a phase I trial as monotherapy and in combination with nivolumab (NCT03829436). The objective response rate was 30% (3/10, all partial responses) in subjects treated at the two highest TPST-1120 doses in combination with nivolumab and included two subjects with renal cell carcinoma previously refractory to anti-PD1 therapy [1]. We performed ctDNA mutational analysis at baseline and quantified lipid and gene expression changes in post-treatment whole blood to identify potential biomarkers of response.

**Methods**

Mutational analysis of ctDNA was assessed using the PredicineCARE™ assay (Predicine Inc.), and lipid analysis was performed by tandem mass spectrometry. Gene expression changes were quantified using the nCounter® PanCancer Immune Profiling panel (NanoString Inc.) supplemented with 30 PPAR-α target genes. Putative clinical response biomarkers were identified as those differentially expressed by patients with partial response (PR) compared to those with progressive disease ($p$<0.05 by Mann-Whitney U Test). Longitudinal lipid change magnitudes were assessed by Wilcoxon paired analysis ($p$<0.05).

**Results**

Baseline ctDNA mutational analysis revealed that patients with PR or stable disease were more likely to bear mutations in isocitrate dehydrogenase *(IDH)* and phosphatase and tensin homolog *(PTEN)* compared to patients with progressive disease. Patients with PR demonstrated significant elevations ($p$<0.05) in multiple genes including those associated with lipid transport *(APOE)*, Th17 development *(RORC)* and down-regulation of CD155, a TIGIT ligand. Lipid analysis demonstrated acute changes in free fatty acids (FFA) four hours after first dose ($p$<0.05). Among patients receiving combination therapy, the highest post-dose elevations in FFA, lysophosphatidylcholine and lysophosphatidylethanolamine levels were observed in a PR patient exhibiting the longest duration of response.

**Conclusions**

TPST-1120 treated patients with PR demonstrated fatty acid oxidation perturbations and immune gene expression changes as potential biomarkers of clinical benefit. Increased frequencies of responding patients bearing PI3K pathway or *IDH* mutations may reveal populations likely to benefit from treatment with TPST-1120.

1. Yarchoan M, et al., "A phase 1 study of TPST-1120 as a single agent and in combination with nivolumab in patients with advanced solid tumors." Journ. Clin. Onc. 2022; 40 (16) suppl.

## INTRODUCTION

**Peroxisome Proliferator Activated Receptor-Alpha (PPAR-α)**

- Nuclear hormone receptor that regulates transcription of fatty acid oxidation (FAO) genes and transrepresses proinflammatory transcription factors including NF-κB, STAT1, STAT3 and AP-1
- Numerous tumors utilize FAO as a primary metabolic pathway, including renal cell carcinoma (RCC), cholangiocarcinoma (CCA) and hepatocellular carcinoma (HCC)

**TPST-1120**

- A first-in-class, orally-bioavailable, competitive, small molecule antagonist of PPAR-α **(Fig. 1)**
- In multiple pre-clinical models, TPST-1120 demonstrated anti-tumor efficacy concomitant with reduced expression of select fatty acid oxidation (FAO) genes and enhanced immune activation

**TPST-1120 clinical findings from completed Ph I trial**

- **53% disease control rate** as a single agent
- **30% objective response rate** in combination with nivolumab at two highest TPST-1120 doses
  - Best objective response (BOR) of partial response (PR) in two RCC previously refractory to anti-PD-1 and one subject with late-line CCA.

**TPST-1120 translational strategy**

- Assess baseline ctDNA mutational status associations with BOR
- Quantify gene expression changes in circulation as a function of TPST-1120 exposure and BOR
- Quantify lipid class changes in circulation over time and as a function of BOR

## METHODS

**Patients**

- Provided informed consent prior to enrollment in trial of TPST-1120 as a single agent or with nivolumab **(Fig. 2)**
  - TPST-1120 doses ranged from 100-600 mg BID as a single agent or 200-600 mg BID in combination with nivolumab

**Baseline tumor mutational burden assessments**

- PredicineCARE™ assay (Predicine Inc.) performed on ctDNA from baseline plasma

**On-treatment differential gene expression analysis**

- nCounter® PanCancer Immune Profiling panel (NanoString Inc.) and 30 additional PPAR-α associated genes
- Quantified gene expression changes between baseline and cycle 1 day 8 and cycle 3 day 1

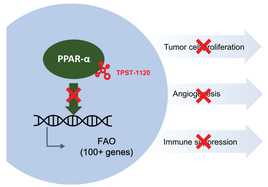
**Lipidomics analysis**

- A modified Bligh and Dyer extraction was used on all samples prior to analysis on Sciex 5500 with DMS device using 70 lipid standards across 17 subclasses. Analysis performed by the UCLA Lipidomics Laboratory

**Data Analysis**

- Associations between TPST-1120 exposure and gene expression changes were assessed by linear regression analysis correcting for false discovery rate (FDR) by Benjamini-Hochberg method
  - Genes exhibiting FDR p-value < 0.05 and effect size > 0.5 on cycle 1 day 8 and similar exposure:expression relationships on cycle 3 day 1 were categorized as potential pharmacodynamic biomarkers
- Linear Discriminant Analysis (LDA) used to identify combinations of genes that distinguish among BOR
  - Genes differentially expressed by PR vs. PD patients (p<0.05 by Mann-Whitney U test) considered predictive biomarkers
- Lipid classes changes were compared using Wilcoxon's Paired Analysis (p<0.05) or in patients stratified by BOR

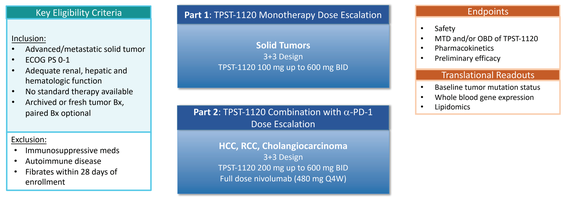
**Figure 1: TPST-1120 is a first-in-class PPAR-a Antagonist that Targets Tumor and Immune Cells**




- **Fatty Acid Oxidation (FAO)**
  - Key cancer metabolic adaptation that supports tumor growth and metastasis
  - Principal metabolic pathway for immune suppressive cells
  - Induces angiogenesis
- **PPAR-α**
  - Transcription factor and master regulator of FAO, controlling > 100 lipid metabolism genes

Inhibiting PPAR-α to reduce FAO is a promising strategy to inhibit tumor growth and relieve immunosuppression.

**Figure 2: TPST-1120-01 Phase I Study Design (NCT03829436)**




ECOG PS: Eastern Cooperative Oncology Group Performance Status; Bx: biopsy; BID: twice daily; RCC: renal cell carcinoma; HCC: hepatocellular carcinoma; CCA: cholangiocarcinoma; MTD: maximal tolerated dose; OBD: optimal biologic dose; DLT: dose limiting toxicity

**Figure 3: Baseline tumor mutation status and BOR in patients enrolled in monotherapy arm as a function of best percent change in target lesion**

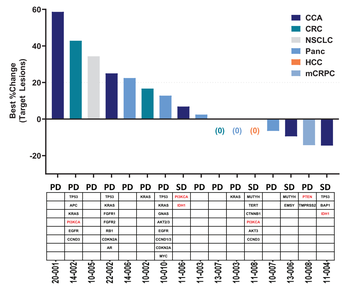



**Figure 4: Baseline tumor mutation status and BOR in HCC, CCA and RCC patients enrolled in combination therapy arm as a function of best percent change in target lesion**

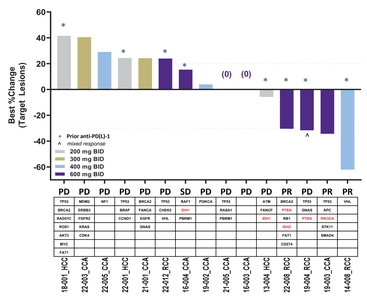
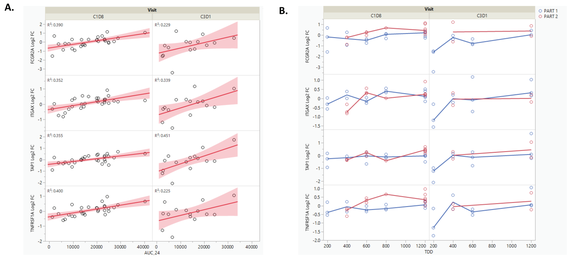



**Figure 5. Genes differentially expressed as a function of TPST-1120 exposure**

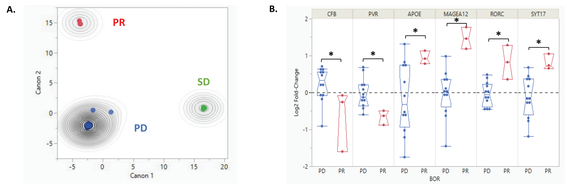



A) Log2 Fold Changes (Log2 FC) in gene expression levels as a function of TPST-1120 AUC0-24 on cycle 1 day 8 (C1D8, left) or cycle 3 day 1 (C3D1, right). Red line and shaded area: linear regression and 95% CI of linear regression. B) Comparison of Log2 FC in patients enrolled in Part 1 monotherapy (blue) vs. Part 2 combination therapy (red) arms on C1D8 and C3D1. TDD: Total Daily Dose.

**Table I. Summary of Genes Associated with TPST-1120 Exposure Levels**

| Gene | Name | Function | FDR p-value, effect size (C1D8) | Direction | PPAR-α Pathway Association |
|---|---|---|---|---|---|
| FCGR2A | Fc-γ RIIa, CD32 | Low affinity Fc-γ receptor | 0.047, 0.732 | Increased | STAT1 & STAT3 binding sites in promoter |
| ITGAX | Integrin α-X, CD11c | Expressed by Mono., Mac., DC & Lymphos | 0.049, 0.566 | Increased | unknown |
| TAP1 | Transporter Associated with antigen Processing-1 | Peptide transporter into ER, loading onto MHC-I | 0.049, 0.550 | Increased | STAT1 & NF-kB-regulated transcription |
| TNFRSF1A | TNF-α R1, CD120a | TNF-α Receptor | 0.047, 0.648 | Increased | STAT3-regulated transcription |

**Figure 6. Identification of differentially expressed genes associated with BOR**



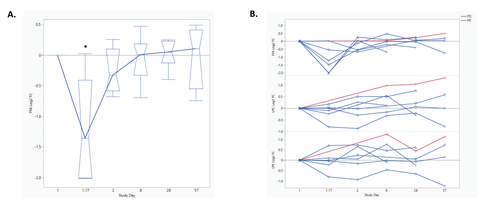

A.) Linear Discriminant Analysis density plot demonstrates differential expression of genes by different BOR groups. B.) Genes down- or up-regulated in PR vs. PD patients enrolled in combination therapy arm on cycle 1 day 8. *p < 0.05 by Mann-Whitney U-test between PD and PR groups.

**Table II. Summary of Genes Associated with PR**

| Gene | Name | Function | Differential Expression in PR patients | PPAR-α Pathway Association |
|---|---|---|---|---|
| RORC | RAR related Orphan Receptor C | Transcriptional regulator of Th17 cell differentiation | Increased | PPAR-α suppresses Th17 development in mice[1] |
| APOE | Apolipoprotein E | Lipid metabolism | Increased | STAT1, AP-1 and NF-κB-regulated transcription |
| MAGEA12 | Melanoma-associated Antigen-12 | Repressor of tumor-suppressor genes | Increased | Unknown |
| SYT17 | Synaptotagmin 17 | Intracellular membrane trafficking | Increased | Unknown |
| PVR | Poliovirus Receptor, CD155 | TIGIT ligand | Decreased | NF-κB-regulated transcription[2] |
| CFB | Complement Factor B | Alternative complement pathway, C3B convertase | Decreased | PPAR-α suppresses C3 transcription |

[1]Chang et al., 2019, *Experimental Cell Research*, 375:22; [2]Kamran et al., 2013, PLoS ONE 8(1): doi:10.1371/journal.pone.0054406

**Figure 7. Lipid analysis of patients receiving TPST-1120 in combination with nivolumab (Part 2)**




A) Log2 Fold Changes (Log2 FC) in baseline-normalized free fatty acid (FFA) quantities over time *p < 0.05 by Wilcoxon pair-wise analysis B) Log2 Fold Changes (Log2 FC) in FFA, lysophosphorylcholine (LPC) or lysophosphorylethanolamine (LPE) over time in patients receiving TPST-1120 + nivolumab. Blue = PD patients, red = PR patient.

## RESULTS

**Baseline tumor mutational burden**

- Trend of increased prevalence of PTEN, PIK3CA, IDH1 or IDH2 mutations in SD and PR patients in patients receiving TPST-1120 as a single agent or in combination with nivolumab (**Fig. 3** and **Fig. 4**, respectively)

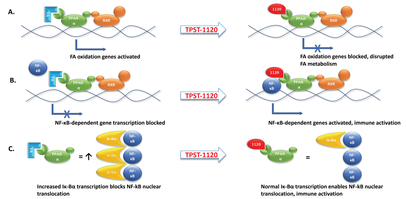
**Gene expression analysis**

- Linear regression analysis revealed genes differentially expressed as a function of TPST-1120 exposure on C1D8 and C3D1 (**Fig. 5** and **Table I**) in patients receiving TPST-1120 as monotherapy or in combination with nivolumab
  - Increased expression levels of FCGR2A (CD32), ITGAX (CD11c), TAP1, and TNFRSF1A (CD120a)
- Linear Discriminant Analysis identified genes differentially expressed by PR vs. PD or SD patients on study day 8 (**Fig. 6A**)
  - Genes increased in PR vs PD patients (p<0.05 by Mann-Whitney U test) include RORC, APOE, MAGEA12 and SYT17 (**Fig. 6B** and **Table II**)
  - Genes decreased in PR vs PD patients (p<0.05 by Mann-Whitney U test) include PVR (CD155) and CFB (**Fig. 6B** and **Table II**)

**Lipidomics analysis**

- Reductions in circulating FFA levels observed 4-hours after first dose of TPST-1120 (**Fig. 7A**)
- PR patient exhibited highest increases in circulating FFA, LPC and LPE levels on day 57 (**Fig. 7B**)

**Figure 8. Model of TPST-1120 effects on PPAR-α activities**




TPST-1120 blocks binding of PPAR-α ligands thereby inducing A) reduced Fatty Acid (FA) oxidation gene transcription B) activated NF-κB binding to DNA, and C) blockade of enhanced Ik-Bα transcription enabling for NF-κB nuclear translocation

## CONCLUSIONS

- Increased frequencies of responding patients bearing PI3K pathway or IDH mutations may reveal populations likely to benefit from treatment with TPST-1120
- TPST-1120 induced on-target changes as a function of exposure in multiple immunologic and metabolic genes and/or pathways, including:
  - NF-kB and STAT-regulated genes
  - Fatty acid biosynthesis/catabolism
- Patients with PR demonstrated gene expression changes that implicate immune activation via TPST-1120 blockade of PPAR-α trans-suppression as potential biomarkers of clinical benefit **(Fig. 8)**

## FUTURE DEVELOPMENT

- Ongoing clinical collaboration with Roche in front-line HCC: a randomized Ph IB/II trial (NCT04524871)