Pharmacodynamic and predictive biomarkers associated with response in cancer patients treated with TPST-1120: a first-in-class small molecule antagonist of Peroxisome-Proliferator Activated Receptor-Alpha

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TEMPEST THERAPEUTICS

ABSTRACT

Background
TPST-1120 is a first-in-class, small molecule antagonist of peroxisome-proliferator activated receptor-alpha (PPAR-α), a transcriptional regulator of fatty acid oxidation and mediator of immune suppression. TPST-1120 was well tolerated and showed signs of activity in a phase I trial as monotherapy (53% disease control rate) and in combination with nivolumab (NCT03829436). In combination cohorts the objective response rate (ORR) was 23%, including 30% (3/10, all partial responses) in subjects treated at the two highest TPST-1120 doses. Responses included two subjects with renal cell carcinoma previously refractory to anti-PD-1 and one subject with late line cholangiocarcinoma [1]. We assessed gene expression changes in post-treatment whole blood and performed baseline mutational analysis on ctDNA to identify potential biomarkers.

Methods
Differential expression of 780 genes in 30 subjects receiving 100 mg to 600 mg TPST-1120 BID was assessed using the nCounter® PanCancer Immune Profiling panel (NanoString Inc.) supplemented with 30 PPAR-α target genes. Associations between expression change magnitudes and TPST-1120 exposure levels on study day 8 were calculated, and genes exhibiting a False Discovery Rate p-value < 0.05 and effect size > 0.5 were categorized as potential pharmacodynamic biomarkers. Putative clinical response biomarkers were identified using linear discriminant analysis (LDA) with best objective response as categorical discriminants to identify genes differentially expressed by partial response (PR) patients (p<0.05 by Mann-Whitney U Test). Mutational analysis of ctDNA was performed using the PredicineCARE™ assay (Predicine Inc.).

Results
Seven of 780 genes assessed were modulated by TPST-1120 exposure (p<0.05), including genes associated with enhanced immune responsiveness (CXCL16, TNFRSF1A), monocytes or macrophages (ITGAX, FCGR2A) and PPAR-α blockade (NCF4). Similar TPST-1120 exposure-biomarker associations were observed among monotherapy and combination therapy patients. LDA performed on combination therapy patients revealed that those with PR demonstrated significant elevations (p<0.05) in multiple genes including those associated with Th17 development (RORC), lipid transport (APOE) and down-regulation of CD155, a TIGIT ligand. 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.

Conclusions
TPST-1120 induces pharmacodynamic changes in circulating blood consistent with PPAR-α blockade and reversal of PPAR-α immune suppressive activities. Patients with PR demonstrated gene expression changes that implicate immune activation and alleviation of immune suppression 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 et al. 2022, *Journal of Clinical Oncology* 40 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, small molecule antagonist of PPAR-α
- In multiple pre-clinical models, TPST-1120 therapy reduced expression of selected fatty acid oxidation (FAO) genes, enhanced immune activation and tumor-specific immune memory

TPST-1120 clinical findings from completed Phase I trial
- **53% disease control rate** as a single agent
- **30% objective response rate** (ORR) at two highest doses of TPST-1120 in combination with nivolumab (23% ORR across all dose levels)
  - Best objective response (BOR) of partial response (PR) seen in two subject with late-line RCC refractory to prior anti-PD-1 and one subject with late-line CCA

TPST-1120 translational strategy
- Quantify gene expression changes as a function of TPST-1120 exposure levels or BOR
- Assess baseline ctDNA mutational status associations with BOR

METHODS

Patients
- Provided informed consent prior to enrollment in trial of TPST-1120 as a single agent or with nivolumab **(Fig. 1)**
- Received TPST-1120 ranging from 100-600 mg BID as a single agent or 200-600 mg BID in combination with full dose nivolumab **(Table I)**

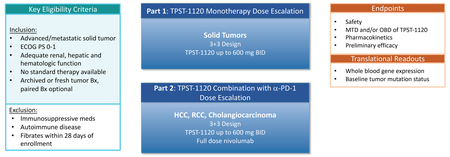
Pharmacokinetic assay
- LC-MS/MS-based assay to quantify TPST-1120 in plasma

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

Predictive biomarker assay (tumor mutational burden)
- PredicineCARE™ assay (Predicine Inc.) performed on ctDNA from baseline plasma

Data Analysis
- Associations between TPST-1120 exposure and gene expression changes 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 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 potential predictive biomarkers

Figure 1: TPST-1120-001 Phase I Study Design



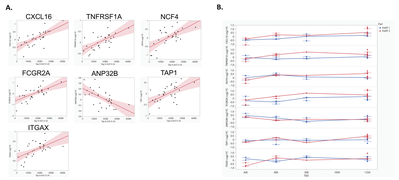
TPST-1120-001 Phase I Study NCT03829436. ECOG PS: Eastern Cooperative Oncology Group Performance Status; Bx: biopsy; BID: twice daily; RCC: renal cell carcinoma; HCC: hepatocellular carcinoma; CCA: cholangiocarcinoma; MTD: maximal tolerated dose; OBD: optimal biologic dose; DLT: dose limiting toxicity

Table I: Patients Enrolled in TPST-1120-01 with Translational Readouts

Subject ID	Indication	Best Objective Response*	Dose BID	Day 8 AUC_{0-24} (ng*hr/ml)
001-10-002	Colorectal Cancer	PD	100 mg	1259.5
001-10-003	Pancreatic Cancer	SD**	100 mg	2067.0
001-11-003	Pancreatic Cancer	PD	100 mg	6629.4
001-11-004	Cholangiocarcinoma	SD**	200 mg	13556.5
001-11-005	Colorectal Cancer	PD	200 mg	10665.5
001-10-008	Prostate Cancer	PD	300 mg	14989.5
001-11-006	Cholangiocarcinoma	SD	300 mg	13297.0
001-11-008	Hepatocellular Carcinoma	SD**	300 mg	9573.5
001-10-010	Pancreatic Cancer	PD	400 mg	20602.5
001-11-009	Pancreatic Cancer	PD	400 mg	23505.5
001-14-002	Colorectal Cancer	PD	400 mg	13939.0
001-13-006	Cholangiocarcinoma	SD	600 mg	14511.0
001-13-007	Colorectal Cancer	PD	600 mg	19929.0
001-14-006	Pancreatic Cancer	PD	600 mg	16498.5
001-14-007	Pancreatic Cancer	PD	600 mg	25003.5
001-20-001	Cholangiocarcinoma	PD	600 mg	23254.0
001-13-004	Hepatocellular Carcinoma	PD	200 mg + nivo. ***	8191.5
001-18-001	Hepatocellular Carcinoma	PD	200 mg + nivo. ***	5828.5
001-22-001	Hepatocellular Carcinoma	PD	200 mg + nivo. ***	3869.0
001-21-001	Cholangiocarcinoma	PD	300 mg + nivo. ***	9537.5
001-22-003	Cholangiocarcinoma	PD	300 mg + nivo. ***	24903.5
001-22-004	Hepatocellular Carcinoma	PD	300 mg + nivo. ***	23230.5
001-14-008	Renal Cell Carcinoma	PR	400 mg + nivo. ***	22226.5
001-13-008	Cholangiocarcinoma	PD	600 mg + nivo. ***	20452.0
001-16-003	Cholangiocarcinoma	PD	600 mg + nivo. ***	28884.0
001-16-004	Cholangiocarcinoma	SD**	600 mg + nivo. ***	40270.0
001-19-003	Cholangiocarcinoma	PR	600 mg + nivo. ***	20047.5
001-19-004	Renal Cell Carcinoma	PD	600 mg + nivo. ***	27228.0
001-22-008	Renal Cell Carcinoma	PR	600 mg + nivo. ***	23239.0
001-22-012	Renal Cell Carcinoma	PD	600 mg + nivo. ***	24369.0

* PR: partial response; SD: stable disease; PD: progressive disease. ** 3 or more consecutive SD read-outs. *** nivolumab dose: 480 mg Q4W

Figure 2: Genes associated with TPST-1120 exposure on study day 8



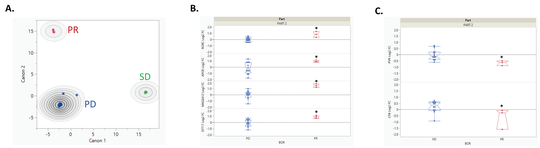
A) Log2 Fold Changes (Log2 FC) in gene expression levels as a function of TPST-1120 (AUC0-24). Red line: linear regression, red shaded area: 95% CI of linear regression. B) Comparison of Log2 FC in patients enrolled in monotherapy (Part 1) vs. combination therapy (Part 2) arms. TDD: Total Daily Dose.

Table II: Summary of Genes Associated with TPST-1120 Exposure Levels

Gene	Name	Function	FDR p-value, Effect size	Direction of change	PPAR-α Pathway Association
CXCL16	CXCL16	Macrophage & neutrophil recruitment	0.025, 0.596	Increased	NF-kB-regulated transcription
TNFRSF1A	TNF-α R1	TNF-α Receptor	0.047, 0.648	Increased	STAT3-regulated transcription
FCGR2A	Fc-γ RIIa, CD32	Low affinity Fc-γ receptor	0.047, 0.732	Increased	STAT1 & STAT3 binding sites in promoter
NCF4	Neutrophil Cytosolic Factor 4	NADPH oxidase component	0.047, 0.632	Increased	Down-modulated by PPAR-α agonist (pemafibrate) in mice[1]
ANP32B	Acidic leucine-rich Nuclear Phospoprotein-32b	Histone chaperone, immuno-suppressive transcription factor	0.049, 0.558	Decreased	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
ITGAX	Integrin α-X, CD11c	Trafficking of innate immune cells & lymphocytes	0.049, 0.566	Increased	unknown

[1]Sasaki et al., 2020, *Scientific Reports* 10:7818

Figure 3: Identification of differentially expressed genes associated with BOR



A) Linear Discriminant Analysis of 780 genes revealed two canons (gene sets) that discriminate patients based on BOR. Canon 2 maximally distinguishes between PR and PD or SD patients. B) Genes upregulated in PR vs. PD patients enrolled in combination therapy arm (Part 2). C) Genes downregulated in PR vs. PD patients enrolled in combination therapy arm (Part 2). *p < 0.05 by Mann-Whitney U-test vs. PD patients

Table III: 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	Unknown
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

Figure 4: Baseline tumor mutation status in patients enrolled in monotherapy arm as a function of best percent change in target lesion

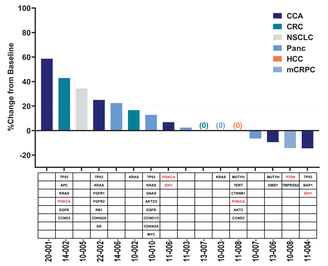
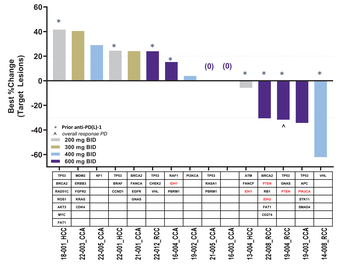


Figure 5: Baseline tumor mutation status in HCC, CCA and RCC patients enrolled in combination therapy arm as a function of best percent change in target lesion.



RESULTS

Pharmacodynamic analysis
- Seven of 780 monitored genes were differentially expressed as a function of TPST-1120 exposure (FDR p-value < 0.05, effect size > 0.5, **Fig. 2A**)
- Gene expression changes were similar between patients receiving TPST-1120 as a single agent or in combination with nivolumab (Part 1 and Part 2, respectively, **Fig. 2B**)
- Differentially expressed genes were predominantly regulated by NF-κB, STAT1 or STAT3 (**Table II**)

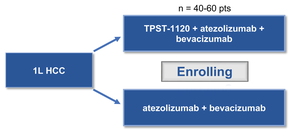
Predictive analysis
- Linear discriminant analysis of gene expression changes on study day 8 using BOR as categorical variables reveals that Canon 2 genes maximally distinguish between PR and SD or PD patients (**Fig. 3A**)
- Canon 2-associated genes increased in PR vs PD patients (p<0.05 by Mann-Whitney U test) include RORC, APOE, MAGEA12 and SYT17 (**Fig. 3B** and **Table III**)
- Canon 2-associated genes decreased in PR vs PD patients (p<0.05 by Mann-Whitney U test) include PVR (CD155) and CFB (**Fig. 3C** and **Table III**)
- Trend of increased prevalence of PTEN, PIK3CA, IDH1 or IDH2 mutations in SD and PR patients receiving TPST-1120 as a single agent or in combination with nivolumab (**Fig. 4** and **Fig. 5,** respectively)

CONCLUSIONS

- TPST-1120 induces pharmacodynamic changes detectable in whole blood consistent with reversal of PPAR-α transrepressive activities on proinflammatory transcription factors NF-κb, STAT1 and STAT3
- Patients with PR demonstrated gene expression changes in whole blood that implicate immune activation and alleviation of immune suppression 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

FUTURE DEVELOPMENT

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



Primary Endpoint: ORR
Secondary Endpoints (include): PFS, OS

Global study: US, EU, Asia

Operationalized by Roche