PKM2 activation modulates the tumor-immune microenvironment and enhances response to checkpoint inhibitors in preclinical solid tumor models

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I. Abstract

Pyruvate kinase is a crucial enzyme responsible for the last step of glycolysis. Cancer cells can use the M2 isoform of pyruvate kinase (PKM2), to better balance respiration and biosynthesis due to allosteric switching between the less active dimeric and fully active tetrameric forms. Additionally, the dimeric form of PKM2 can translocate to the nucleus, altering transcription to enhance cancer cells' ability to grow and evade immune detection. Inducing tetramerization presents an opportunity to target PKM2 resulting in the metabolic reprogramming of tumor-immune microenvironment (TME). TP-1454 is a potent PKM2 activator with low nanomolar PKM2 activation in biochemical assays (AC50 = 10 nM) and multiple cell types (AC < 50 nM), tolerated in mice, rats and dogs after repeat doses as high as 1000 mg/kg/day and has recently entered a Phase I clinical trial (NCT04328740).

We hypothesize that PKM2 activation may reverse the immune-suppressive TME. To test this hypothesis, we examined the activity of TP-1454 combination with immunotherapy (I/O) in multiple mouse syngeneic tumor models. TP-1454 and anti-PD-1 combination therapy in colorectal cancer models resulted in tumor growth inhibition versus vehicle (53% in CT26; 99% in MC38, P < 0.001). We observed decreases in multiple glycolytic intermediates in TP-1454-treated tumors versus vehicle.

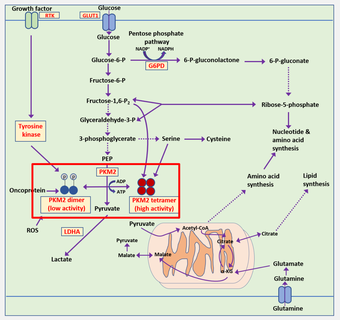
We conducted immunophenotyping of the TME in multiple models to identify targets of PKM2 activation. TP-1454 treatment reduced the CD4+ Foxp3+ T-regulatory (Treg) population in MC38, 4T1, RENCA models. Further, we assayed TP-1454 induced PKM2 activation in different immune cell types. To confirm the effect of PKM2 activation on Treg cells we conducted an in vitro assay to explore TP-1454 treatment response on polarization of Tregs and/ or toxicity and proliferation. We further utilized LCMS to explore metabolic intermediates that play a critical role in Treg regulation, including regulation of the O-linked β-N-acetylglucosamine (O-GlcNac) post-translational modification, which is reported to stabilize Foxp3 in CD4+ cells. We are currently exploring the effect of TP-1454 treatment on O-GlcNacylation of Foxp3 and its stability in HEK293 cells, to support the link between PKM2 activation and stabilization of Foxp3.

TP-1454 effects on tumor-specific immunity were validated using tumor rechallenge studies. The results of a tumor rechallenge study will be presented using murine MC38 or RENCA xenograft models that are treated with TP-1454 and I/O combination therapies that exhibited a complete response (CR) and were re-implanted. These preclinical studies indicate a unique mechanism modulating tumor metabolism and the TME to improve the response of cancer patients to immunotherapy.

II. Background

Figure 1: PKM2 function

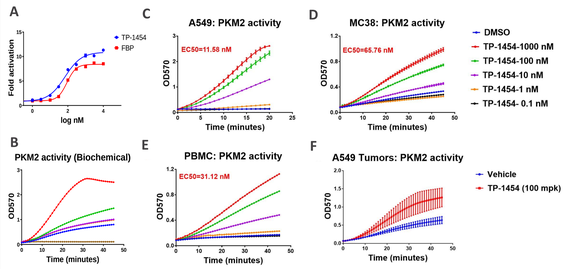
- PKM2 (Pyruvate Kinase Muscle 2)
- Converts a glucose metabolite (phosphoenolpyruvate) and ADP into **ATP** and **pyruvate**
- Balance between synthetic process and glycolytic energy production
- PKM2 active tetramer favors energy production pathway
- PKM2 inactive dimer favors synthetic process pathway
  - allows continued biosynthesis
  - Alters transcription to enhance cell growth and immune evasion
- Targeting PKM2 tetramerization could result in metabolic reprogramming of the TME to allow improved targeting of solid tumors.



Adapted from Tamada et al. ,Clin Cancer Res, 2012

III. Results

Figure 2: TP-1454 potently activates PKM2 in biochemical assays, cells, and tissue



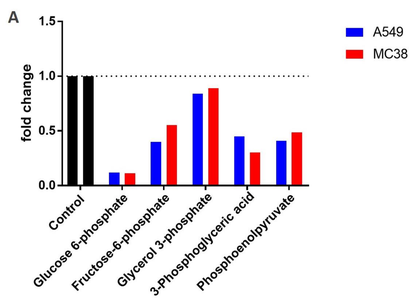
TP-1454 displays 1.4-fold stronger PKM2 activation than fructose-1,6-bisphosphate (FBP), a potent endogenous PKM2 activator (A). TP-1454 displays PKM2 activation at nanomolar concentrations in biochemical assays using recombinant PKM2 (1h treatment) (B), A549 cells (24h treatment) (C), MC38 cells (24h treatment) (D), and human PBMC (24h treatment) (E). TP-1454 also displays PKM2 activation in A549 tumors at 4h post dose(F).

Table 1: TP-1454 is well tolerated in mice, rats, and dogs

Type of Study	Species / Strain	Duration of Dosing	Daily Doses (mg/kg[a])	Toxicity
Single-Dose Toxicity	CD-1 Mice	Acute	500, 1000, 1500, 2000	None observed
	Beagle Dogs	Acute	50, 250, 1000, 1500	None observed
	Sprague-Dawley Rats	Acute	100, 250, 500, 2000	None observed
Repeat-Dose Toxicity	CD-1 Mice	7 days	100, 300, 1000	None observed
	CD-1 Mice	28 days	100, 300, 1000	None observed
	Sprague-Dawley Rats	7 days	200, 1000	None observed
	Beagle Dogs	7 days	20, 200, 2000	None observed
	Beagle Dogs	28 days	100, 300, 1000	None observed

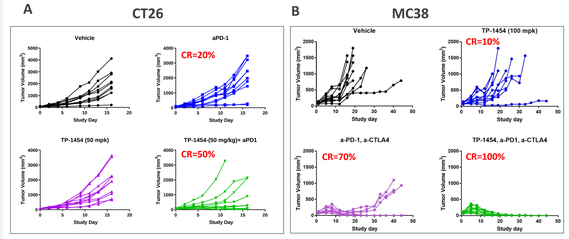
a. Unless otherwise specified. For Repeat-Dose Toxicity, the highest NOAEL (No Observed Adverse-Effect Level) is underlined.

Figure 3: TP-1454 reduces intermediate metabolites of glycolysis



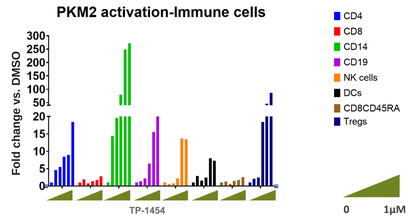
Treatment of mice bearing A549 (Blue) or MC38 (Red) with 100 mg/kg TP-1454 qdx21 (A549) or qdx8 (MC38) results in the reduction of several intermediate glycolysis metabolites

Figure 4: TP-1454 improves efficacy of I/O therapy in colorectal syngeneic models



Combination with TP-1454 (50 mg/kg qd) improves the efficacy of anti-PD-1 in CT26 syngeneic colorectal tumor model, resulting in 50% complete response (CR)(CR=20% for a-PD-1 alone) (A). In MC38 syngeneic colorectal tumor model, double combination of aPD-1+aCTLA4 results in 70% CR, whereas triple combination of TP-1454(100 mg/kg qd)+aPD-1+aCTLA4 results in 100% CR (B).

Figure 5: TP-1454 differentially activates PKM2 in immune cells

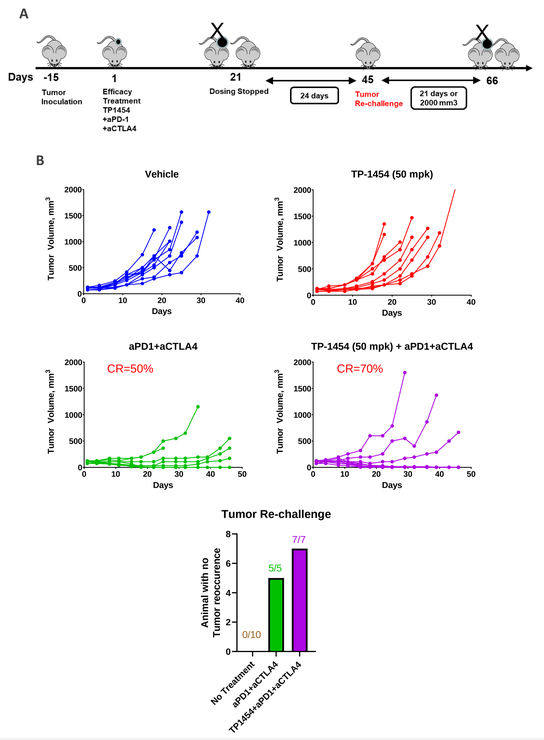


In vitro treatment of different immune cell populations with TP-1454 reveals differential levels of PKM2 activation in these populations. One immune cell type strongly activated by TP-1454 (AC50 = 123 nM) is Treg cells..

IV. Conclusions

- TP-1454: First in clinic PKM2 allosteric activator, activates PKM2 in tumors as well as immune cells.
- TP-1454 alters immune and tumor cell metabolism.
- Combination of TP-1454 with I/O therapies can enhance tumor efficacy response
- TP-1454 differentially activates immune cell types.
- Immune memory is retained after TP1454 and IO therapy combinations.
- Phase I clinical trial currently ongoing (clinicaltrials.gov, NCT04328740)

Figure 6: TP-1454 protects against tumor reoccurrence in tumor rechallenge studies



TP-1454 effects on tumor-specific immunity were validated using tumor rechallenge studies. Mice were implanted with either MC38 or RENCA cells and treated with TP-1454 and I/O combination therapies, and mice that exhibited a complete response (CR) were re-implanted (A). MC38 tumor growth curves from the efficacy portion of the study show 50% CR in the double combination group, and 70% CR in the triple combination group (B). In the RENCA and MC38 models, combination treatment of TP-1454 with I/O showed an improvement in initial treatment response and tumor reoccurrence (TR) following rechallenge, compared to I/O alone (RENCA: I/O 50% CR, 2/5 TR, TP-1454+I/O 70% CR, 2/6 TR; MC38: I/O 50% CR, 0/5 tumor reoccurrence, TP-1454+I/O 70% CR, 0/7 TR). The data from the tumor rechallenge studies is shown in Table 2.

Table 2: Summary of results from tumor rechallenge studies

Re-challenge groups	No. of animals with No Tumor Reoccurrence	
	MC38	RENCA
Naïve mice	1/10 (10%)	0/10 (0%)
aPD1+aCTLA4	5/5 (100%)	3/5 (60%)
TP-1454 (50 mpk) + aPD1 + aCTLA4	7/7 (100%)	2/4 (50%)
TP-1454 (100 mpk) + aPD1 + aCTLA4	4/4 (100%)	4/6 (67%)