The PKM2 activator and molecular glue TP-1454 modulates tumor-immune responses by destabilizing T-regulatory cells

Salah Sommakia, Satya Pathi, Yuta Matsumura, Curtis A. Allred, Ethika Tyagi, Matthew S. Lalonde, Jason M. Foulks, Adam Siddiqui, Clifford J. Whatcott, David J. Bearss, Steven L. Warner
Sumitomo Pharma Oncology, Inc., Lehi, UT

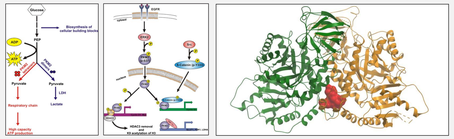
Sumitomo Pharma
Sumitomo Pharma Oncology

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 an investigational potent PKM2 activating molecular glue with with low nanomolar PKM2 activation in biochemical assays (AC50 = 10 nM) and multiple cell types (AC50 < 50 nM), tolerated in mice, rats and dogs after repeat doses as high as 1000 mg/kg/day and and is currently being investigated in 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 in combination with immunotherapy (I/O) in multiple mouse syngeneic tumor models. TP-1454 and anti-PD-1 combination therapy in colorectal cancer models showed 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. We observed 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 Liquid Chromatography Mass Spectrometry 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 activity of TP-1454 treatment on O-GlcNacylation of Foxp3 and its potential stability in HEK293 cells, to support the link between PKM2 activation and stabilization of Foxp3.
TP-1454 activity in 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 potentially improve the response of cancer patients to immunotherapy.

II. Background

Figure 1: Dimeric PKM2 supports biosynthesis and protumor transcriptional programs

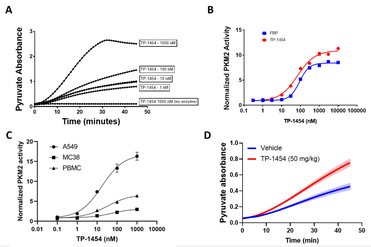


Wong et al. *Cancer Letters (2015)*

- PKM2 is an isozyme of pyruvate kinase which mediates the conversion of phosphoenolpyruvate into pyruvate, which can then either enter the TCA cycle or be converted to lactate, making it an important regulator of cellular metabolism and biosynthesis.
- In cancer cells, the lower activity dimeric form of PKM2
  - Allows continued biosynthesis
  - Alters transcription to enhance cell growth and immune evasion
- TP-1454 is an investigational small molecule which activates PKM2 by gluing two PKM2 dimers into the tetrameric form.
- Targeting PKM2 tetramerization may result in metabolic reprogramming of the TME to allow improved targeting of solid tumors.

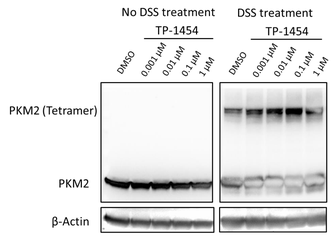
III. Results

Figure 2: TP-1454 is an investigational potent activator of PKM2 in cell lines and *in vivo*



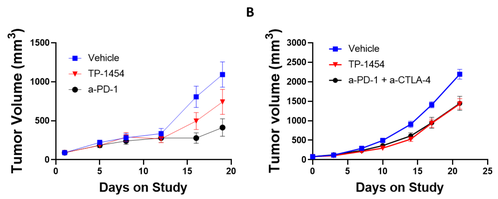
(A) TP-1454 shows PKM2 activation at nanomolar concentrations (AC50= 10 nM) in biochemical assays. (B) TP-1454 displays 1.4-fold stronger PKM2 activation than fructose 1,6-bisphosphate (FBP), a potent endogenous PKM2 activator. (B) TP-1454 displays PKM2 activation at nanomolar concentrations in multiple cell lines and human PBMCs *in vitro* with treatment times between 16 and 45 minutes. (C) TP-1454 also displays PKM2 activation in tumor tissue 4 hours after oral administration (CT26 syngeneic mouse tumors).

Figure 3: TP-1454 observed to increase PKM2 tetramerization in vitro



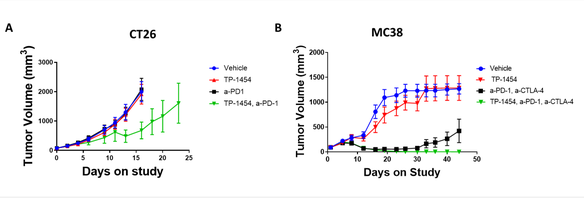
In vitro treatment of A549 cells with TP-1454 for 24 hours shows a dose-dependent decrease in PKM2 levels as seen on the left side of the blot. Using the crosslinking agent Disuccinimidyl suberate (DSS), we see an increase in PKM2 tetramer levels up to 1 µM. β-actin was used as loading control.

Figure 4: TP-1454 shows single agent activity in syngeneic tumor models



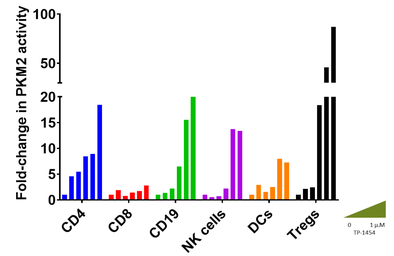
TP-1454 administered orally at 50 mg/kg q.d. shows single agent activity in the strongly I/O-sensitive MC38 syngeneic colorectal cancer model (TGI=42%, n.s.) (A) and the weakly I/O-sensitive KLN205 syngeneic lung cancer model (TGI=35%, p = 0.04) (B)

Figure 5: TP-1454 observed to improve efficacy of I/O therapy in colorectal syngeneic models



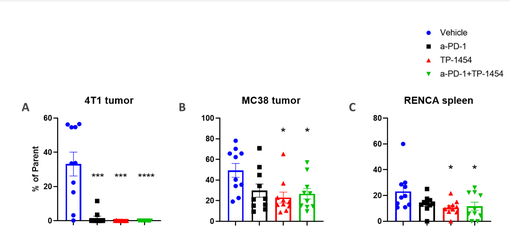
Combination with TP-1454 (100 mg/kg qd) observed to improve the efficacy of a-PD-1 (10 mg/kg BIW x 3 weeks) in CT26 syngeneic colorectal tumor model, resulting in 55% TGI vs. Vehicle (TGI=5% for a-PD-1 alone) (A). In MC38 syngeneic colorectal tumor model, double combination of a-PD-1+a-CTLA-4 (results in TGI > 98% with 70% CR, whereas triple combination of TP-1454 (100 mg/kg qd)+a-PD-1+a-CTLA4 results in 99% TGI with 100% CR (B).

Figure 6: TP-1454 shown to differentially increase PKM2 activation in immune cell population



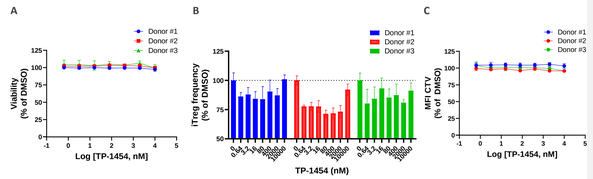
In vitro treatment of different human immune cell populations with TP-1454 for 24 hours reveals differential levels of PKM2 activation in these populations. One immune cell type that exhibits strong sensitivity to TP-1454 mediated PKM2 activation is Tregs.

Figure 7: TP-1454 shown to suppress CD4 Tregs in multiple syngeneic models



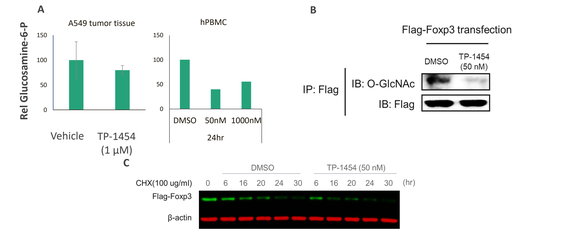
Immunophenotyping using flow cytometry revealed treatment with TP-1454 (50 mg/kg q.d.) for 10-12 days reduces CD4+ CD25+ Foxp3+ Treg population in 4T1 tumors (p = 0.002, Kruskal-Wallis test with Dunn's post-hoc comparison) (A), MC38 tumors (p = 0.0131, Kruskal-Wallis test with Dunn's post-hoc comparison) (B), and CD4+ CD25+ Foxp3+ PD-1+ Tregs in RENCA spleens (p = 0.0186, Ordinary one way ANOVA with Dunnett's post-hoc comparison (C).

Figure 8: TP-1454 observed to inhibit Treg polarization without cytotoxicity or inhibition of proliferation



In vitro treatment of naïve CD4+ T-cells from human donors with TP-1454 in presence of CD3/28, TGF-β1 and IL-2 for 7 days showed no negative effect on viability (A) but was observed to reduce the proportion of $CD4^+CD25^+FOXP3^+CD127^-$ induced Treg (iTregs) as assayed by flow cytometry (B). Treatment of iTregs with TP-1454 for 3 days showed no effect on proliferation as assayed by Cell Trace Violet (CTV) (C).

Figure 7: TP-1454 shown to inhibit O-GlcNac- modification of Foxp3 and destabilizes Foxp3



Metabolomic analysis revealed that TP-1454 reduces levels of Glucosamine-6-P, a precursor of the O-GlcNac posttranslational modification which is reported to stabilize Foxp3 in CD4+ cells, in both tumor tissue and human PBMCs (A). TP-1454 treatment of HEK cells transfected with a flag-tagged Foxp3 for 20 hours was observed to reduce O-GlcNacylation of Foxp3 as shown by Co-IP assay (B). TP-1454 also shown to results in faster degradation of Foxp3 in the presence of cycloheximide (CHX), indicating a destabilization of the Foxp3 protein. β-actin was used as loading control.

IV. Conclusions

- TP-1454, the first clinical stage PKM2 activating molecular glue, is shown to activate PKM2 in tumor as well as immune cells.
- TP-1454 is observed to increase PKM2 tetramerization.
- TP-1454 shows single agent activity as well as combination activity with checkpoint inhibitors in nonclinical models.
- Tregs are a prominent immune cell type with which TP-1454 treatment shows activity.
- TP-1454 observed to inhibit-Treg polarization without affecting viability and proliferation.
- One potential mechanism of Treg inhibition is mediated by suppression of O-GlcNacylation of Foxp3 and its subsequent destabilization.
- Phase I clinical trial currently ongoing (clinicaltrials.gov, NCT04328740).