Abstract #1345

Pharmacodynamic biomarkers for Pim-1 inhibition

Curtis Allred, Yuta Matsumura, Ethika Tyagi, Mark Wade, Clifford J. Whatcott, Jason M. Foulks, Adam Siddiqui, David J. Bearss, and Steven L. Warner

Sumitomo Dainippon Pharma Oncology, Inc. Lehi UT

I. Abstract

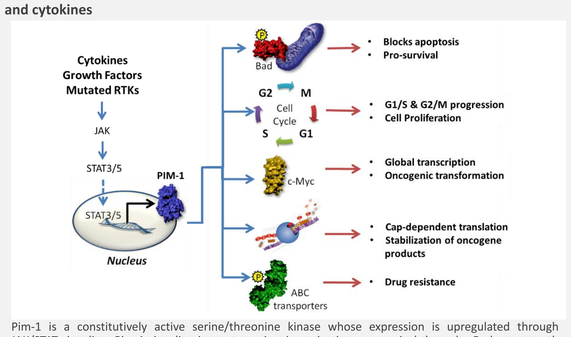
Proviral integration site for Moloney murine leukemia virus-1 (Pim-1) is a serine/threonine kinase downstream of Jak/Stat signaling which promotes cell growth, survival, and drug resistance. Pim-1 kinase is an important driver of tumorigenesis and tumor survival through its role in a number of downstream pathways, including inhibition of apoptosis through phosphorylation of the BH3-only protein BAD. Pim-1 is expressed at very low levels in most normal tissues, but is overexpressed in many cancers, such as prostate, colorectal, and many hematologic malignancies. Pim-1 kinase activity is constitutive and therefore directly proportional to protein expression. As such, Pim-1 is an attractive therapeutic target.

TP-3654 is a second-generation, oral Pim inhibitor currently in Phase I clinical trials in solid tumors and myelofibrosis (NCT03715504 and NCT04176198). TP-3654 inhibits all three Pim kinases, with Ki values for Pim-1 (5nM), and Pim-2 and Pim-3 <250 nM in a biochemical assay. Treatment with TP-3654 in Jurkat and HEL cell lines showed dose-dependent modulation of the downstream targets of PIM, such as pS6K and pBad. In a murine pharmacokintetic (PK) analysis, a dose-dependent increase of TP-3654 Cmax was observed in plasma and subcutaneous PC-3 xenograft tumors. Animals were dosed orally at 50, 100 and 200 mg/kg and time to maximum concentration (Tmax) was reached at 1 hour. Animals bearing HEL tumors were dosed similarly and Cmax values increased in a dose-dependent manner with Tmax at 2 hours. Plasma and tumor TP-3654 concentrations were above the minimum measurement threshold out to 24 hours post dose. We further explored pharmacodynamic (PD) biomarkers of Pim inhibition (pBad, pS6K, and pS6RP) in flash frozen (FF) tumor tissue at various time points in both models by western blot and saw a maximum of 48% decrease in phosphorylated S6K at 24 hours, a 35% decrease in pS6RP at 24 hours, and an 51% decrease in pBad at 8 hrs in the PC3 xenograft tumors.

We hypothesized that treatment with TP-3654 would modulate Pim signaling in both cancer cells and peripheral blood mononuclear cells (PBMCs). This allows for detection of pharmacodynamic markers using repeated peripheral blood draws instead of invasive biopsies. We treated PBMCs from multiple healthy human donors with TP-3654 ex vivo at 0.3 and 3 µM to assess the movement of Pim biomarkers. Two phosphorylation markers, pS6K and pS6RP) consistently exhibited dose-dependent decreases up to 65% as measured by western blot. Inhibition of pBad was dependent on pretreatment phosphorylation state. These results were confirmed in an automated western blot system.

II. Background

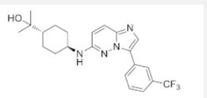
Figure 1: Pim-1 is a major effector of JAK/STAT signaling from multiple growth factors and cytokines



Pim-1 is a constitutively active serine/threonine kinase whose expression is upregulated through JAK/STAT signaling. Pim-1 signaling is pro-tumorigenic, activating pro-survival through pBad, pro-growth through S6RP, and oncogenic transformation through c-Myc

Figure 2: TP-3654 selectively binds Pim-1

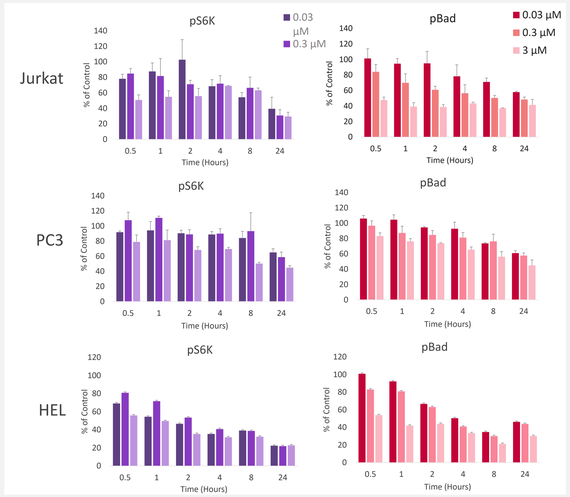
	Pim-1	Pim-2	Pim-3
Ki (nM)	1.8	183	6.4



TP-3654 has a 4-fold selectivity for Pim-1 over Pim-3 and a 100-fold selectivity over Pim-2.

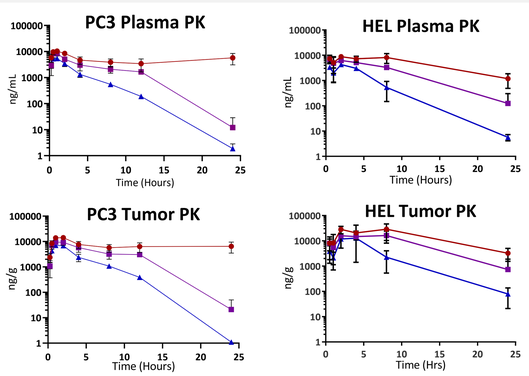
III. Results

Figure 3: TP-3654 inhibits pBad and pS6K in a dose and time dependent manner in three cancer cell lines.



Jurkat (T-ALL), PC3 (prostate cancer) and HEL (erythroleukemia) cancer cells were treated with 0.03, 0.3 or 3 µM TP-3654 for 0.5, 1, 2, 4, 8, or 24 hours. Cells were lysed and pBad and pS6k levels were measured by ELISA. Samples were normalized to 0-hour control. TP-3654 inhibits pBad and pS6k in a dose and time dependent manner The maximal inhibition in Jurkat cells was 63% and 71%, in PC3 cells 55% and 55%, and in HEL 80% and 79% for pBad and pS6K, respectively

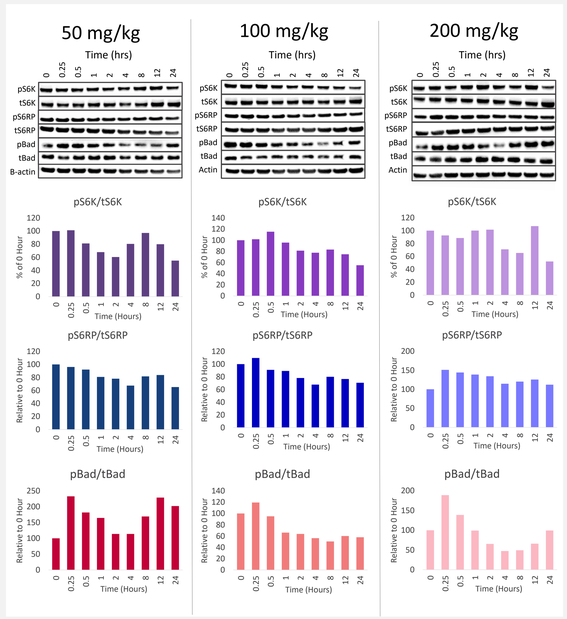
Figure 4: The PK profile of orally dosed TP-3654 in PC3 and HEL tumor-bearing mice



		PC3 Model				HEL Model			
		C_{max} (ng/mL)	T_{max} (Hrs)	AUC_{0-24} (Hr*ng/mL)	$T_{1/2}$ (Hrs)	C_{max} (ng/mL)	T_{max} (Hrs)	AUC_{0-24} (Hr*ng/mL)	$T_{1/2}$ (Hrs)
Plasma	50 mg/kg	5360	1.0	19600	1.92	4300	2.0	24100	2.27
	100 mg/kg	8570	1.0	48400	2.03	6940	0.5	65800	3.63
	200 mg/kg	10300	1.0	116000	-	11600	8.0	170000	6.56
Tumor	50 mg/kg	7032	1.0	32000	3.07	12804	4.0	82900	2.87
	100 mg/kg	9493	1.0	78700	2.05	16232	8.0	249000	4.27
	200 mg/kg	14226	2.0	171000	-	28747	8.0	459000	6.60

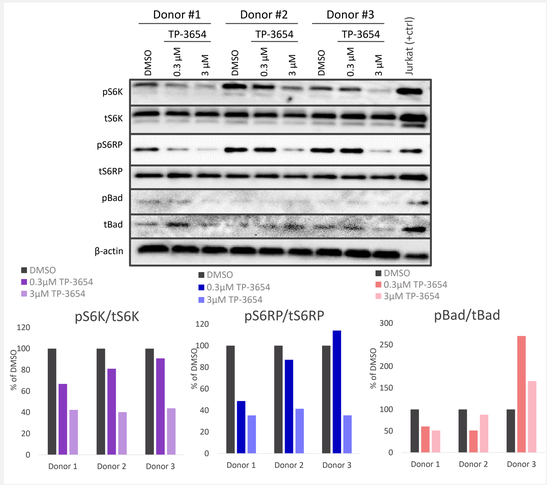
PC3 or HEL were implanted subcutaneously on the flank of male Balb/c nude or athymic nude mice, respectively. Mice were orally dosed with 50, 100, or 200 mg/kg of TP-3654 and plasma/tumor samples were collected at indicated timepoints. The 200 mg/kg animals did not reach the half life after 24 hours in the plasma and tumor of the PC3 model. The HEL model had longer half-lives and overall exposures in both plasma and tumor.

Figure 5: TP-3564 inhibits pBad, pS6k and pS6RP in PC3 xenograft model



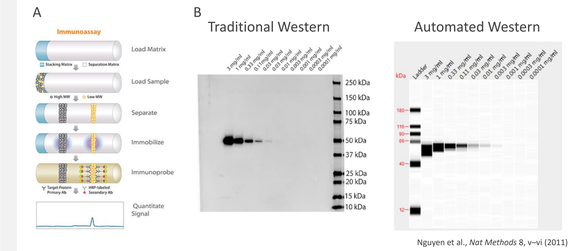
PD biomarker analysis in the tumor tissues from PC3 PK analysis. pBad, pS6k and pS6RP were inhibited in tumor tissues after TP-3654 administration. Samples were homogenized and pooled in each treatment group (n=3) and protein expression was analyzed by western blot. Total S6K, S6RP, and Bad (tS6K, tS6RP, tBad) were used as a loading controls for corresponding phospho-protein. The greatest inhibition for pS6K was observed at 48% in the 200mg/kg group at 24 hours, pS6RP was 35% in the 50 mg/kg group at 24 hours, and pBad at 51% in the 200 mg/kg group at 8 hours.

Figure 6: TP-3654 inhibits pS6K, pS6RP and pBad in human healthy donor PBMCs



TP-3654 inhibits pS6K, pS6RP and pBad in multiple healthy donor PBMCs(donor #1, #2, and #3) in a dose dependent manner after 24 hr treatment. The maximal inhibition for pS6K was observed in donor #2 of 60%, pS6RP in Donors #1 and #3 of 65%, and pBad in Donor#1 of 50%.

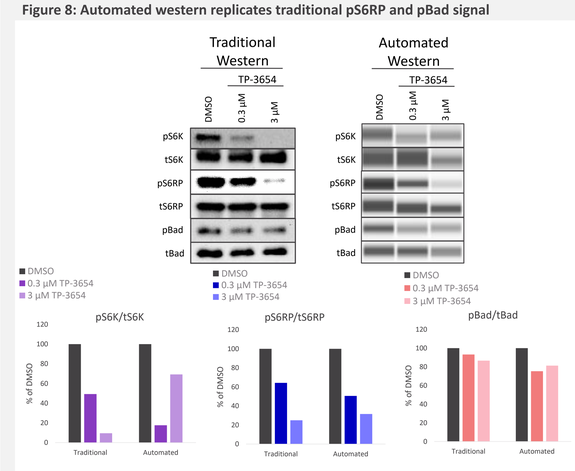
Figure 7: Automated western blot systems as an alternative to traditional western



Nguyen et al., *Nat Methods* 8, v–vi (2011)

The automated western systems from ProteinSimple, allows for faster, less hands-on protein analysis than traditional western blots. The assay flow is shown (A). Briefly, the electrophoresis takes place in capillaries, where the protein is immobilized, removing the need for transfer. The antibody probe flows through the capillaries and binds the protein of interest. The example of comparison test (Nguyen et al., Nature Methods, 2011) where serially diluted K562 cell lysate were analyzed using a glycogen synthase kinase-3α antibody is shown (B).

Figure 8: Automated western replicates traditional pS6RP and pBad signal



Healthy donor PBMCs were treated with 0.3 or 3 µM TP-3654 (or DMSO) for 24 hours. And the lysates were aliquoted and compared in both assays to assess the reliability of automated western blots. pS6RP and pBad closely correlated between the two assays, but the pS6K did not in the 3 µM treatment group.

IV. Conclusions

- TP-3654 specifically inhibits Pim-1
- TP-3654 inhibits targets of Pim-1, pBad and pS6K in cancer cell lines *in vitro*
- Long TP-3654 exposure *in vivo* in two models ($t_{1/2}$ > 6 hrs in 200 mg/kg)
- TP-3654 inhibits phosphorylation of S6K, S6RP, and Bad *in vivo*
- TP-3654 inhibits pS6K, pS6RP, and pBad in healthy donor PBMCs
- Traditional and automated western blots are equivalent for pS6RP and pBad
- Current phase I clinical trials (NCT03715504 and NCT04176198)