

The Hedgehog inhibitor IPI-926 increases tumor perfusion and enhances drug delivery in a preclinical pancreatic cancer xenograft model



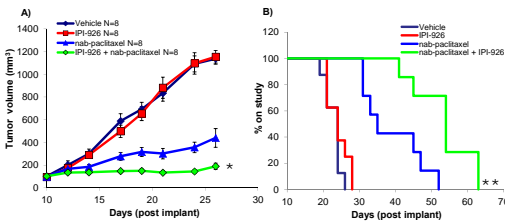
Veronica T. Campbell, John Coco, Igor Deyneko, Jennifer Proctor, Kip West, Karen McGovern, John MacDougall
Infinity Pharmaceuticals Inc., Cambridge, MA

Abstract

Ligand-dependent activation of the Hedgehog (Hh) pathway is associated with multiple tumor types. In certain cancers, such as pancreatic, a paracrine role for the Hh pathway has been described, wherein cancer cells produce Hh ligand that activates the Hh pathway in the surrounding tumor microenvironment. Pancreatic cancer has a dense, fibrous stroma that may decrease delivery of chemotherapy to the tumor cells and targeting the Hh pathway may facilitate chemotherapy delivery. IPI-926, a potent and selective Smoothened inhibitor, blocks Hh signaling in preclinical pancreatic tumor models. In a KRAS, p53 genetically engineered mouse model of pancreatic cancer (KPC), IPI-926 treatment led to increased vascular perfusion and enhanced delivery of gemcitabine, which resulted in increased overall survival (Olive, Science 2009). When the combination of IPI-926 and nanoparticle albumin bound (Nab)-paclitaxel (Abraxane®) was tested in the L3.6pl and ASPC-1 subcutaneous pancreatic xenograft models similar results were obtained. IPI-926 enhanced delivery of nab-paclitaxel in tumors, leading to an increase in cells arrested at G2/M as measured by phospho-histone-H3 (PH3) staining, and increased anti-tumor activity resulting in improved survival.

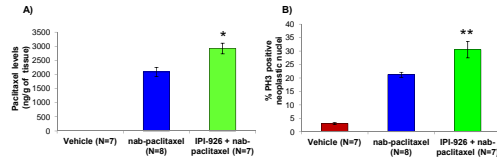
To further explore the effects of IPI-926 on the surrounding tumor microenvironment that lead to enhanced drug delivery, tumor perfusion and vasculature were studied by ultrasound, vascular casting and immunohistochemical (IHC) methods in the L3.6pl subcutaneous xenograft model. Tumor perfusion was directly measured in IPI-926 treated and untreated animals using contrast enhanced ultrasound. In tumor bearing animals treated with IPI-926 for 7 days, the ultrasound data revealed greater tumor perfusion with IPI-926. On average, the peak time for contrast agent infiltration into the tumor decreased from 11.0 seconds to 4.75 seconds in the vehicle versus IPI-926 treated animals respectively, (p=0.0321). Vascular casting methods were also employed to study IPI-926 effects on tumor vasculature and perfusion. Analysis of micro CT images shows that L3.6pl tumors treated with IPI-926 have 5-8 fold increased vascular volume compared to control but no change in vascular radius, implying that the number of vessels increased but not the vessel size. In support of these results, IHC analysis of the vessel endothelial marker, MECA-32, also demonstrated an approximate 2-fold increase in vessel density with IPI-926 treatment. Taken together, these data suggest that IPI-926 has an effect on the surrounding tumor vasculature that increases tumor perfusion and leads to enhanced drug delivery. These preclinical data provide the rationale for evaluating the Hh inhibitor IPI-926 in pancreatic cancer in combination with not only with the current standard of care gemcitabine, but also with emerging therapies like nab-paclitaxel. IPI-926 is currently in a randomized Phase 2 trial in metastatic pancreatic cancer in combination with gemcitabine vs gemcitabine alone.

Enhanced activity of nab-paclitaxel when co-administered with IPI-926



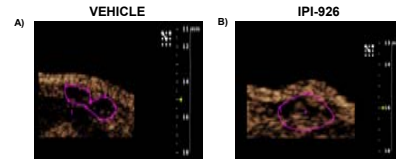
Combination treatment effect in the L3.6pl pancreatic xenograft model. The L3.6pl human pancreatic cell line was implanted subcutaneously. IPI-926 was administered orally at 40mg/kg QOD and nab-paclitaxel was administered i.v. at 20mg/kg QW1. A) On day 26, compared to the vehicle control, nab-paclitaxel alone showed 61% tumor growth inhibition, while the combination with IPI-926 resulted in 83% tumor growth inhibition (**p=0.0048). B) Mice remained on treatment and time to reach 1000mm³ was recorded. The combination showed an increase in median % on study (day 54), versus nab-paclitaxel alone (day 35) (**p<0.001), while IPI-926 had no effect as a single agent.

Increased paclitaxel levels and late G₂M arrest in IPI-926 treated tumors



Increased paclitaxel levels and phosphorylated histone H3 (PH3) staining in L3.6pl tumors treated with the combination of IPI-926 and nab-paclitaxel. 24 hours after the last dose of IPI-926 and nab-paclitaxel, tumors were collected for PK analysis and PH3 immunostaining. A) The combination of IPI-926 and nab-paclitaxel resulted in 28% higher paclitaxel levels in the tumors compared to the tumors treated with nab-paclitaxel alone (*p<0.001). B) PH3 quantitative whole section analysis revealed a 33% increase of tumor cells accumulating in the late G₂M phase, in the combination group versus the nab-paclitaxel alone group (**p=0.0014).

IPI-926 increases tumor perfusion



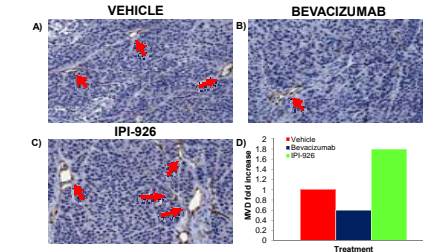
Treatment	* Time to Peak (s)
Vehicle N=4	16
	13
	8
	7
IPI-926 N=4	3
	4
	6

* Mean IPI-926 values are statistically significant compared to mean control values (p<0.05, Student's t test)

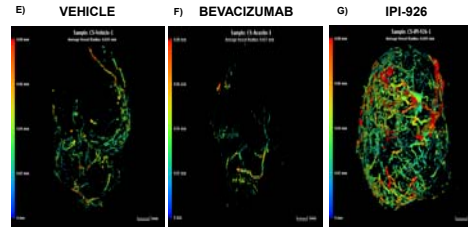
IPI-926 treatment increases tumor perfusion in the L3.6pl model. The L3.6pl tumor cell line was injected subcutaneously and treatment with IPI-926 was initiated. IPI-926 or vehicle was administered orally at 40mg/kg for seven consecutive days. Mice were subjected to ultrasound image analysis using perfusion contrast enhancement (microbubbles) during the imaging procedure. A, B) Vehicle-treated animals imaged via ultrasound show less contrast agent in the tumors than the IPI-926-treated. The time to reach peak contrast was measured and showed a decrease in the IPI-926 treated animals compared to vehicle (table 1).

IPI-926 has a significant effect on tumor vasculature

IPI-926 increases vessel density



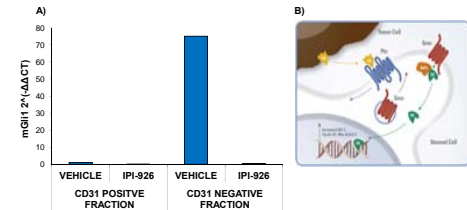
IPI-926 increases functional tumor vascular volume



Treatment Group	Avg. Volume (mm ³)	Avg. Surface Area (mm ²)	Avg. Vessel Radius (mm)
Vehicle	0.7	34.	0.04
Bevacizumab	0.3	16.3	0.03
IPI-926	4.6	165	0.05

In L3.6pl tumors, IPI-926 treatment increases microvessel density, vessel volume and vessel function. Mice bearing L3.6pl bilateral tumors were treated for 20 days with IPI-926 @ 40mg/kg, QOD, for a total of 10 doses, bevacizumab QW1 @ 5mg/kg, for a total of 4 doses or Vehicle control. A, B & C) Tumors were collected, fixed and stained with the mouse vessel marker, MECA32. D) The Aperio microvessel algorithm quantification demonstrates ~ 2-fold increase in microvessel density (MVD) in the IPI-926 group. E, F & G) A three part perfusion of the mouse was performed to clear, fix and inject a lead-oxide resin into the vasculature. Tumors were then collected and micro CT imaged. Compared to Control and Avastin, the IPI-926 image clearly shows a large increase in vessel area and volume. The calculated values for each group are listed in Table 1.

IPI-926 blocks Gli1 signaling in the non-endothelial stromal compartment



IPI-926 treatment down regulates stromal Gli1 in a non-endothelial cell.

Whole tumor analysis showed that IPI-926 modulates Gli1 expression in the tumor stroma but not the tumor cells (data not shown). To specifically evaluate Gli1 expression in endothelial cells +/- IPI-926 treatment, we set up L3.6pl sorting experiments. Tumors cells were depleted by sorting onto the EPCAM+ cells and endothelial cells were sorted based on CD31 expression. A) Q-RT-PCR analysis revealed inhibition of murine Gli1 mRNA expression, following a single dose of IPI-926, in the CD31 negative fraction. B) Schematic of Hh paracrine signaling between pancreatic tumor cells and stromal cells.

Summary/Conclusions

- Combination administration of IPI-926 and nab-paclitaxel (Abraxane®) leads to increased tumor growth inhibition, increased paclitaxel tumor levels and concomitant arrest of tumor cells in late G₂/M.
- Administration of IPI-926 leads to an increased vasculature volume assessed by vascular casting and analysis of vessel density and tumor perfusion.
- Analysis of Hh pathway signaling indicates that IPI-926 blocks Hh signaling in the non-endothelial stromal compartment; thus, the effects on tumor vasculature are indirect.
- This preclinical data provide a rationale for combining IPI-926 with a variety of chemotherapies in pancreatic cancer.
- A randomized Phase 2 trial is ongoing in patients with metastatic pancreatic cancer to evaluate IPI-926 in combination with gemcitabine vs gemcitabine alone**.

Acknowledgments: We thank Gohar Mushtaq and Neil Desai from Abraxis Bioscience, LLC for help with the tumor PK analysis.

**Cintrials.gov