

The Hh inhibitor IPI-926 enhances tumor perfusion and nab-paclitaxel activity in a pancreatic xenograft model



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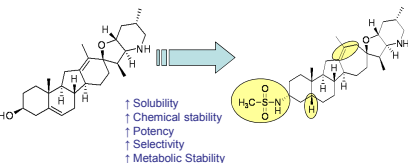
#LB-374

Abstract

Malignant activation of the Hedgehog (Hh) pathway is associated with multiple tumor types. In certain cancers, such as pancreatic, a paracrine role for the Hh ligand has been described, wherein cancer cells produce Hh ligand that activates the Hh pathway in the surrounding stroma. Consistent with this model, IPI-926, a potent and selective Smoothened (Smo) inhibitor, blocks Hh signaling in the mouse stroma but not in the cancer cells of several pancreatic xenograft models. We recently published (Olive, Science 2009) that IPI-926 increases vascular perfusion and enhances gemcitabine drug delivery to tumors in a genetically engineered mouse model of pancreatic cancer (KPC) leading to an increase in overall survival. To determine if similar effects could be observed in xenograft models of human pancreatic cancer, experiments were designed to study the combination of IPI-926 with nab-paclitaxel, an agent that has recently demonstrated anti-tumor activity in pancreatic cancer (Von Hoff, ASCO 2009). While IPI-926 had no single agent activity in the L3.6pl human pancreatic xenograft model, it enhanced the activity of nab-paclitaxel from 61% tumor growth inhibition (nab-paclitaxel alone) to 83% tumor growth inhibition (nab-paclitaxel plus IPI-926, p<0.0048). Tumor IHC analysis of phosphorylated histone H3 (PH3) showed a higher frequency of cells arrested at the late G₂/M phase in the IPI-926 plus nab-paclitaxel group versus nab-paclitaxel alone (p=0.0014). One possible explanation for the synergistic effect of a combination of IPI-926 and nab-paclitaxel is that IPI-926 affects the mouse stroma and increases tumor perfusion and nab-paclitaxel accessibility to the tumor. 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 showed greater tumor perfusion with IPI-926. On average, the peak time for contrast agent levels decreased from 11.0 seconds to 4.75 seconds in the vehicle versus IPI-926 treated animals, respectively. (p=0.0321).

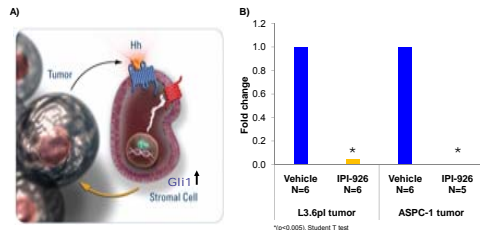
These data suggest that the mechanism of synergy between IPI-926 and nab-paclitaxel is likely enhanced drug delivery to the tumor through the effect of IPI-926 on the stroma. Studies are ongoing to measure nab-paclitaxel and paclitaxel levels in IPI-926 treated and untreated tumors, and to investigate these findings with the KPC *in situ* mouse model of pancreatic cancer. These preclinical data provide a strong rationale for evaluating the Hh inhibitor IPI-926 not only with the current standard of care, gemcitabine, but with emerging new potential therapies like nab-paclitaxel in pancreatic cancer.

IPI-926



	C3H10 Diff. IC50 (nM)	Smo binding (nM)
cyclopamine	260	114
IPI-926	7	2

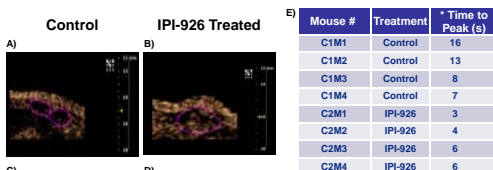
IPI-926 inhibits Hh paracrine signaling in pancreatic xenograft models



IPI-926 treatment down regulates stromal Gli1 in pancreatic xenograft models.

A) Schematic of Hh paracrine signaling between tumor cells and stromal cells. B) The L3.6pl and ASPC-1 human pancreatic cell lines were implanted subcutaneously. IPI-926 was administered orally at 40mg/kg and tumors were collected 24 hours later. Q-RT-PCR analysis revealed inhibition of murine Gli1 mRNA expression with IPI-926 treatment. Human Hh ligand expression was detected and human Gli1 mRNA levels were not modulated with treatment (data not shown).

IPI-926 increases tumor perfusion

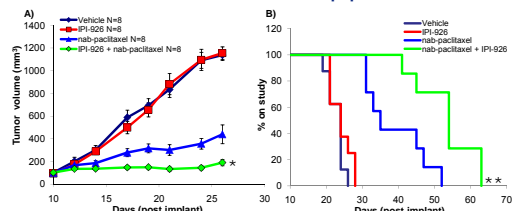


IPI-926 treatment increases the time to peak tumor levels of the injected contrast agent.

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. Vehicle treated animals imaged via ultrasound show less contrast agent in the tumors than the IPI-926 treated (figures A and B). The time to reach peak contrast was measured and showed a decrease in the IPI-926 treated animals compared to vehicle (figures C and D and tabulated in E).

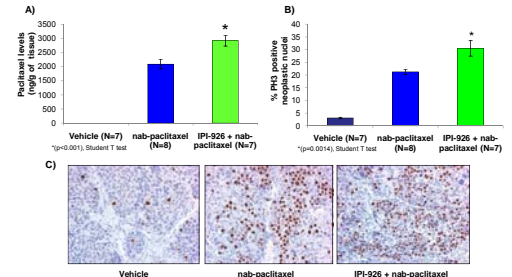
IPI-926 enhanced activity of nab-paclitaxel in L3.6pl tumor bearing mice

In vivo Combination effect in the L3.6pl pancreatic model



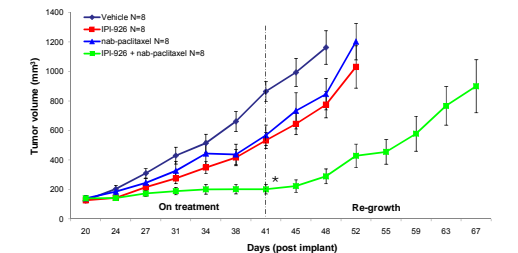
Increased tumor growth inhibition in the IPI-926 and nab-paclitaxel combination group in a pancreatic xenograft model. The L3.6pl human pancreatic cell line was implanted subcutaneously and treatment was initiated on Day 10 after implant. IPI-926 was administered orally at 40mg/kg QOD and nab-paclitaxel was administered i.v. at 20mg/kg QW1. A) On day 26, compared to the vehicle control, the nab-paclitaxel alone group showed 61% tumor growth inhibition, while the combination of IPI-926 and nab-paclitaxel resulted in an 83% tumor growth inhibition (*p<0.0048). B) Mice remained on treatment and time to reach 1000mm³ was recorded. Once tumors reached 1000mm³, mice were taken off study. The combination group 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.

Higher paclitaxel levels and increased late G₂/M arrest was detected in the IPI-926 and nab-paclitaxel 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. On day 27, 24 hours after the last dose of IPI-926 and nab-paclitaxel, tumors were collected for PK analysis and PH3 immunostaining. A) The combination treatment 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 & C) PH3 quantitative whole section analysis revealed a 33% increase of tumor cells accumulating in the late G₂/M phase, in the combination treatment group versus the nab-paclitaxel alone group (p<0.0014, 20x).

IPI-926 and nab-paclitaxel combination therapy delays tumor re-growth in ASPC-1 tumor bearing mice



Increased tumor growth inhibition and delay in tumor re-growth of the IPI-926 and nab-paclitaxel combination group. The ASPC-1 human pancreatic cell line was implanted subcutaneously and treatment was initiated on day 20 after implant. IPI-926 was administered orally at 40mg/kg QOD and nab-paclitaxel was dosed i.v. at 20mg/kg QW1. The final i.v. dose of nab-paclitaxel was administered on day 34 and the final dose of IPI-926 was administered on day 41. On day 41, compared to the vehicle control, both IPI-926 and nab-paclitaxel showed single agent activity resulting in 38% and 34% tumor growth inhibition, respectively. In combination, IPI-926 and nab-paclitaxel resulted in a 77% tumor growth inhibition (*p=0.0048). Re-growth was monitored after the cessation of treatment.

Conclusions

Hedgehog (Hh) signaling can occur in a paracrine manner in pancreatic xenograft models, where the human tumor cells provide Hh ligand and activate murine Gli1 in the stromal cells. IPI-926 treatment inhibits murine Gli1 expression in the stromal cells.

Inhibition of the Hh pathway in tumor stroma with IPI-926 leads to increased tumor perfusion in a subcutaneous xenograft model of pancreatic cancer.

Combination treatment of IPI-926 and nab-paclitaxel leads to increased tumor growth inhibition, and increased paclitaxel tumor levels and late G₂/M tumor cell accumulation. These data, along with the increase in tumor perfusion, show that IPI-926 can enhance tumor drug delivery in a subcutaneous pancreatic tumor model.

The preclinical data provide rationale for combining IPI-926 with a variety of chemotherapies in the pancreatic cancer setting. Efforts are ongoing to study IPI-926 in combination with gemcitabine + nab-paclitaxel in the KPC *in situ* mouse model of pancreatic cancer.

Infinity has initiated a randomized clinical trial evaluating IPI-926 in combination with gemcitabine in patients with metastatic pancreatic cancer.

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