

Activity of IPI-926, a novel inhibitor of the Hh pathway, in subcutaneous and orthotopically implanted xenograft tumors that express SHh ligand

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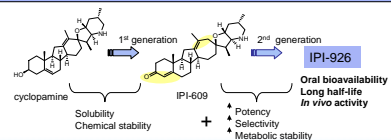
Abstract

Background: IPI-926 is a novel, potent and selective inhibitor of the Hedgehog pathway and functions as a Smoothened (Smo) antagonist. The Hedgehog (Hh) signaling pathway is known to be important in the development of several organ systems, most notably the gastrointestinal tract and lungs. Moreover, Hedgehog signaling is also important for the growth and survival of cancers of these organs. Herein, the *in vivo* efficacy of IPI-926 was evaluated in pancreatic cancer tumor models. **Results:** We observed a significant inhibition of xenograft tumor growth which was mediated, at least in part, through inhibition of the Hh pathway in the stroma of tumors that express Hedgehog ligand. Thus, daily dose administration of IPI-926 in a subcutaneous (Bx-PC3) or orthotopic (Panc1) pancreatic cancer model at 40 mg/kg resulted in significant tumor growth inhibition after a 28 day treatment course. When a single dose of IPI-926 was administered in these human tumor models, the result was rapid Hh pathway inhibition, as measured by Gli-1 expression, in the murine cells, but not in the human tumor cells themselves. Consistent with inhibition of Hedgehog signaling by IPI-926, similar results were observed with a single administration of the mAb 5E1, a neutralizing antibody targeted to both SHh and Ihh, strongly implicating a role for ligand produced by tumor cells. These data extend from pancreatic cancer to include a number of other Hh expressing cancers, notably colon cancer in which IPI-926 treatment resulted in a similar pattern of stromal response, presumably driven by tumor derived Hh ligand. Expression of Hh ligand appears to be a common feature of a number of cancer types, including pancreatic, colon, breast and ovarian cancer. Finally, efforts to elucidate the identity of the IPI-926 responsive stromal cells have revealed that these cells reside in a non-CD31 expressing subset of cells, suggesting that the anti-tumor effect of IPI-926 is not directly related to the tumor vasculature. **Conclusion:** These data suggest that tumor-stromal interactions, mediated by Hh ligand, are an important attribute for the growth of pancreatic cancer, and may be important for other cancers as well.

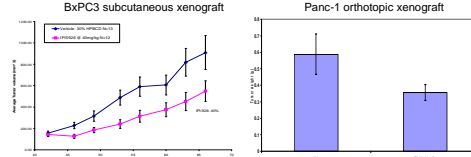
Methods

In vivo studies – Tumor bearing animals were administered single doses of IPI-926 (40 mg/kg, p.o.) or mAb 5E1 (6 mg/kg, i.p.) for studies measuring pathway modulation. In efficacy studies, animals were administered daily doses of IPI-926 (40 mg/kg, p.o.) for 28 consecutive days. Tumor size was measured twice weekly with calipers. For the orthotopic study, terminal tumor weights were recorded. **Immunohistochemistry** – Formalin fixed and paraffin embedded tissue samples were processed and embedded according to standard procedures. 5µm sections were blocked and stained with a primary antibody raised against SHh protein. Slides were subsequently washed, probed with HRP conjugated secondary antibody and developed using DAB. **Stromal cell enrichment** – L3.6pl tumors were harvested from animals after a single dose of vehicle or IPI-926 (40 mg/kg, p.o.). Tumors were enzymatically dissociated into single cell suspensions and subjected to the indicated magnetic bead based cell separation. Resulting populations of enriched cells were then processed for RNA or immunofluorescence analysis for Gli-1 expression. **RT-PCR analysis** – RNA was purified from tissue samples using standard procedures and analyzed using primer/probe sets specific for murine and human Gli-1, GAPDH and murine CD31. **Immunofluorescence** – Murine stromal cells isolated from xenograft tumors were fixed to cytopsin onto glass slides. Immunofluorescent staining with a Gli-1 antibody followed fixation of samples in 4% PFA. An Alexa-568 labeled secondary was used to reveal Gli-1 antibody binding, and DAPI was used as a counterstain.

Discovery of IPI-926

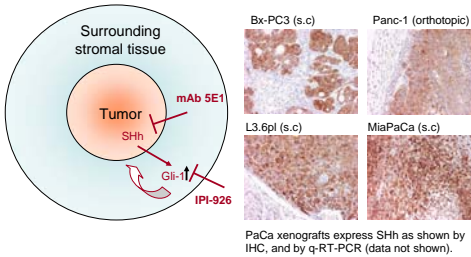


IPI-926 is efficacious in PaCa Xenografts

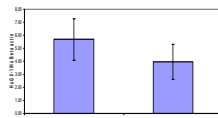


IPI-926 inhibits the growth of pancreatic xenografts. Bx-PC3 or Panc-1 tumors were grown subcutaneously (Bx-PC3) or orthotopically (Panc-1) in nude mice. Tumor size (volume measurement or tumor weight) was evaluated at the end of 28 consecutive days of daily administration of IPI-926 (40 mg/kg, p.o.). A significant inhibition of tumor growth was observed with IPI-926 treatment when compared to vehicle treated control.

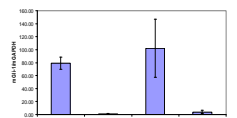
Stromal Hh pathway modulation accompanies efficacy



Bx-PC3 derived human GLI-1



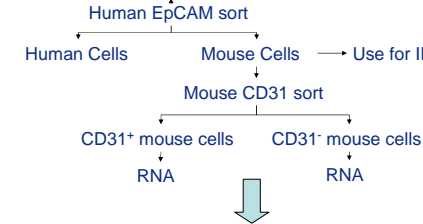
Stromal derived murine Gli-1 response to IPI-926 or 5E1 mAb



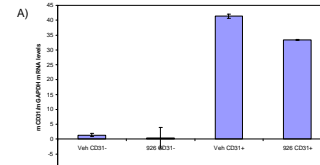
Murine Gli-1, not human Gli-1, is down-regulated after IPI-926 or 5E1 administration. Bx-PC3 tumor samples were collected 24 hours after administration of IPI-926 (40mg/kg, p.o.) or 5E1 mAb (6mg/kg, i.p.). Maximum mGli-1 inhibition, with a single dose of IPI-926 (40mg/kg, p.o.) extends to at least 48 hours (data not shown).

IPI-926 stromal responsive cell is not CD31+

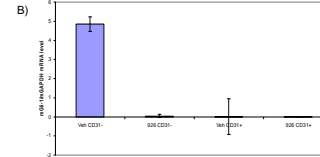
L3.6pl Tumor (Vehicle or IPI-926 treated)



mCD31 transcript levels are higher in mCD31+ sorted fractions

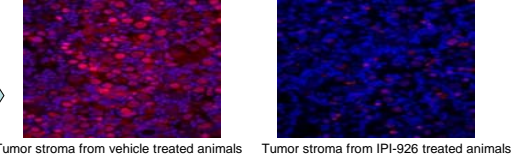


mGli-1 levels 24 hours post IPI-926 treatment in CD31+ and CD31- fractions



The CD31 negative fraction, of murine stromal cells, expresses Gli-1 mRNA levels that respond to IPI-926 treatment. L3.6pl tumors were grown *in vivo*, extracted, and dissociated for sorting. Human tumor cells were removed by a Human EpCAM sort. The eluted mouse cells were bound with CD31 MACS beads and sorted to obtain CD31+ and CD31- fractions. A) CD31 expression in +/- fractions was confirmed by q-RT-PCR. B) mGli-1 RNA levels in CD31 +/- were analyzed by q-RT-PCR.

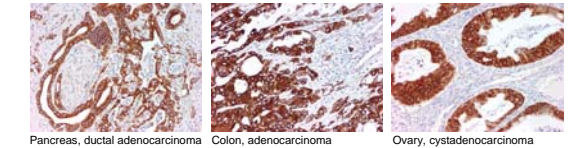
Gli-1 protein levels in murine stromal cells is modulated by IPI-926



Stromal cells isolated from vehicle and IPI-926 treated tumors. Stromal cells isolated from IPI-926 treated tumors reveals that Gli-1 protein (red fluorescence) is modulated in a subset of tumor associated stromal cells. This coupled with our RNA analysis indicates that a non-endothelial cell population is the direct target of IPI-926 in these models.

SHh expression is a common occurrence in human tumors

| Cancer Type | Total # of Tumor samples | Positive | Negative | Percent positive |
|-------------|--------------------------|----------|----------|------------------|
| Pancreatic | 92 | 65 | 27 | 70% |
| Colon | 69 | 58 | 11 | 84% |
| Ovarian | 68 | 28 | 36 | 44% |



Immunohistochemical staining of patient samples IHC staining of clinical samples reveals a high proportion of pancreatic, colon, and ovarian cancer specimens staining positive for SHh expression.

Summary/Conclusions

Summary

- Oral administration of IPI-926 inhibits tumor growth in pancreatic cancer xenografts
- IPI-926 treatment modulates Hh pathway signaling in surrounding tumor stroma
- The stromal cells that respond to IPI-926 are non-endothelial cells
- A high percentage of human pancreatic (70%), colon (84%), and ovarian (44%) tumors express SHh ligand

Conclusions

- Treatment of mice bearing human pancreatic tumors with IPI-926 results in inhibition of SHh-dependent tumor-stromal signaling, thereby inhibiting tumor growth
- Expression of Hedgehog ligand appears to be a common feature in pancreatic, colon, and ovarian cancer, suggesting anti-cancer potential of IPI-926 in these settings