IPI-926 delays metastatic spread in an experimental model of pancreatic liver metastases

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Abstract

Metastases, or the spread of disseminated tumor cells to and colonization of distal sites, is the major cause of cancer mortality in numerous cancer types. The Hedgehog (Hh) signaling pathway is normally involved in embryogenesis; however recent evidence demonstrates a key role for this pathway in cancer and metastases. Thus, pharmacologic agents that inhibit Hh signaling have the potential to be used as novel targeted anti-tumor therapies and the management of metastatic disease. IPI-926, a novel, oral molecule that antagonizes the Hh pathway by binding to Smoothened, is currently in Phase 2 clinical trials for pancreatic cancer, chondrosarcoma and primary myelofibrosis. We have previously demonstrated that administration of IPI-926 in combination with gemcitabine inhibits metastatic spread in a transgenic model of pancreatic ductal adenocarcinoma (Olive et al., 2009), supporting an integral role for the Hh pathway in this process.

To further investigate the activity of IPI-926 in the setting of metastases, we developed a novel experimental model of pancreatic cancer liver metastases where luciferase-tagged tumor cells were intra-splenically implanted followed by splenectomy, resulting in frank liver metastases. Tumor burden as assessed by bioluminescence and survival were employed to evaluate IPI-926 efficacy and different dosing regimens were explored. While treatment of tumor-bearing animals with established disease provided no survival benefit, IPI-926 administered on the day of cell implant provided modest but reproducible increases in survival. Importantly, pre-implantation administration of IPI-926 beginning 14 days prior to cell implant resulted in significant decreases in disease burden which translated to enhancement of survival. This effect was intimately dependent on the timing of IPI-926 initiation because drug treatment begun 7 days prior to cell implant was less effective than at 14 days, and treatment started only 2 days prior to cell implant was no different than when begun on the day of cell implant. Molecular analysis of serum harvested from IPI-926 14 day pre-treated versus control animals showed down-regulation of components of the VEGF, PDGF and MMP pathways, suggesting that IPI-926 modulation of these pathways may be involved in the observed effects on metastatic spread.

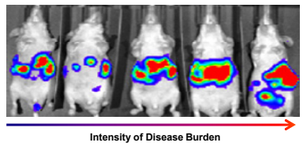
In support of a role of IPI-926 in interfering in metastatic progression we also observed an effect of IPI-926 on sunitinib-induced enhancement of metastases. Indeed, as has been shown previously (Ebos et al., 2010), short, high-dose sunitinib administration resulted in striking increases in disease burden and shortening of survival compared to control. However, when IPI-926 was administered 14 days prior to cell implant and sunitinib treatment, disease burden and survival were returned to the levels observed in the control group.

Taken together, these data demonstrate that IPI-926 is able to delay metastases in a novel experimental model of pancreatic cancer liver metastases and in response to high-dose anti-angiogenic therapy. These results suggest that IPI-926 could potentially be useful as adjuvant/neoadjuvant therapy in helping control metastatic spread post surgical resection.

Development of a novel experimental liver metastases model

L3.6pl pancreatic cancer tumor cells, engineered to express the firefly luciferase gene, were injected directly into the spleen of Ncr/Nu mice, immediately followed by a splenectomy to limit the formation of a primary tumor at the injection site. Disease is then followed via IVIS imaging system, a non-invasive system that utilizes firefly luciferase, which when bound to a reporter within the implanted cells emits a photon flux (bioluminescence), which is directly proportional to the number of cells within the tumor.

At 3 Weeks, massive metastatic infiltration of the liver with a >95% take rate.



Pre-implantation treatment with IPI-926 leads to the delay of metastatic growth

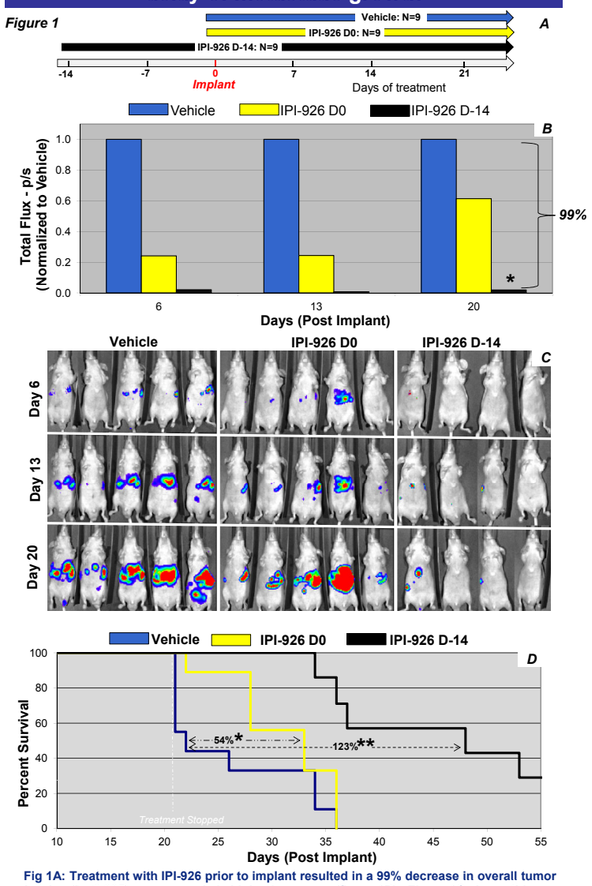
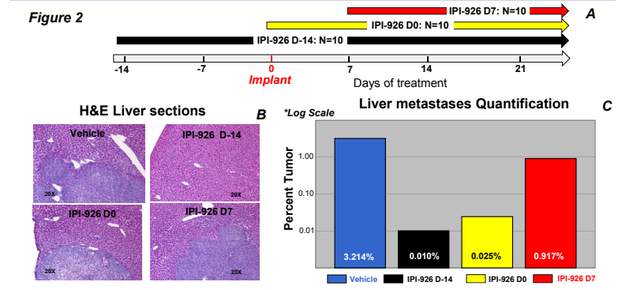


Fig 1A: Treatment with IPI-926 prior to implant resulted in a 99% decrease in overall tumor burden (*p<0.005) as assessed via bioluminescence (figure 1B). Figure 1C shows this decrease in tumor burden.

Figure 1D: Mice were followed after the 3-week dosing period ended for survival (*p<0.05, **p<0.005).

Histological analysis of IPI-926 mediated decrease of metastatic burden



Mice implanted with L3.6pl cells were treated with IPI-926 according to the schedule outlined in figure 2A. Livers were harvested at the conclusion of the study and subjected to histological analysis using Hematoxylin & Eosin (figure 2B).

Fig 2C: The tumor content of the liver was quantified by H&E staining using the Aperio digital pathology platform.

IPI-926 decreases the levels of mediators of metastases

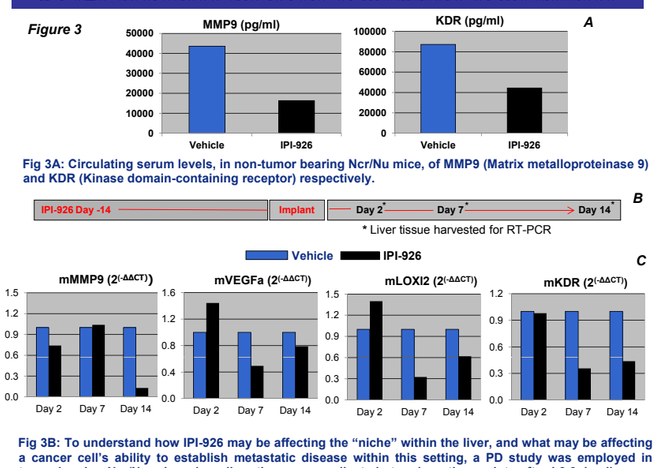
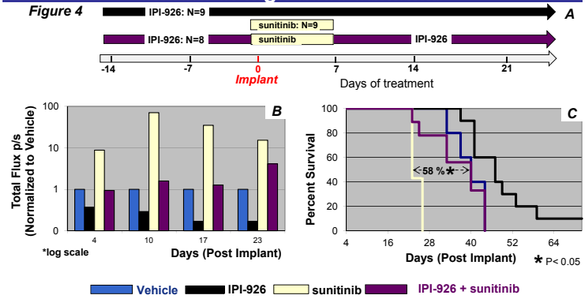


Fig 3A: Circulating serum levels, in non-tumor bearing Ncr/Nu mice, of MMP9 (Matrix metalloproteinase 9) and KDR (Kinase domain-containing receptor) respectively.

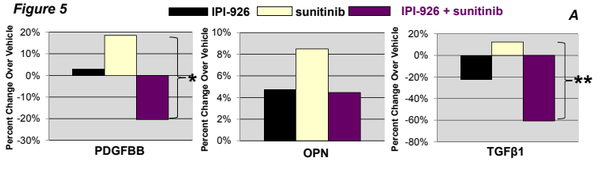
Fig 3B: To understand how IPI-926 may be affecting the "niche" within the liver, and what may be affecting a cancer cell's ability to establish metastatic disease within this setting, a PD study was employed in tumor-bearing Ncr/Nu mice where liver tissue was collected at various time points after L3.6pl cells were intrasplenically implanted and analyzed for numerous mediators of metastases (figure 3C).

IPI-926 pre-implantation treatment abrogates the effects of high dose sunitinib



Multiple studies in the literature have shown an increase of metastasis caused by angiogenesis inhibitors. Pre-implantation treatment with IPI-926 was combined with post implant-high dose sunitinib (figure 4).

IPI-926 regulates sunitinib induced mediators of metastases



Pre-implantation treatment with IPI-926, in non-tumor bearing immune competent mice regulates certain circulating mediators of metastases that are observed to change when subject to high dose sunitinib treatment. (*p<0.05, **p<0.005)

IPI-926 as a single agent also mitigates levels of MMP9 and KDR (data not shown) in this model, correlating with data seen in figure 3.

Summary / Conclusions

- Pre-implantation treatment with IPI-926 decreases metastatic burden and increases survival in an experimental model of pancreatic liver metastases.
- IPI-926 pretreatment abrogates the increase in metastases induced by high dose sunitinib.
- The effects of IPI-926 in both settings correlate with decreases in the mRNA of several proteins involved in the metastatic process.
- Taken together, these data support the further investigation of IPI-926 in the setting of pancreatic metastases.
- IPI-926 is currently being evaluated in three Phase 2 clinical trials: 1) Randomized Ph2 in combination with gemcitabine in patients with metastatic pancreatic caner 2) Randomized Ph2 in patients with unresectable or metastatic chondrosarcoma and 3) Single arm Ph2 in patients with myelofibrosis.

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