

Direct targeting of the Hedgehog pathway in primary chondrosarcoma xenografts with the Smoothened inhibitor IPI-926

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Abstract

Chondrosarcoma is a malignant cartilage tumor in which there is constitutive activation of Hedgehog-mediated signaling. Pharmacologic agents that inhibit Hedgehog (Hh) signaling have the potential to be used as novel targeted anti-tumor therapies. IPI-926, a novel, selective, molecule that antagonizes the Hh pathway by binding to Smoothened, is currently in clinical trials. The activity of IPI-926 was assessed in human primary chondrosarcoma tumors obtained at surgery from six different donors and grown as subcutaneous xenografts in NOD/SCID mice. Studies were conducted in primary chondrosarcoma xenografts to assess the activity of IPI-926 either at time of implant or in established tumors and to compare the anti-tumor activity of IPI-926 to chemotherapy and targeted agents. Tumor tissue was collected post-treatment at the end of each study for histopathological analysis or for evaluation of expression of Hh pathway genes by RT-PCR for GLI1, PTCH1, and SMO mRNA.

The chondrosarcoma tumor sizes were significantly smaller in the IPI-926 treated groups, compared to either control or chemotherapy-treated groups. There was also less cellularity, with cells appearing more differentiated, in the IPI-926 treated group. The tumors from mice treated with IPI-926 had significantly reduced expression of Hedgehog target genes GLI1 and PTCH1 compared to those from mice treated with vehicle or other chemotherapies. In addition, inhibition of GLI1 and PTCH1 gene expression was detected in the human tumor cells, a finding that has not been previously demonstrated in carcinomas, where the Hedgehog blockade seems to affect primarily the murine-derived stem cells. As expected, inhibition of Hh target gene expression was also detected in the tumor stroma in these xenografts. Initial results from gene expression profiling of the tumor cells suggest that several additional genes may be affected by IPI-926 treatment.

In summary, IPI-926 administration to mice bearing tumors derived from primary human chondrosarcoma tumors results in down-modulation of the Hh pathway in the tumor cells, as demonstrated by evaluation of the Hh-dependent genes GLI1 and PTCH1. Hh pathway gene expression is also inhibited in the tumor stroma. Down-modulation of the Hh pathway with IPI-926 results in inhibition of growth of both newly implanted and established chondrosarcoma tumors. Decreased tumor growth is accompanied by histopathological changes, including loss of cellularity and calcification. Thus, IPI-926 directly targets the Hh pathway in chondrosarcoma tumor cells and results in growth inhibition and changes in the tumor histopathology. These studies provide strong scientific rationale for further evaluation of Hh pathway inhibition with IPI-926 in humans with chondrosarcoma.

Chondrosarcoma background

- Chondrosarcomas are malignant tumors of the cartilage
- “Conventional” histology – 85%
- Multidisciplinary approach to treatment, surgery primary modality
- Indolent course followed by transition to more aggressive behavior
- Incurable when metastatic / unresectable
- Lack of established systemic standard of care
- Evidence for Hh pathway as a rational therapeutic target

Chondrosarcoma model development

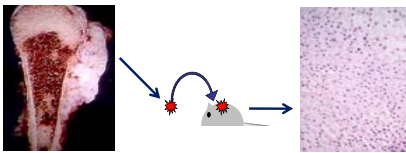


Figure 1. Primary chondrosarcoma xenograft models were established by implanting human patient derived tumor subcutaneously, into NOD.SCID or NSG mice. The morphology and poor vascularity of the xenograft tumor resembles that of clinical chondrosarcoma.

Treatment with IPI-926 leads to tumor growth inhibition in 1^o chondrosarcoma xenografts

Table 1

Donor	Mouse strain	N/group	Start treatment	IPI-926 schedule	Length of treatment
Patient A	NOD.SCID	8-11	Day of implant	40mg/kg, QDx5, po	6 weeks
Patient B	NOD.SCID	11-15	4 weeks post implant	40mg/kg, QDx5, po	10 weeks
Patient C	NSG	7-10	4 weeks post secondary implant	40mg/kg, QDx5, po	4 weeks

Table 1 describes the different study designs carried out in the 1^ochondrosarcoma xenografts treated with IPI-926.

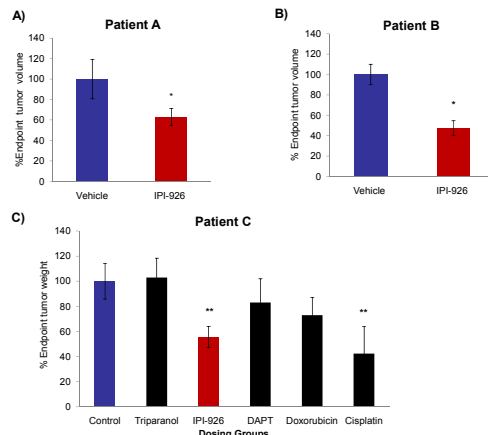


Figure 2. Subcutaneous 1^ochondrosarcoma xenografts were treated with IPI-926 at the time of implant A) or after tumor establishment B), which led to a 37% and 52% decrease in endpoint tumor volume, compared to the vehicle control ($p < 0.03$, respectively). C) IPI-926 treatment was compared head to head with other targeted therapies (Triparanol and DAPT) and chemotherapies (doxorubicin and cisplatin). Only IPI-926 or cisplatin treatment led to decreased endpoint tumor weight, when compared to vehicle control ($**p < 0.001$).

IPI-926 inhibits Hh signaling in chondrosarcoma tumor cells

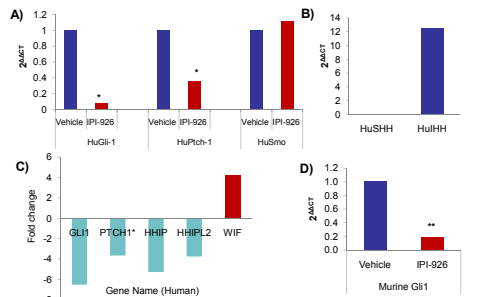


Figure 3. RNA was extracted from selected tumors in studies described in table 1, collected 24 hours after the final dose, for q-RT-PCR analysis, or human 44K Agilent array analysis. A) Human GLI1 and PTCH1 were significantly down regulated by IPI-926 treatment compared to control ($p < 0.005$), while human SMO levels remained unchanged. B) Human IHH mRNA levels were predominantly detected over SHH mRNA levels. C) Human 44K Agilent chip array data showed a set of Hh pathway genes regulated by IPI-926 treatment, relative to the vehicle control, including GLI1 and PTCH1. D) Although detected at lower levels than human Gli1, murine Gli1 mRNA levels were also down regulated by IPI-926 treatment ($**p < 0.03$).

IPI-926 treatment leads to a decrease in cellularity and to evidence of calcification

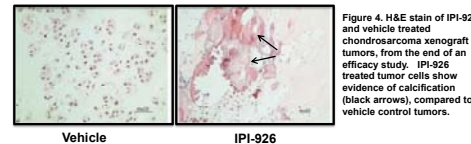


Figure 4. H&E stain of IPI-926 and vehicle treated chondrosarcoma xenograft tumors, from the end of an efficacy study. IPI-926 treated tumor cells show evidence of calcification (black arrows), compared to vehicle control tumors.

Human chondrosarcoma tumor cells stain for cilia, an essential structural component for Hh signaling

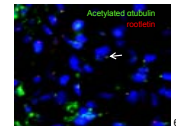
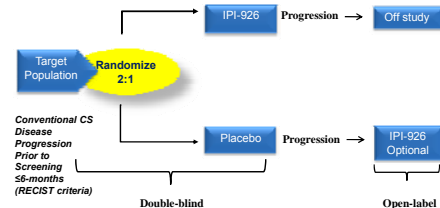


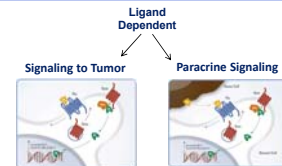
Figure 5. A human bone and cartilage tumor tissue array (Biomax) was stained for cilia by immunofluorescence. To the left is a representative image from a chondrosarcoma tissue spot. The majority of the cilia (white arrow) are found on the chondrosarcoma tumor cells.

A Phase 2, Placebo-Controlled Study Evaluating the Efficacy and Safety of IPI-926 in Patients with Metastatic Or Locally Advanced (Unresectable) Chondrosarcoma



- 1^o objectives: Progression free survival and safety
- 2^o objectives: TTP, OS, and ORR; PK
- Exploratory objectives: Predictive biomarker analyses

Conclusions



• Chondrosarcoma tumors have constitutive activation of the Hh signaling pathway, which is ligand dependent.

• IPI-926 directly inhibits cell autonomous Hedgehog signaling in chondrosarcoma tumor cells.

• In 1^o chondrosarcoma xenograft model that histologically resemble the human disease, IPI-926 treatment leads to significant tumor growth inhibition, a decrease in tumor cellularity and evidence of calcification.

• A randomized, controlled Phase 2 trial of IPI-926 in patients with metastatic or locally advanced chondrosarcoma is ongoing.