The Hh inhibitor IPI-926 delays tumor re-growth of a non-small cell lung cancer xenograft model following treatment with an EGFR targeted tyrosine kinase inhibitor

#5045

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Abstract

The Hedgehog (Hh) pathway plays a key role in multiple types of cancer, either by mutational activation of the pathway in tumor cells or by ligand-dependent signaling to the tumor cells or, by paracrine signaling between tumor cells and the tumor microenvironment. We have previously shown in a primary model of small cell lung cancer (SCLC) that the Hh pathway inhibitor IPI-926 delays tumor re-growth after regression of the tumor with chemotherapy (Travaglione *et al.*, AACR 2009). To investigate whether tumor regression with a targeted agent could elicit a similar response as that seen with chemotherapy, two non-small cell lung cancer (NSCLC) cell lines (NCI-H1650 and HCC827), with activating mutations in EGFR were evaluated. NSCLC cell lines bearing mutant EGFR are sensitive to EGFR kinase inhibitors. Upon treatment with the EGFR inhibitor gefitinib in vitro, HCC827 increased the expression of Indian and Sonic Hedgehog ligands 10-fold, as measured by qRT-PCR. When grown in mice as subcutaneous xenografts, H1650 tumors regressed following treatment with the EGFR inhibitor gefitinib (40 mg/kg every day) for 7 days. The gefitinib treated mice were then administered either vehicle or IPI-926 at 40 mg/kg every other day for three weeks, and in this setting IPI-926 treatment led to a 65% decrease (p=0.0104) in tumor volume compared to vehicle-treated mice. Conversely, tumors that had not been regressed with gefitinib showed no response to the Hh pathway inhibitor, similar to our previous results in SCLC with chemotherapy. Studies are ongoing to define the mechanism of action of IPI-926 in this setting. When grown in mice as subcutaneous xenografts, these tumor cells produce ligand in conjunction with stromal Gli1 expression in the murine stroma. Stromal Gli1 expression is inhibited by treatment with IPI-926, indicating that Hh ligand produced by NSCLC tumor cells signals to stroma in a paracrine manner. Together with our previous results in SCLC, these data suggest that therapeutic inhibition of the Hh signaling pathway might be an important strategy to extend progression free survival in patients who initially respond to therapy but later relapse, and that this paradigm is applicable to multiple cancer types and treatment modalities.

Introduction

IPI-926 is a novel, selective, potent, semi-synthetic Hh pathway inhibitor that directly blocks the activity of Smoothend (Smo) and is currently being investigated in a Phase 1 clinical trial. Because Smo plays a critical role in malignant activation of the Hh pathway, Smo may be a target for the management of a broad range of cancers. In previous reports we have shown that orally administered IPI-926 leads to the inhibition of the Hh pathway, and tumor re-growth inhibition in Hh ligand-producing human tumor models following *cytoreduction by chemotherapy*. In the following studies we investigated IPI-926 activity against xenograft models following *cytoreduction by targeted therapy*. NCI-H1650 and HCC827 tumor cells were isolated from metastatic pleural effusion and lung tumor respectively, from patients with non-small lung cancer (NSCLC). These cells have an acquired mutation in the EGFR tyrosine kinase domain (E746 - A750 deletion). This mutation makes them sensitive to targeted therapy with gefitinib, a tyrosine kinase inhibitor.

Methods

Cell lines **– HCC827*wt* is a lung adenocarcinoma cell line that harbors a mutant form of EGFR (Del E746-A750). HCC827GR5* was derived from HCC827*wt in vitro* and harbors both the mutant EGFR allele (Del E746-A750) and an amplification of c-Met. NCI-H1650 is a lung adenocarcinoma cell line that harbors a mutant form of EGFR (Del E746-A750).**

***In vitro* studies** **– HCC827 cells were plated in six well plates and allowed to grow for 72 hours. Gefitinib or DMSO control was then added to each well in duplicate. These cells were washed and collected by scraping 72 hours later. RNA was isolated from the pellets using the Qiagen RNAEasy kit and RT-PCR was performed for human GAPDH, SHh, IHh, and DHh on the ABI-7300 system with ABI primer/probes. Relative quantification of the expression data was obtained using the ΔΔCT method as described by Applied Biosystems.**

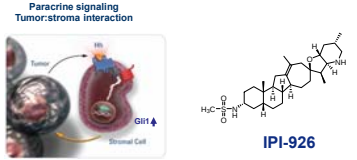
***In vivo* studies** **– Ncr nude mice were subcutaneously implanted with either NCI-H1650 or HCC827 cells. In the HCC827 efficacy study, cells were implanted at 5 x10^6 cells/mouse + Matrigel (1:1, v/v). In the NCI-H1650 efficacy study, cells were implanted at 2 x10^6 cells/mouse. Once tumors reached ~150-200 mm^3, the tumor-bearing animals were randomized into treatment groups. In efficacy studies measuring tumor growth inhibition, animals received IPI-926 (40 mg/kg, p.o.) every other day post therapy with gefitinib (40 mg/kg, p.o). In pharmacodynamic studies measuring pathway modulation, NCI-H1650 cells were implanted at 1 x10^7 cells/mouse. Randomized tumor-bearing mice were then administered IPI-926 (40 mg/kg, p.o.) daily post therapy with gefitinib (40 mg/kg, p.o). Tumor size was measured twice weekly with calipers.**

RT-PCR analysis – RNA was purified from tissue samples using standard procedures and analyzed using primer/probe sets specific for murine or human genes of interest. Fold RNA expression are calculated by normalizing gene of interest to housekeeping gene GAPDH, and then to naïve control tumor (value=1).

Statistical analysis – All values were statistically determine by JMP 7 program using the "Student's t test" method of analysis.

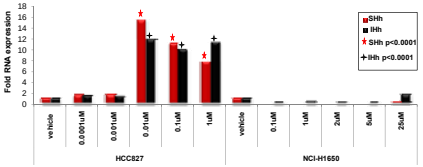
HCC827GR5 cell line was obtained from Dr. Pasi Jänne, Dana Farber Cancer Institute, Boston, MA.

Paracrine Signaling Tumor:Stroma Interaction

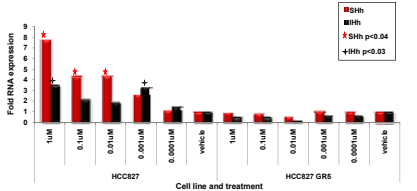


IPI-926

In vitro, gefitinib treatment up-regulates human Hh ligands in HCC827 cells but not NCI-H1650 or HCC827GR5 cells

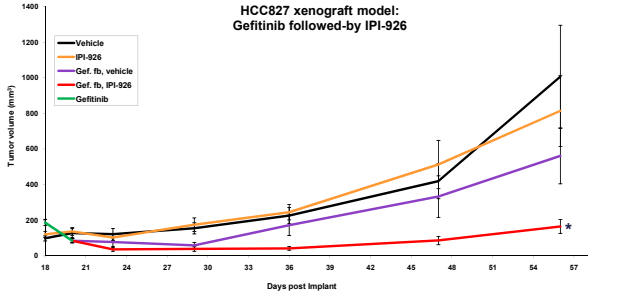


***In vitro* HCC827 cells up-regulate ligand upon treatment with gefitinib.** At low micro molar concentrations of gefitinib HCC827 cells up-regulate the ligands SHh and IHh (p<0.0001). NCI-H1650 cells show no up-regulation of ligands with gefitinib treatment *in vitro*.

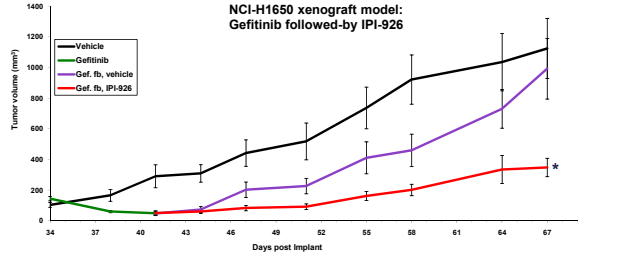


***In vitro* HCC827 cells are sensitive to gefitinib treatment compared to HCC827GR5 cells.** At low micro molar concentrations of gefitinib, HCC827 cells significantly up-regulate the ligands SHh (p<0.04) and IHh (p<0.03). No up-regulation was observed in HCC827GR5 cell line with c-MET amplification.

IPI-926 delays tumor re-growth post gefitinib therapy in NSCLC xenograft models

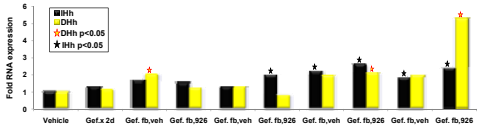


IPI-926 delays tumor re-growth in a HCC827 xenograft model post gefitinib therapy. HCC827 cells were grown subcutaneously in nude mice. Tumor bearing mice were administered gefitinib (10 mg/kg, p.o) for 3 days then followed-by (fb) IPI-926 (40 mg/kg, p.o) every other day for 34 days. A 70% inhibition *(p<0.03) of tumor re-growth post regression with gefitinib was observed with IPI-926 treatment compared to vehicle treated mice post regression.



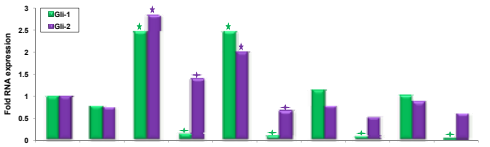
IPI-926 delays tumor re-growth in a NCI-H1650 xenograft model post gefitinib therapy. NCI-H1650 were grown subcutaneously in nude mice. Tumor bearing mice were administered gefitinib (40 mg/kg, p.o) for 7 days then followed-by (fb) IPI-926 (40 mg/kg, p.o) every other day for 24 days. H1650 sensitivity (regression) to gefitinib in vivo was followed by a 65% inhibition *(p<0.02) of tumor re-growth with IPI-926 treatment compared to vehicle treated mice post regression.

In vivo, gefitinib treatment up-regulates human Hh ligands in NCI-H1650 tumors



NCI-H1650 xenograft up-regulates human ligands in vivo. Human IHh and DHh ligands are up-regulated (p<0.05) post gefitinib treatment in NCI-H1650 xenograft. (*No modulation of human SHh ligand and no modulation of human Gli was observed. Data not shown.*)

Gli1 and Gli2 are up-regulated post gefitinib therapy and inhibited by IPI-926 treatment



IPI-926 inhibits the up-regulation of stromal cell Gli1 and Gli2, post gefitinib treatment in the NCI-H1650 xenograft. Murine Gli1 is up-regulated ★(p<0.05) post therapy compared to vehicle treated tumor and down modulated +(p<0.0001) by IPI-926 treatment. Murine Gli2 is up-regulated ★(p<0.01) post target therapy when compared to vehicle, and down modulated + (p<0.03) with IPI-926 treatment. (*No modulation of murine ligands. Data not shown.*)

Conclusions

In NSCLC xenograft models HCC827 and NCI-H1650, IPI-926 significantly inhibits tumor re-growth post gefitinib targeted therapy.

Gefitinib results in an up-regulation of human DHh and IHh ligands as measured by qRT-PCR. Tumor-derived Hh ligands signal to the surrounding stromal cells to up-regulate Gli1 and Gli2 levels which are significantly down-modulated by IPI-926.

These data suggest that therapeutic inhibition of the Hh signaling pathway might be an important strategy to extend progression free survival in patients who initially respond to therapy but later relapse, and that this paradigm is applicable to multiple cancer types and treatment modalities.