

A novel Hh pathway inhibitor, IPI-926, delays recurrence post-chemotherapy in a primary human SCLC xenograft model

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ABSTRACT# 4611

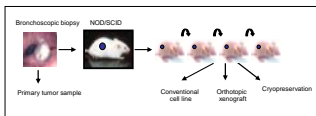
Abstract

Small cell lung cancer (SCLC) is a highly aggressive thoracic malignancy that is rapidly fatal when untreated. While SCLC has a high initial response rate to platinum-based chemotherapy, it almost invariably recurs and is fatal within months. One potential explanation for this phenomenon is that while the majority of tumor cells are highly chemo-sensitive, a subset of cells exist within the tumor that are intrinsically resistant to chemotherapy. After chemotherapy these resistant cells (a potential stem cell population) repopulate and ultimately lead to death. Recent studies have suggested that hedgehog (Hh) signaling may act to maintain cancer "stem cell" function in melanoma and glioblastoma; moreover, deregulation of this pathway has been implicated in SCLC. To test the hypothesis that the Hh pathway is important in maintenance of a chemo-resistant subset of tumor cells within SCLC, a primary human xenograft SCLC model was developed with tissue taken directly from a chemo-naïve patient and passaged in immunodeficient mice. Modulation of the Hh pathway was achieved with IPI-926, a potent and selective inhibitor of SMOOTHENED (SMO). *In vitro*, the clonal capacity of a small population of aldehyde dehydrogenase expressing SCLC cells was markedly reduced by treatment with SMO antagonists. Moreover, cells able to survive treatment with carboplatin demonstrated a marked up regulation of Hh signaling, which could be modulated by Hh pathway agonists and antagonists. In a mouse model using patient-derived primary SCLC xenografts, no response to IPI-926 alone was noted. However, after tumor debulking with carboplatin and etoposide (a similar regimen to that used clinically in SCLC), IPI-926 treatment resulted in a substantial and significant tumor growth delay, when compared to animals receiving vehicle following chemotherapy. These data suggest that the Hh pathway is important in SCLC regrowth after chemotherapy. Moreover, treatment with the potent SMO inhibitor IPI-926 after completion of chemotherapy may delay or prevent tumor recurrence in SCLC.

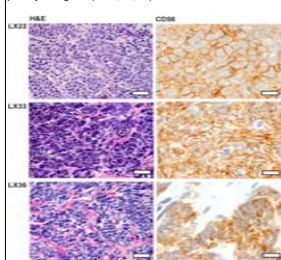
SCLC and Hh Background

- ~33,000 new cases of SCLC per year in the US
- Limited disease: median 2 year survival 20 to 40%
- Extensive disease: median 2 year survival <5%
- Platinum-based therapy can lead to robust tumor regression, but high % relapse within months
- No significant advances in treatment for 20+ years
- Hypothesis: Chemo resistant "progenitor cell" component relies on Hedgehog signaling

SCLC Tumor Model Development



Characterization of 3 Primary SCLC Xenografts. Hematoxylin and Eosin (H&E) and CD56 immunostaining of three SCLC primary xenografts (LX22, 33, 36).



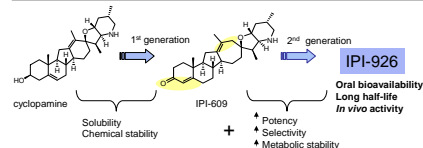
Methods

In vitro, SCLC cell lines were tested for Aldehyde dehydrogenase (ALDH) activity by Flow cytometry +/- Cyclopamine. The ALDH^{hi} and ALDH^{lo} were expanded for a total of two passages in single cell cloning assays (Figure 1).

In vitro, the H82 cell line was treated with Carboplatin. Cells surviving Carboplatin treatment, were treated +/- 1µM IPI-609. mRNA levels of Hh pathway members were measured by q-RT-PCR analysis (Figure 2).

In vivo, LX22 tumor bearing mice were treated on days 34, 41, 48 iv with Carboplatin (60mg/kg) and days 34, 35, 36, 48 iv with Etoposide (12mg/kg) (E/P). Once tumors regressed down to <40mm³, the mice were randomized to receive follow up treatment with orally administered IPI-926 @ 40mg/kg/day or Vehicle control. (Figure 3). At the end of the follow up treatment, the groups were pooled and implanted into primary and secondary transplants (Table 1).

Discovery of IPI-926



In Vitro Results

Figure 1 Survival of clonogenic ALDH^{hi} subpopulation is inhibited by cyclopamine

Expansion of A) H82 and B) LX22 ALDH^{hi} and ALDH^{lo} fractions, in primary and secondary single cell cloning assays. C) Reduction in % ALDH positive cells in response to treatment with cyclopamine.

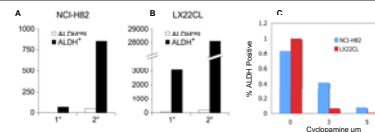
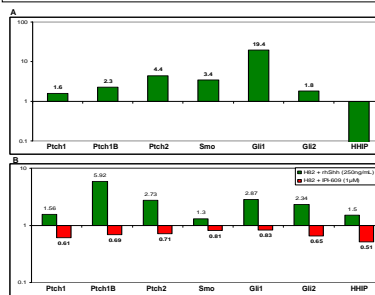


Figure 2 H82 Cells surviving Carboplatin treatment have higher mRNA levels of Hh pathway members, which can be modulated

A) Compared to untreated control. B) Treated with SHH ligand or the SMO inhibitor IPI-609 for 18 hours, compared to ETOH treated control.



In Vivo Results

Figure 3 IPI-926 delays tumor re-growth, post E/P treatment, in the LX22 primary SCLC xenograft model

LX22 tumors were implanted subcutaneously. Carboplatin and Etoposide (E/P) were dosed during a 15 day period. After completion of E/P treatment, the mice received either IPI-926 or Vehicle control as follow up treatment. After 35 consecutive oral doses of IPI-926, there was an 82% tumor growth inhibition as compared to the vehicle control (p<0.01).

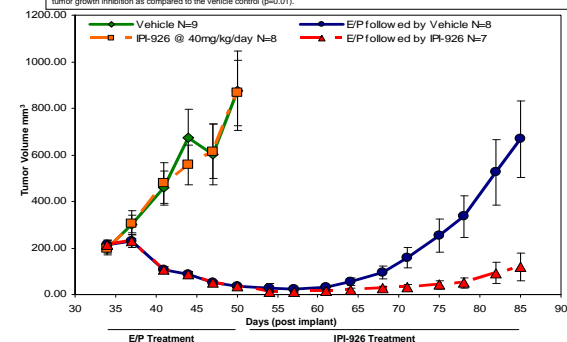
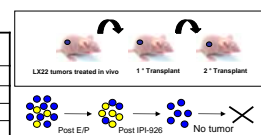


Table 1 LX22 tumors initially treated with E/P followed by IPI-926, show a significant decrease in tumor-take during the 2nd Transplant

Transplant Data	1 st Transplant	2 nd Transplant			
Group	Cell no.	Day	Tumor take	Day	Tumor take
Naïve	2000	N/A	N/A	111	5/5
Vehicle control	2000	76	5/6	111	12/14
IPI-926 treated	2000	76	5/6	111	4/16



Summary/Conclusions

- Identified a candidate Hh pathway-dependent subpopulation of cells with clonogenic properties.
- Cells that survived Carboplatin treatment *in vitro*, had higher transcript levels of Hh pathway members, which could be modulated by Hh pathway activation/inhibition.
- Developed a primary model of SCLC that closely mimics the disease response in the clinical setting.
- Following E/P treatment *in vivo*, oral administration of IPI-926 at 40mg/kg/day for 35 days, led to a significant delay in tumor re-growth, compared to vehicle control (p=0.01).
- IPI-926 may impact a subpopulation of "progenitor-like" cells that are chemo-resistant and Hh responsive.
- Results with IPI-926 merit further investigation of the impact of Hh pathway inhibition on relapse-free survival in SCLC.