

ABSTRACT

Medulloblastoma is the most common malignant brain tumor in children, and brain tumors are the leading cause of cancer deaths during childhood. Average risk medulloblastoma patients have a 70-80% survival rate while high risk patients only have a 30% chance of survival. The current standard of care includes neurosurgical resection, cytotoxic chemotherapy, and radiation therapy. New therapies are needed to improve cure rates and to reduce long-term toxicity in survivors.

The Sonic hedgehog (Shh) pathway plays a central role in both mouse medulloblastoma models and in human disease. Mutations in several genes in the Shh pathway result in medulloblastoma formation. In addition, Shh upregulation is known to occur in tumor cells and in the microenvironment of many tumor types, where it drives tumor proliferation. For these reasons, drugs that interfere with the Shh pathway are prime candidates for medulloblastoma therapy. One such candidate is IPI-926, a novel cyclopamine derivative that inhibits hedgehog signaling by interacting with Smoothened.

To further evaluate the role of hedgehog inhibition in medulloblastoma, we determined the efficacy of IPI-926 against medulloblastomas in conditional Patched-1 (Ptc1) null mice. When Ptc1 is inactivated by MATH1-Cre in this model, cerebellar granule neuron precursor cells give rise to early and aggressive medulloblastomas, which are often evident by the time of weaning. Symptomatic 21-day old mice were identified by the presence of a bulging calvarium. Mice were then randomized to receive either IPI-926 or vehicle via daily intraperitoneal (IP) injection. Vehicle-treated mice developed ataxia and neurologic deficits, then died from their disease. In contrast, mice treated with 19 days of IPI-926 showed dramatic improvement in their bulging skulls and were neurologically normal. Pharmacodynamic activity of IPI-926 in these tumors was confirmed by analysis of *gli1* mRNA by RT-PCR, which was down-modulated following IPI-926 treatment. IPI-926 was well tolerated over the course of these studies.

We have shown that the novel hedgehog pathway inhibitor IPI-926 ameliorates the clinical symptoms of advanced medulloblastomas and effectively reduces bulk disease in the Ptc1-null medulloblastoma model. Additional studies are in progress to determine whether hedgehog pathway inhibitors may be suitable for future clinical trials in childhood medulloblastoma patients.

The Hedgehog Inhibitor IPI-926 leads to dramatic regression of mouse medulloblastoma

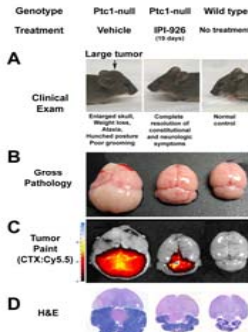


FIGURE 1: The hedgehog inhibitor IPI-926 leads to dramatic regression of mouse medulloblastoma and complete resolution of advanced clinical symptoms.

We evaluated the efficacy of IPI-926 in Ptc1-null mice in a pilot study. 21-day old Ptc1-null mice displaying bulging skulls from medulloblastoma were randomized to receive daily intraperitoneal IPI-926 (20 mg/kg/dose, n=3) or vehicle (n=2) for 19 days. Compared to a representative vehicle-treated mouse with a large tumor (left panels) and a wild-type littermate with no tumor (right panels), a representative mouse treated with IPI-926 (center panels) showed resolution of clinical symptoms after 19 days of IPI-926 treatment (Figure 1A). Arrow denotes bulging skull due to tumor. A very good partial response to IPI-926 was apparent by decreased cerebellar tumor size using gross pathology (Figure 1B), *ex vivo* imaging with Tumor Paint (CTX-Cy5.5), a tumor-tracking molecular imaging agent developed by our laboratory (Figure 1C), and hematoxylin and eosin (Figure 1D).

IPI-926 down-regulates Gli-1 in tumor

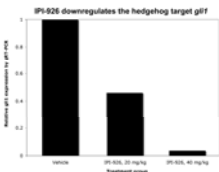


FIGURE 2: IPI-926 treatment downregulates the canonical hedgehog target gene *gli1*. Ptc1-null mice were treated with daily intraperitoneal injections of vehicle (n=3) or IPI-926 (n=2). Mice were treated either with 20 mg/kg/dose IPI-926 for 2 weeks, or were treated for 3 days at 40 mg/kg/dose followed by 20 mg/kg/dose for the remaining time. RNA was isolated from cerebellar tumors. Expression of *gli1* and *gapdh* were analyzed by quantitative RT-PCR. The dose-dependent downregulation of *gli1* confirms that IPI-926 interferes with hedgehog signaling.

IPI-926 prolongs survival in a dose-dependent manner

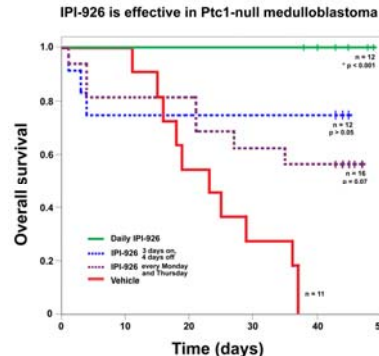


FIGURE 3: IPI-926 prolongs survival in a dose-dependent manner.

Four- to six-week-old Ptc1-null mice with bulging skulls due to medulloblastoma were randomized to receive vehicle (red line) or intraperitoneal IPI-926. IPI-926 was administered either daily (green line, 15-30 mg/kg/dose); in cycles of 3 days on IPI-926 followed by 4 days off drug (blue dashed line, 20 mg/kg/dose); or twice weekly IPI-926, administered every Monday and Thursday (purple dashed line, 20 mg/kg/dose). Kaplan-Meier analysis demonstrates that all mice treated with daily IPI-926 for six weeks survived, while all vehicle-treated mice died.

Conclusions

The novel cyclopamine derivative IPI-926 induces dramatic regression of medulloblastomas in the Patched1-null mouse model. Striking resolution of nearly all symptoms of advanced medulloblastoma occurred within the first 19 days of daily IPI-926 therapy in pilot studies. In a six-week trial, 100% of vehicle-treated mice died from aggressive medulloblastoma, whereas 100% of mice that received daily IPI-926 were alive at the end of the study. Murine MRI is feasible and effective method that we used to measure response to IPI-926 therapy. IPI-926 is a promising new agent for medulloblastoma therapy and may be considered for future human trials.

MRI scans are feasible and effective for monitoring response in mouse brain tumors

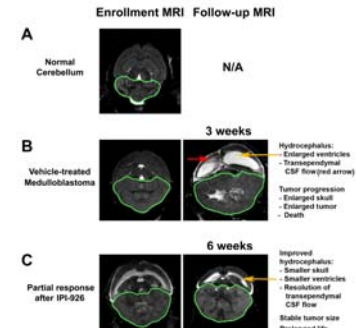


FIGURE 4: MRI scans are a feasible and effective method for monitoring response to therapy in mouse brain tumor models

Serial MR scans of each mouse were performed at enrollment and after 3 and 6 weeks of treatment. T2-weighted axial images were acquired at 3 Tesla, using a Philips MRI system with a custom mouse coil. A wild-type mouse is shown in Figure 4A, with its normal cerebellar contour outlined in green. Medulloblastoma is evident in a Ptc1-null mouse by the enlarged cerebellum demonstrating loss of normal foliation pattern in the cerebellar parenchyma (Figure 4B, left). The natural history of Ptc1-null medulloblastoma includes rapid tumor enlargement, hydrocephalus, and death (Figure 4B, right). Hydrocephalus is diagnosed by enlarged lateral ventricles (orange arrows) and third ventricles, and the presence of transpendymal fluid of cerebrospinal fluid (hazy periventricular region, red arrow). A representative mouse treated with IPI-926 showed a large tumor and evidence of hydrocephalus at study enrollment (Figure 4C, left). After 6 weeks of daily IPI-926 treatment the mouse was alive without evidence of neurologic deficits. MRI showed interval resolution of transpendymal CSF flow, decreased ventricle size, decreased skull size, and no evidence of tumor progression (Figure 4C, right). Mice were sacrificed at the end of the trial, and corresponding sections of tumor were stained with hematoxylin and eosin (not shown). Validation studies are in progress to ascertain that MRI-based tumor volumetric measurements correlate with histopathologic evidence of tumor burden.

Acknowledgements

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