The Hedgehog Pathway in Human Cancer

Luc Y Dirix
Iridium Kankernetwerk
Antwerp
Hedgehog Signaling Pathway

- Fundamental signal transduction pathway in embryogenesis
- Originally delineated in *Drosophila*
- Named for mutant fruit fly embryos covered with bristles (normally only in stripe on anterior segment)
- Vertebrate homologues (Sonic, Desert and Indian Hh)
- Mutations may lead to congenital anomalies and to human cancers
Hedgehog Signaling Pathway

- Under normal conditions, Hh pathway is actively repressed.

- In absence of Hh, patched (PTCH) represses smoothened (SMO) and inhibits activation of the pathway.
Hedgehog Signaling

- Binding of Hh removes inhibition and activates pathway
- SMO acts down-stream from PTCH and activates the GLI transcription factors when released from PTCH inhibition
The Hedgehog Signaling Pathway.

The Hedgehog Signaling Pathway Inhibitors.
Hedgehog Inhibitors in Human Cancer

- BCC
- Gorlin
- Medulloblastoma
- Pancreatic Carcinoma
- Colorectal Carcinoma
- Ovarian Carcinoma
Basal Cell Nevus Syndrome (Gorlin)  
First Link Between Hh Signaling and Cancer

• Multiple BCCs
  – Also medulloblastoma, ovarian fibroma, fibrosarcoma, rhabdomyosarcoma, meningioma, cardiac fibroma

• Positional cloning and subsequent screening identified a spectrum of PTCH mutations in BCNS patients

• Gene mapped to chromosome 9q22-31: deleted in high % of BCCs and other syndrome tumors

• BCCs develop secondary to activation of target genes of Hh pathway in cells that have lost both normal copies of PTCH

• Autosomal dominant
Sporadic BCCs

• Majority show allelic loss for chromosome 9q22 and inactivating mutations of PTCH

• Activating mutations of SMO in 10-20% sporadic BCCs

• Suggests abnormal Hh signaling involved in most (all?) BCCs - high levels of Hh target genes such as GLI1
Corn Lilies, Sheep and Hedgehog

- Cyclopia in lambs found to be due to pregnant ewes ingesting corn lily in western USA
- Chemical identified: “cyclopamine”
  - No effect in adult sheep
- Effect due to inhibiting Hh
  - Probable site of action is SMO
- Holoprosencephaly and cyclopia may be due to genetic defects related to blocking of Hedgehog (Hh) pathway or environmental effects
- If cyclopamine blocks Hh signaling, could cyclopamine or similar drugs be used to treat BCCs?
Small Molecule Inhibitors Targeting Hh Pathway

• Hh responsive reporter cell line developed and used in high-throughput screen against ~100,000 small synthetic molecules

• Identified small molecule compounds that directly inhibited SMO activity (strongly inhibited Hh signaling in cell lines with inactive PTCH)

• A topical Hedgehog Antagonist was used in a Phase 1 study in patients with nodular BCC (N=66)
  – Preliminary data revealed no significant safety concerns in four weeks of topical treatment. However, clinical activity was far less than anticipated.
  – Topical treatment with this formulation did not down-regulate Gli.
GDC-0449

- GDC-0449 is a small molecule hedgehog pathway inhibitor (HPI) that binds to SMO\textsuperscript{1-3}
  - Developed for oral delivery
  - Structurally distinct from cyclopamine

\textsuperscript{1}Epstein EH. Nature Rev Cancer 2008;8:743-754
Hedgehog binding to PTCH1 stops PTCH1 from inhibiting SMO signaling.

In the absence of PTCH1 (e.g. loss-of-PTCH1 mutations), SMO signaling occurs constitutively.

GDC-0449 inhibits SMO signaling through direct interaction with SMO.

Background: Advanced Basal Cell Carcinoma

- Basal-cell carcinoma, the most common skin cancer in the United States, has an estimated annual incidence of 0.1 to 0.5%.  
  1,2

- A recent report estimates that there were approximately 3.5 million NMSCs treated in the US in 2006.  
  3 (Approximately 80% of NMSCs are BCC.)

- Surgery effectively treats most cases of basal-cell carcinoma, but in a small percentage of patients there is progression to life-threatening, unresectable, locally advanced  
  4 or metastatic  
  5 tumors.

- There is no standard therapy for locally advanced or metastatic basal-cell carcinoma.
Phase I Study

- Safety, preliminary efficacy, and pharmacokinetics of GDC-0449 assessed in an open-label, multicenter, two-stage Phase I trial.¹
- Patients with solid tumors refractory to therapy, and expansion cohort for patients with locally advanced or metastatic BCC.
- Eligibility: tumors evaluable on physical examination or imaging.
- Patients with BCC received continuous daily administration of GDC-0449 at 150 (n=17), 270 (n=15) or 540 mg/day (n=1).
- Treatment until disease progression, intolerable toxic effects, or withdrawal from study.
- No dose-limiting toxic effects were observed.
- The recommended Phase 2 dose was 150 mg per day. Pharmacokinetic analyses indicated that higher doses did not result in higher plasma concentrations of the drug.

# Baseline Characteristics of Patients with BCC

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age – yr</strong></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>53</td>
</tr>
<tr>
<td>Range</td>
<td>38–84</td>
</tr>
<tr>
<td><strong>Sex – no (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25 (76)</td>
</tr>
<tr>
<td>Female</td>
<td>8 (24)</td>
</tr>
<tr>
<td><strong>Race or ethnic background – no (%)</strong></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>32 (97)</td>
</tr>
<tr>
<td>Latino</td>
<td>1 (3)</td>
</tr>
<tr>
<td><strong>ECOG score – no (%)</strong></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>14 (42)</td>
</tr>
<tr>
<td>1</td>
<td>19 (58)</td>
</tr>
<tr>
<td><strong>Type of disease – no (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Metastatic</td>
<td>18 (55)</td>
</tr>
<tr>
<td>Locally advanced</td>
<td>15 (45)</td>
</tr>
<tr>
<td><strong>Previous therapies – no (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>28 (85)</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>19 (58)</td>
</tr>
<tr>
<td>Systemic therapy</td>
<td>15 (45)</td>
</tr>
</tbody>
</table>

* Race or ethnic background was reported by investigators.

† Eastern Cooperative Oncology Group (ECOG) scores range from 0 to 5, with higher scores indicating a greater severity of illness.
Response Assessment

- In patients with radiologically measurable disease (generally, those with metastatic tumors):
  - Tumor assessment was conducted per Response Evaluation Criteria in Solid Tumors (RECIST) (version 1.0) to determine stable disease, progressive disease, and best overall response. A complete or partial response was determined on two consecutive occasions 4 or more weeks apart.

- In patients with locally advanced tumors (and no radiologically measurable disease):
  - Tumors were assessed on physical examination. A complete response was defined as disappearance of a palpable or visible tumor, and a partial response was defined as a reduction of more than 50% in the diameter of a palpable or visible tumor.
Responses to GDC-0449 Treatment and Duration on Study – Feb 2009

MET = metastatic disease, LA = locally-advanced disease

* Patients 15 and 29 were the only patients with radiologically-evaluable, locally-advanced disease and were evaluated by RECIST.
† Patients with metastatic disease were evaluated by RECIST, except for patients 10 and 24, who did not have RECIST-evaluable disease and were evaluated by clinical parameters.
‡ Patient 20 was evaluated by both RECIST and clinical parameters.

The overall response rate for the 18 patients with metastatic tumors was 56% (95% confidence interval [CI], 31 to 78).

The overall response rate in patients with locally advanced tumors was 60% (95% CI, 33 to 83).

The Kaplan–Meier estimate for median time of participation in the study was 9.8 months and ongoing. Median duration of response was 8.8 months and ongoing.
GDC-0449 Activity

- Patient 24, a 60-year-old man with the basal-cell nevus syndrome with lesions of the posterior scalp, at baseline and after 5 months of GDC-0449 therapy.

- This patient had a partial response to treatment, which was ongoing at the time of data cutoff.
Patient 29, a 41-year-old woman with facial lesions

At time of data cutoff, this patient had stable disease as assessed on imaging, and continued to receive therapy.
Before Treatment

After 3 Months

- Patient 7, a 49-year-old male with locally advanced BCC shown with multiple large lesions on upper right back at baseline and at the onset of a clinical partial response after 3 months of GDC-0449 administration.
Patient 2, a 67-year-old male with BCC metastatic to lung, soft tissue and bone, with PET and CT (coronal view) scans at baseline and at 8 months, at time of confirmed radiologic partial partial response.
Patient 11, a 83-year-old male with BCC in the right external ear and invasion into the parotid gland, shown with PET/CT scans at baseline and at the onset of a clinical partial response after 2 months of GDC-0449 administration.
Adverse Events in BCC Patients*

No dose-limiting toxic effects or grade 5 events were observed during the study period.

A single grade 4 adverse event (asymptomatic hyponatremia) occurred.

* All grade 3 and 4 events are listed. Grade 2 events are listed only if investigators considered them to be related to GDC-0449 treatment. The highest grade of event is reported for each patient.

<table>
<thead>
<tr>
<th>Event</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical Event</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Hyponatremia</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Muscle spasm</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Dysgeusia</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anorexia</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Weight loss</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Aspiration</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Back Pain</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Corneal abrasion</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Dehydration</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Keratitis</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Electrocardiographic event (prolonged QT interval)</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Laboratory Event</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypocalcemia (calcium &lt; 8.4 mg/mL [2.1 mmol/L])</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Increase in serum alkaline phosphatase (&gt;120 U/L)</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Increase in serum potassium (&gt; 5.1 mmol/L)</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
• *GLI1* was overexpressed in tumors obtained from 25 of 26 patients with metastatic or locally advanced tumors, compared with control samples of normal skin and lung tumor (p<0.001 for all comparisons).

• *GLI1* mRNA expression levels were consistent with expression levels previously observed in cutaneous basal-cell carcinoma.
Identification of Mutations in *PTCH1*

- The entire coding region of the *PTCH1* gene and an exon encoding a previously identified activating mutation of *SMO* (SMO-M2) were sequenced from evaluable patient stored tumor samples.

- Results: Mutations in the *PTCH1* gene, including nonsense and missense mutations, were found in 9 of 10 such specimens. A SMO-M2 mutation was identified in 1 patient of 10 sequenced.
Pharmacodynamics

• Pharmacodynamic assessments of GLI1 expression were conducted on RNA extracted from 4-mm biopsy specimens of noninvolved skin at baseline and at 7 and 21 days after the start of daily drug therapy.

• Results: Pharmacodynamic down-modulation in the hedgehog pathway was shown by a >two-fold decrease in GLI1 expression, compared with pretreatment biopsy-sample analysis.
Conclusions

• In this study, a tumor response to GDC-0449 was seen in a number of patients with advanced basal-cell carcinoma.
  – Of 33 patients with locally advanced or metastatic tumors, 18 had a response to GDC-0449.
  – Of the remaining 15 patients, 11 had stable disease for up to 10.8 months, and 4 had progressive disease.

• There were no dose-limiting toxicities.

• The recommended Phase 2 dose was 150 mg per day because pharmacokinetic analyses indicated that higher doses did not result in higher plasma concentrations of the drug.

• The patients treated in this study had advanced tumors that were no longer amenable to conventional treatment options, including surgery, radiotherapy, or systemic therapy.
Phase II Study in Patients with Advanced BCC

• An ongoing single-arm, two-cohort, multicenter phase II study is evaluating the efficacy and safety of GDC-0449 in locally advanced inoperable BCC and metastatic BCC. Study is closed to enrollment.

  – 104 enrolled patients with a histologically confirmed diagnosis of advanced BCC (metastatic or locally advanced).

  – Patients with mBCC have histologic confirmation of a distant BCC metastasis (e.g., lung, liver, lymph nodes, or bone).

  – Patients with laBCC have disease that is considered inoperable, or have disease that recurred following radiotherapy (unless contraindicated).

  – Patients with nevoid BCC syndrome (Gorlin syndrome) may enroll in this study but must meet the criteria for laBCC or mBCC listed above.
Hedgehog Inhibitors in Human Cancer

- BCC
- Medulloblastoma
- Pancreatic Carcinoma
- Colorectal Carcinoma
- Ovarian Carcinoma
Hedgehog Inhibitors in Medulloblastoma

- Common brain tumour in childhood
- Cerebellar granule neuron precursor
- Gorlin syndrome mutations in *PATCHED* (PTCH), receptor Sonic Hedgehog (5% medulloblastomas)
- Turcot’s syndrome WNT pathway APC
- Role of *Oncomir* (*miR-17-92*)
- Mouse model: heterozygous deletion PTCH1 medulloblastoma in 1/3 25 weeks
- Suppression of Shh eliminates medulloblastoma in *Ptc1(+/−)* *p53(−/−)* mice
- One case report
Hedgehog Inhibitors in Medulloblastoma
Tumor Response on Positron-Emission Tomographic (PET) Scanning.

Tumor-Specific Hedgehog Pathway Activation.

Hedgehog Inhibitors in Human Cancer

- BCC
- Medulloblastoma
- **Pancreatic Carcinoma**
- Colorectal Carcinoma
- Ovarian Carcinoma
Roles of the Hedgehog Pathway in Pancreatic Cancer.

Phase II Study in Patients with Pancreatic Cancer

- Overexpression of Smo activates the Sonic Hedgehog pathway in pancreatic fibrblasts (CCR 2010; 1781)

- Tuveson lab (Science 2009; 1457):
  - Increase in MVD
  - Increase in perfusion
  - Increase in drug penetration (gem-dox)

- Feldman (Mol Cancer Ther 2008; 2725)
  - Oral HHI results in decrease tumor initiation
Hedgehog Inhibitors in Human Cancer

- BCC
- Medulloblastoma
- Pancreatic Carcinoma
- Colorectal Carcinoma
- Ovarian Carcinoma
THANK YOU FOR YOUR ATTENTION