Targeting the Hedgehog Pathway in Cancer: Can the Spines Be Smoothened?

Laurie Ailles and Lillian L. Siu

Aberrant Hedgehog (Hh) pathway signaling has been suggested to play a role in the development of multiple solid tumors and hematologic malignancies. GDC-0449 is a novel first-in-human, first-in-class smoothened (SMO) inhibitor, which has completed its phase I evaluation and achieved proof of concept in tumors with Hh pathway mutations. *Clin Cancer Res;* 17(8); 2071–3. ©2011 AACR.

In this issue of *Clinical Cancer Research*, 2 companion reports by LoRusso and colleagues (1) and Graham and colleagues (2) present the safety, pharmacokinetic, and pharmacodynamic results of the phase I trial of the novel first-in-class smoothened (SMO) inhibitor GDC-0449 in patients with advanced solid malignancies. Hedgehog (Hh) signaling supports the development of many organs and tissues and regulates tissue homeostasis and wound repair in postnatal tissues, such as the central nervous system and skin (3). The mechanisms by which Hh signaling can be aberrantly activated in cancer can be divided into 2 classes (3, 4). The first is through mutation of members of the Hh pathway leading to hyperactivation, increased cell proliferation, and tumor formation [e.g., *PTCH1* mutations in Gorlin syndrome, sporadic basal cell carcinomas (BCC), and a significant fraction of medulloblastomas]. In these cases, mutational Hh deregulation is thought to be the initiating event. The second class displays excessive and/or inappropriate expression of Hh ligands, resulting in autocrine or paracrine stimulation of cancer cells, or, in some cases, overexpression of Hh ligands by tumor cells leads to paracrine activation in adjacent stromal fibroblasts, which in turn contribute to tumor cell growth and survival via a number of mechanisms (5). Conversely, it has been reported in other cancers (leukemias, glioma) that Hh ligands secreted by the stroma stimulate the cancer cells (6). In contrast to cancers with mutational activation, in these cases Hh signaling plays a secondary role in tumor maintenance and growth (3). As an added layer of complexity, there is accumulating evidence that aberrant Hh activation may act via regulation of the putative cancer stem cell (CSC) population (4). CSCs are a subpopulation of cancer cells within tumors that can self-renew, extensively proliferate, and differentiate to recapitulate the phenotype of the primary tumor. They are functionally defined on the basis of their expression of specific biomarkers and their ability to initiate tumors in immunocompromised mice. They have been identified in many cancers, and evidence is accumulating that they may be responsible for treatment resistance and relapse (7). Hh activation may influence CSCs, either directly (e.g., by mediating CSC self-renewal; ref. 4) or indirectly (e.g., paracrine activation of the stroma may provide a supportive microenvironment for CSC). Hh inhibition may, therefore, be beneficial, either through direct targeting of the CSCs or through targeting stromal cells that support them.

The clinical development of GDC-0449 poses interesting pharmacokinetic and pharmacodynamic challenges. In mutation-driven tumors such as locally advanced or metastatic BCCs and Gorlin syndrome, GDC-0449 has shown unequivocal clinical activity, with tumor regression observed in a substantial proportion of patients, thus providing proof of concept (1, 8). Beyond this unique target population, objective tumor response has yet to be demonstrated among patients with cancers lacking driver mutations of the Hh pathway. Indeed, in tumors in which aberrant Hh signaling is secondary to genetic changes in multiple other signaling pathways, targeting Hh is less likely to be a successful strategy unless used in combination with other appropriately targeted or cytotoxic therapies. Limitations are inherent in the search for early efficacy signals in phase I trials, including their small sample size, heterogeneous patient populations with often heavily pretreated advanced malignancies that harbor multiple drug resistance mechanisms, and the delivery of potentially subtherapeutic doses during dose escalation. The recommended phase II dose of GDC-0449 was established on the basis of pharmacokinetic futility, rather than toxicity. It is uncertain whether relevant biological concentrations to achieve antitumor activity differ between cancers with different mechanisms of Hh activation. The mechanistic pharmacokinetic model of GDC-0449 proposed by Graham and colleagues has eloquently elucidated drug disposition (2). The pharmacokinetic properties described, such as limited solubility affecting gut absorption, high
degree of protein binding especially to alpha-1-acid glycoprotein, and slow metabolic elimination, are not easily surmountable if a higher free plasma drug level is required for the class of tumors without Hh pathway mutations. From a pharmacodynamic perspective, although GLI1 mRNA is downregulated in normal skin and hair follicles, these surrogate tissues may not be reflective of Hh pathway inhibition in the tumors themselves, whether it be the stromal cells, the cancer cells, or the CSCs that are the relevant cellular target.

Ideally, the availability of serial tumor biopsies pre- and post-treatment with GDC-0449 would enable a better appreciation of intratumor pharmacodynamic effects. This availability would allow a fairly straightforward assessment of the activity of Hh pathway inhibitors in specific malignancies before and after treatment through Hh...
biomarker profiling on paraffin-embedded tissue. In addition, such material could also be used to address biological mechanisms, such as whether the drug is affecting stromal cell activation or cancer cell activation, and in the latter case, are the cells affected within the CSC subset? Mechanistic studies are challenging, particularly with respect to assessing effects on CSCs. Clinical responses due to CSC targeting may be delayed, and most phase I trials are not designed to detect differences in long-term outcomes. Thus, correlational studies that are designed to quantify CSCs pre- and post-treatment may more rapidly reveal whether they are being affected by Hh pathway inhibition. This research can be done by phenotypic analysis using flow cytometry for tumor types with a defined CSC phenotype (9). Ideally, functional assays, which are a more accurate measure of CSC activity, could also be done using in vitro and/or in vivo systems. In vitro assays exist for some cancers (e.g., breast, brain, pancreatic, colon) in which "tumorsphere" formation under specific culture conditions is thought to represent CSCs (7, 9). These assays can be made quantitative by performing them at limiting dilution (Fig. 1) and are feasible with the small numbers of cells obtained from biopsies (7). Ultimately, however, the true measure of CSCs is their ability to initiate tumors in immunocompromised mice. Injection of serial dilutions of cells into mice allows calculation of the tumor-initiating cell frequency. Performing this assay directly on cells derived from biopsies is not feasible due to a lack of sufficient cell numbers. However, xenografts can be initiated from tumor biopsies, and within 2 to 3 passages, cohorts of animals can be treated with the Hh pathway inhibitor to determine whether CSCs are affected by drug treatment in comparison to controls, in parallel with assays to determine the status of the Hh pathway within the CSC and non-CSC compartments (Fig. 1).

Disease-specific phase II evaluations of GDC-0449 and other Hh pathway inhibitors in oncology are ongoing. In mutation-driven malignancies, such as advanced BCCs and some medulloblastomas in which there is early evidence of anticancer activity, the developmental strategy is relatively straightforward, and orphan drug designation will likely be sought for these rare tumors. In cases that demonstrate initial tumor response and subsequent regrowth, efforts should be made to obtain tumor tissues at the time of disease progression, as they may offer useful insights into the mechanisms of acquired resistance to Hh pathway inhibition (10). For other malignancies that are not driven by mutational activation of the Hh pathway, patient selection is challenged by the absence of predictive biomarkers that signify effective targeting of the tumor–stroma interaction or eradication of CSCs. Adverse events such as muscle cramps, dysgeusia, and alopecia, which have been commonly observed among Hh pathway inhibitors, may represent on-target modulation of normal tissues (1, 11, 12), but the value of these events as systemic biomarkers of activity is yet to be explored. The combination of Hh pathway inhibitors with cytotoxic chemotherapy or targeted agents should be based on preclinical rationale, as in the example of improved drug delivery when this class of agent is combined with gemcitabine in a mouse pancreatic cancer model (13). The introduction of Hh pathway inhibitors, such as GDC-0449, into the clinic has led to extensive translational cross-talk between bench and bedside. Metaphorically, the challenges of interrogating this developmental signaling pathway, similar to the thorny spines of the hedgehog, will require continued collaborative research efforts to be smoothened.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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