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Smoothing Out Drug Resistance

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Smoothened inhibitors have emerged as successful treatment alternatives for Hedgehog pathway-dependent tumors, but they are linked with the appearance of drug resistance. In this issue of *Cancer Cell*, Kim and colleagues overcome such resistance by combining approved drugs itraconazole and arsenic trioxide, thus opening a path toward new treatment options.

Inappropriate reactivation of key developmental signaling pathways in cancer is a common theme, with Hedgehog (Hh), Wnt, and Notch pathways being prime examples. Consequently, focused efforts to develop inhibitors of these pathways are an important part of the broader drive toward targeted therapy for cancer. Aberrant activation of Hh signaling, most often caused by mutational inactivation of the Hh receptor Patched 1 (PTCH1), is a feature of basal cell carcinoma of the skin (BCC) and a subgroup of medulloblastomas (MBs). The importance of the Hh pathway in controlling growth and differentiation in tissue stem and progenitor cells is consistent with the pathway's role as a driver in these tumor types. Unfortunately, as with most cancer drug treatments, a major limitation of agents that block Hh signaling is the presence of preexisting or acquired drug resistance, which prevent cure.

Presently, all drugs in advanced stages of development for the treatment of Hh pathway-dependent tumors target Smoothened (Smo), a seven transmembrane G protein coupled receptor. Smo is present at the cell membrane and is required for ligand-dependent activation of the Hh pathway. One Smo inhibitor, vismodegib (GDC-0449), is FDA-approved for the treatment of locally advanced and metastatic BCC. Another four Smo inhibitors have progressed into phase II clinical trials (NVP-LDE225, IPI-926, XL-139, and LY2940680). Two adult patients with end-stage MB showed excellent responses to vismodegib treatment with tumor shrinkage lasting up to five months; however, tumor relapse eventually occurred in both cases (Asklund et al., 2012; Rudin et al., 2009). More recently, reports of vismodegib resistance in BCC patients have also appeared (Atwood et al., 2012). Smo inhibitor resistance may develop by several different mechanisms, including de novo mutations in *SMO*, amplification of *Gli2* and *Ccnd1* (the cyclin D1 gene), PI3K-mTOR pathway activation, and induction of drug efflux by P-glycoprotein (PGP) (Atwood et al., 2012; Buonamici et al., 2010; Yauch et al., 2009) (Figure 1). Hence, with the anticipated increase in the clinical use of Smo inhibitors, an increase in the prevalence of Smo inhibitor resistance is expected.

In this issue of *Cancer Cell*, Kim et al. (2013) present a highly relevant study showing that two FDA-approved drugs, itraconazole and arsenic trioxide (ATO), inhibit Hh pathway activation and tumor growth associated with acquired resistance to Smo antagonists. The group had shown earlier that itraconazole, an anti-fungal agent, inhibits Hh signaling at the level of Smo by a mechanism different from cyclopamine and prevents the ciliary Smo accumulation normally resulting from Hh activation. Functioning at a different part of the Hh pathway, ATO antagonizes Gli effectors downstream of Smo by inhibiting GLI1 transcriptional activity and blocking Hh-induced Gli2 ciliary accumulation (Beauchamp et al., 2011; Kim et al., 2010).

Since itraconazole and ATO inhibit the Hh pathway at distinct levels, Kim et al. (2013) tested the two compounds alone and in combination to assess efficacy in tumors with acquired resistance to Smo inhibitor. First, they showed that itraconazole alone blocked proliferation to similar degrees in parental (Smo^{WT}) and vismodegib-resistant (Smo^{D447G}) MB cells growing in spheroids derived from $Ptch^{+/-};p53^{-/-}$ mice. However, a Gli-dependent reporter-based system revealed that itraconazole only partially

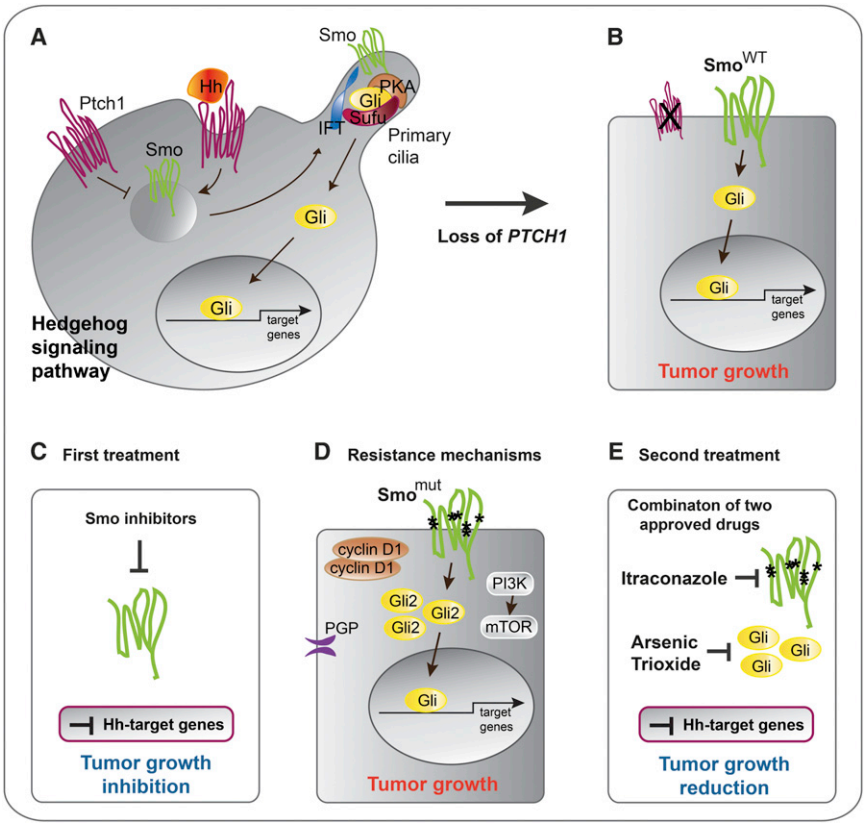

Figure 1. Itraconazole and ATO Are Effective in Treating Smo Inhibitor-Resistant Tumor Growth by Targeting Two Different Levels of the Hh Pathway

(A) The Hedgehog signaling pathway: a simplified model. In its off state, Ptch1 inhibits Smo activity. Hh-ligand binding to Ptch1 abrogates its inhibitory effect on Smo, allowing Smo to translocate to the primary cilium and induce accumulation of the Gli/Sufu complex followed by nuclear translocation of Gli activators initiating Hh-target gene transcription.

(B) Biallelic loss of *PTCH1* leads to ligand-independent Smo (Smo^{WT}) activation, promoting tumor growth.

(C) Smo inhibitors have emerged as a successful treatment alternative for Hh-pathway-dependent tumors, with five small molecule inhibitors currently in phase II trials: vismodegib/GDC-0449, NVP-LDE225, IPI-926, XL-139, and LY2940680.

(D) Resistance to Smo inhibitors may develop by several different mechanisms, such as de novo mutations in *SMO* (Smo^{mut}), *Gli2* and *Ccnd1* (*cyclin D1*) amplifications, PI3K-mTOR activation, and P-glycoprotein (PGP) induction.

(E) Kim et al. (2013) demonstrate that the combination of itraconazole and ATO significantly suppresses Smo inhibitor-resistant tumor growth by inhibiting the Hh pathway at the level of Smo and Gli. IFT, intraflagellar transport; PKA, protein kinase A.

inhibited Smo^{D447G}, whereas Smo^{WT} was fully inhibited. Next, the authors used a combination of itraconazole and ATO in an allograft model of $Ptch^{+/-}$; $p53^{-/-}$ Smo^{WT} MB. Simultaneous treatment not only inhibited tumor growth but also reduced tumor volume by 72%. As expected, Hh pathway activity was most efficiently inhibited after treatment with both itraconazole and ATO. The authors then confirmed the beneficial combinatorial effect using a second Hh pathway-dependent tumor model based on allografted BCC cells derived from $Ptch^{+/-}$;$K14\text{-}Cre^{ER/+}$;$p53^{fl/fl}$ mice.

To answer the key question if combined itraconazole and ATO treatment is able to block growth of tumors with acquired Smo inhibitor resistance, the authors treated mice bearing allografts of $Ptch^{+/-}$; $p53^{-/-}$ Smo^{D447G} MB. Unlike the result with vismodegib, itraconazole and ATO treatment substantially reduced tumor growth, causing a 48% reduction in the original tumor size and a 66% reduction in Hh pathway activity.

Since medulloblastoma is an intracranial malignancy, which is potentially not well represented by subcutaneous allografts, Kim et al. (2013) also tested treatment efficacy in an orthotopic Smo^{D447G} MB model. They observed that mice receiving single agent treatment with itraconazole or ATO had significantly improved survival over control mice (22 and 18 days versus 14 days). Importantly, the combination of the drugs further improved the median survival to 29 days before death due to an excessive tumor burden. Of note, no indications of activation of previously described resistance mechanisms, specifically PI3K-mTOR activity or increased Gli2 mRNA expression, was observed, and drug efflux via PGP is unlikely since itraconazole is a potent inhibitor of PGP. This leaves incomplete pathway inhibition as a likely reason underlying continued tumor growth.

In sum, the combination of itraconazole and ATO showed a significant suppressive effect on tumor growth in preclinical models and, very encouragingly, the suppressive effect overcomes all known drug-resistant Smo mutations and is sustained in the context of Gli2 overexpression.

Both itraconazole and ATO have several additional cellular targets that may play a role in the inhibition of tumor growth. In the case of itraconazole, this includes VEGFR2 and cytochrome P450 retinoic acid metabolizing enzymes, and PML, NF-κB, and microtubules in the case of ATO. Future studies utilizing more selective means to target the downstream components of the Hh pathway, such as RNAi or small molecules, should be informative. A key question in this context is the extent to which resistant tumors will remain dependent on high levels of canonical Hh-signaling. Given the now well-established heterogeneity and plasticity of tumor cells, mechanisms to evade exclusive dependence on a single signaling pathway are likely to evolve. This possibility is supported by response rates in the range of 30%–40% and a limited duration of response in clinical trials treating BCC with Smo inhibitors (Atwood et al., 2012).

The best option to combat Hh-dependent tumors may therefore be to inhibit the Hh pathway very effectively from the start, hoping to prevent development of resistance. In this scenario, the combinatorial use of inhibitors targeting different steps in the signaling pathway, as in the study by Kim et al. (2013), is attractive.

Given the existence of multiple Smo-independent mechanisms of Hh pathway activation, such as gene amplification or increased activity of the Gli transcription factors as well as inactivation of the intracellular repressor Sufu, inhibition of Gli transcriptional effectors appears appealing. Of relevance, activation of the S6K1 kinase in the PI3K/mTOR pathway has recently been shown to increase active GLI1 levels (Wang et al., 2012), which may contribute to the observed Smo inhibitor resistance caused by increased PI3K/mTOR signaling.

A concern with regard to the clinical use of Smo inhibitors is side-effects such as weight loss and muscle cramping that have no known connection to canonical Hh-signaling. Smo-dependent activation of AMPK and Ca^{2+} influx in brown fat and muscle cells elicited by several Smo inhibitors is now proposed as an underlying mechanism (Teperino et al., 2012). Investigation of the presence or absence of such activity induced by itraconazole is therefore warranted.

The findings by Kim et al. (2013) are, despite unanswered questions, very encouraging in that they suggest immediately available second-line treatment options for Smo inhibitor-resistant tumors to be evaluated in the clinical setting.

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Sphingosine 1-Phosphate Is a Missing Link between Chronic Inflammation and Colon Cancer

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In this issue of *Cancer Cell*, Liang and colleagues demonstrated that sphingosine kinase 1, the enzyme that catalyzes formation of the biologically active lipid sphingosine 1-phosphate, drives a malicious amplification loop involving sphingosine 1-phosphate receptor 1 and the NF-κB/IL-6/STAT3 pathway. This appears critical for progression from chronic inflammation to colon cancer.

There is substantial evidence linking inflammatory bowel disease, particularly ulcerative colitis (UC), with colon cancer, termed colitis-associated cancer (CAC). In this regard, mucosal inflammatory cell types can promote (regulatory T cells [Tregs], type 2 macrophages, $CD4^+$ T helper [Th-17 cells]) or inhibit ($CD8^+$ T cells, natural killer cells) CAC (Monteleone et al., 2012). The regulatory effects of inflammatory cells on the growth and survival of cancer cells is dependent on cytokines that can act directly or indirectly on these cells. For instance, cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) provide a supportive tumor microenvironment by activating the transcription factors NF-κB and STAT3, and these are involved in the development of CAC. Activation of NF-κB in a tumor microenvironment is associated with enhanced inflammation and release of IL-6, which promotes CAC (Monteleone et al., 2012).

In this issue of *Cancer Cell*, Liang et al. (2013) elegantly demonstrate that the sphingosine 1-phosphate receptor 1 (S1PR1) and sphingosine kinase 1 (SphK1), the enzyme that catalyzes the formation of sphingosine 1-phosphate (S1P, the natural ligand of S1PR1), are a missing link between chronic inflammation and CAC. Indeed, there is substantial evidence for a role of SphK1 and S1P in cancer (Pyne and Pyne, 2010). There were two important findings that preceded the current study. First, Kawamori et al. (2009) demonstrated that *Sphk1* knockout mice exhibit reduced