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NEWS AND VIEWS

Repurposing an old anti-fungal drug as a Hedgehog inhibitor

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It requires approximately 800 million dollars (US) and 10 years to successfully bring a drug from the laboratory bench into the clinic (DiMasi et al., 2003). Thus, the breakthrough discovery of today will not be available in the clinic for many years, and many dollars, after this initial discovery. One potential way to speed up this drug developmental cycle is by repurposing drugs that have been previously approved for an alternate clinical indication. With this goal in mind, Beachy and colleagues set out to identify a drug with efficacy against tumors that were dependant on the Hedgehog (HH) signaling pathway. They reported in the April issue of a leading cancer research journal that Itraconazole, a commonly used anti-fungal drug, can be turned against HH's signaling function (Kim et al., 2010). It has been estimated that 25% of all human tumors may be HH dependent (Teglund et al, 2010). Thus, getting such an inhibitor into the clinic even a few years earlier has the potential to affect the mortality and morbidity of hundreds of thousands of cancer patients worldwide.

The HH signal transduction pathway regulates numerous aspects of embryonic development. In adult tissues the HH pathway plays an important role in tissue homeostasis, remaining largely inactive until re-activated by tissue injury in order to facilitate tissue regeneration. In tumors, HH acts as a survival factor for cancer cells and helps shape the microenvironment that facilitates tumor development. Many positively acting HH pathway components are classified as human oncogenes, while some of the negatively acting components function as tumor suppressors. Numerous lines of evidence suggest that the rate determining component in the HH signaling cascade is the G-protein-coupled membrane protein Smoothened (SMO) (Teglund et al, 2010). The engagement of SMO initiates a cascade of intracellular events that culminate in the activation and stabilization of the GLI family of transcription factors, which in turn regulate the expression of specific target genes important for apoptosis, cell cycle regulation, or cell renewal. Consistent with the pivotal role SMO plays in HH signaling, many screens for small molecule inhibitors of HH signaling have isolated SMO antagonists—a number of which are currently in different stages of clinical trials (Teglund et al, 2010).

In an effort to more rapidly get a HH inhibitor into the clinic, Beachy and colleagues used an *in vitro* assay to screen through 2400 compounds that had been previously approved for use in humans or had successfully made it through phase I clinical trials, a major hurdle in the drug development cycle (DiMasi et al, 2003). The most promising drug isolated from this library of compounds was the triazole antifungal agent itraconazole, which has been used in the clinic for 25 years. Itraconazole inhibits 14-α-lanosterol demethylase (14-α-LDM), which is critical for the biosynthesis of ergosterol in fungi. Interestingly, some members of the azole class of antifungal agents did not exhibit significant potency as HH inhibitors. This result was the first indication that the mechanism of action of itraconazole as an anti-fungal agent, inhibition of 14-α-LDM, would be different from how it acts as a HH inhibitor.

The Beachy group demonstrated that the likely mechanism of action of itraconazole is via attenuation of SMO activity. Interestingly, itraconazole acted on SMO in a non-competitive manner with the commonly used SMO antagonist cyclopamine. This pharmacological distinction probably underlies the observation that these two compounds acted in a synergistic

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manner to inhibit HH signaling. The simultaneous use of mechanistically different SMO inhibitors would have the practical consequence of lowering the effective concentration of SMO inhibitors needed in patients and would reduce the likelihood that such patients would develop resistance to such inhibitors. Resistance to a SMO inhibitor has already been described in the clinic, during phase I trials of the SMO inhibitor GDC-0449. A mutation in SMO that prevented the binding of GDC-0449 was found responsible for this acquired resistance (Yauch et al., 2009). The use of a second SMO antagonist, such as itraconazole, might significantly decrease the chances of selecting such specific SMO mutations.

This study determined the efficacy of itraconazole *in vivo*, using mouse models of two distinct HH-activity dependent tumors—medulloblastoma and basal cell carcinoma (BCC). In both models, itraconazole therapy suppressed tumor growth. The combination of itraconazole and cyclopamine *in vivo* had an even greater inhibition of tumor growth, consistent with the synergistic effect of the two SMO-antagonists *in vitro*. The mRNA level of the HH target gene *GLI1* decreased after treatment with either itraconazole or cyclopamine, consistent with these drugs attenuating HH signaling. Of relevance to its potential clinical use, the serum concentrations of itraconazole in the experimental animals were comparable to those of patients treated for severe fungal infections. In this high-dose indication, itraconazole therapy consists of 600 mg/d for 3–16 months and this dosing schedule resulted in manageable side effects (Kim et al, 2010). Based on the results summarized here, Stanford University is now recruiting patients for a phase II clinical trial to determine the ability of itraconazole to reduce various BCC biomarkers.

This report serves as another example of the clinical importance and cost-effectiveness of repurposing drugs that are, or were, clinically approved for other indications. While the identification of novel targeted therapies will continue to be of paramount clinical importance, using existing drugs in distinct ways will also continue to provide a novel way to treat many clinically intractable human ailments. Such repurposing of drugs may also provide a way for Pharmaceutical companies to recover some of the exorbitant costs associated with bringing a new compound to the clinic. One recent example of this strategy is the Pfizer compound Sildenafil, which was initially studied for use in hypertension and angina pectoris (Ghofrani et al, 2006). Its potent, unexpected effect on erectile dysfunction was subsequently discovered during clinical trials. Sildenafil was rebranded as Viagra, and is now a billion dollar (US) selling drug. Thus, there are great incentives for all the stakeholders, pharmaceutical companies, clinicians, and cancer patients, for itraconazole to reemerge as an effective agent against HH activity dependent tumors.

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