


Discussion

**Dr Jules Lin (Ann Arbor, Mich).** Dr Nguyen, congratulations on your work and an excellent presentation.

You have identified potential therapeutic targets for MPM in the Hh pathway, SMO and Gli, and identifying a novel target is a good first step. We are often frustrated by the lack of inhibitors, and this approach you have taken is exciting, to find a commonly used drug that has been extensively tested in humans that happens to inhibit the targets you have identified to treat MPM.

I have 3 questions for you. ITRA has many different effects, and it affects other pathways, including the mammalian target of rapamycin pathway. Did you confirm that the effects that you see here are from the inhibition of the Hh pathway? For example, did you inhibit a downstream mediator, such as Gli, with a small hairpin RNA to see whether that eliminates the effect of ITRA?

**Dr Nguyen.** That is a very good point. ITRA has been shown to target the mammalian target of rapamycin in signaling of the vascular endothelial growth factor receptors in endothelial cells. I did a lot of searching. I could not really find good studies on the effect of ITRA in cancer cells. It will be the next step for us to determine what ITRA does in cancer cells. Your comment about selectively targeting an Hh pathway component such as Gli to determine the relative contribution of Hh inhibition in mediating the growth inhibitory effect of iraconazole is valid. In this case, I would knockdown SMO because it is the target of ITRA. I agree with you that ITRA has other effects that inhibit cancer cell growth. The antiproliferation effect we observed might not all be coming from SMO inhibition. Also, even if the anticancer effect is SMO independent, we can always use Gli knockdown as a marker of the drug effect on the cells. That is a very good point.

**Dr Lin.** My second question is, in basal cell carcinoma, as you mentioned, PTCH was decreased and that was the mechanism for Hh overexpression in the basal cells. In MPM, what is the exact alteration? In your cell lines, did you see certain genes that were overexpressed and that correlated with the treatment response? Which would be the best biomarker for the treatment response?

**Dr Nguyen.** I appreciate those comments a lot. A recently published study in the International Journal of Oncology (2012;41:1751-61) examined the antiproliferation effect of cyclopaamine—a prototypic SMO inhibitor—in a large collection of cancer cell lines of epithelial origin. The investigators screened for activating mutations of PTCH or SMO, and they observed none. It only seems to occur in basal cell carcinoma or medulloblastoma. However, the Hh pathway can be activated by other mechanisms, including overexpression of the ligands. More importantly, evidence has shown that the Hh pathway can be activated by a paracrine effect with ligands coming from the tumor microenvironment (low Hh activity in vitro cultures but high levels of activation in tumor xenografts). We do not see that in the in vitro system. The other report has shown that the method we use to grow cells can affect Hh signaling. We grow cells as a monolayer on plastic ware. So, my next step would be to study cells grown as spheroids. Other investigators have shown that in the spheroid-grown condition, pathways such as Notch, Wnt, or Hh are activated. Thus, that would be another way of looking at it. What you referred to in your last question is the identification of a biomarker or gene signature predictive of the treatment response. That is the next step of our research project. I appreciate that insightful comment.

**Dr Lin.** Then, my final question, Dr Jablons’ group at the University of California, San Francisco, found that Gli2 was overexpressed, and they thought that resulted from a SMO-independent pathway, perhaps transforming growth factor-β. Did you consider that at all, and did you find any synergistic effects if you inhibited both SMO and Gli at the same time?

**Dr Nguyen.** Can you repeat that question?

**Dr Lin.** Did you find any synergistic effects if you inhibited both SMO and Gli simultaneously?

**Dr Nguyen.** Yes. A report was published by Beachy’s group from Stanford studying combining ITRA and ATO (Cancer Cell. 2013;23:23-34). Yes, that would be something that we can do, is to combine, targeting different levels of the pathway. As you know, when you treat cells with GDC, SMO mutants emerge that are resistant to the drug. We must find a method to overcome that. One way of doing it would be to target downstream of SMO, such as at the Gli level.

**Dr Lin.** Congratulations on your work.