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The broad stroke of Hsp90 inhibitors: painting over the RAF inhibitor paradox

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Abstract

The novel Hsp90 inhibitor XL888 is undergoing clinical investigation for use in conjunction with the RAF inhibitor vemurafenib to treat unresectable melanoma. The addition of XL888 to current regimens may serve an additional purpose by blocking the RAF inhibitor paradox. Such activity could reduce adverse events in patients and provide a biomarker for the successful inhibition of Hsp90 target proteins.

In this issue of *Journal of Investigative Dermatology*, Phadke and colleagues (Phadke *et al.*, 2015) report results from a phase I dose-escalation study of XL888, a novel hsp90 inhibitor, in combination with the Raf inhibitor, vemurafenib, in patients with unresectable BRAF-mutant melanoma (NCT01657591). Fifty percent of patients with melanomas harbor an activating valine to glutamic acid substitution (V600E) in the serine/threonine kinase BRAF that signals through the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway. Despite initial efficacy, BRAF inhibitor monotherapy and newer BRAF/MEK inhibitor combination therapy inevitably yield to therapy resistance. Mechanisms driving resistance include the acquisition of NRAS mutations, expression of BRAF splice-variants, amplification of BRAF V600E, and the upregulation of receptor tyrosine kinases leading to ERK and AKT pathway activation (Hartsough *et al.*, 2013). The use of Hsp90 inhibitors in conjunction with vemurafenib is being pursued to provide a broad stroke of inhibition against these resistant pathways. Here the authors report a second potential clinical benefit, namely the reduction of paradoxical ERK1/2 activation that occurs as a result of treatment with first generation RAF inhibitors.

In addition to limited durable treatment responses, vemurafenib and other first generation RAF inhibitors display paradoxically activating ERK1/2 signaling in BRAF wild-type cells. As reviewed by Gibney *et al.*, this activation occurs via enhanced dimerization of RAF monomers, either through the relief of BRAF autoinhibition or through conformational changes that stabilize protein-protein interaction when a single RAF monomer is bound to an inhibitor (Gibney *et al.*, 2013). Ultimately, dimerization with and activation of CRAF

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Conflict of Interest

The authors state no conflict of interest.

enables phosphorylation of downstream MEK and ERK1/2 targets (Poulikakos *et al.*, 2010). In patients, this paradoxical activation causes the generation of a number of hyperproliferative cutaneous lesions, including squamous cell carcinomas, keratocanthomas, papillary lesions, and verrucous keratosis, as well as nevi and new primary melanomas. These lesions have been treated by excision and no patients with metastases have been encountered; however, the potential for malignancies at other less accessible locations remains a concern. The observation that rare cases of colonic adenomas, gastric polyps, and a single case of myelomonocytic leukemia have been encountered during RAF inhibition highlight the potential for paradoxical ERK1/2 activation in other tissues (Gibney *et al.*, 2013).

The Hsp90 chaperone protein binds to target proteins to support refolding and thereby can promote cell survival during times of stress. Many proteins integral to melanoma progression and therapy resistance represent Hsp90 “clients” including mutant BRAF (but not wild type BRAF), CRAF, COT, PDGFR, IGF1R, and AKT; it is through these interactions that Hsp90 exhibits pro-tumorigenic activity (Grbrovic *et al.*, 2006). First-generation Hsp-90 inhibitors derived from the natural geldanamycin product studied within the last decade have demonstrated promising pre-clinical results but have ultimately failed to garner FDA-approval. In human melanoma cell lines, the Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG) induced ubiquitination and proteasome-mediated degradation of BRAF V600E and caused BRAF V600E degradation and growth inhibition in SK-MEL-28 xenografts (Grbrovic *et al.*, 2006). 17-AAG progressed to phase I and phase II clinical trials and was implemented at the highest tolerated dose (450mg/m^2 i.v. once weekly). Significant increases in Hsp70 protein levels at a post-treatment biopsy (median 44 hours) compared to pre-treatment biopsy indicated successful Hsp90 inhibition; however, BRAF and CRAF levels remained unchanged, as did phospho-ERK1/2, a measurement of ERK1/2 pathway activity (Solit *et al.*, 2008). These findings contrasted with the phase I results that showed reductions in client proteins at 24 hours (Banerji *et al.*, 2005). Taken together with the measured increase in Hsp70 in the phase II studies, this finding suggests that the biologic effect of 17-AAG was short lived and that more potent Hsp90 inhibitors capable of chronic dosing could improve treatment efficacy.

The FDA approval of targeted therapies with diverse resistance mechanisms has rekindled interest in Hsp90 inhibitors. 17-AAG required bioreduction to a hydroquinone 17-$AAGH_2$ to elicit its full effects and the expression of P-glycoprotein or loss of NAD(P)H dehydrogenase, quinone 1 (NQO1) could prevent this metabolic event. XL888 is a novel non-benzoquinone, ATP-competitive inhibitor of Hsp90 (Catalanotti *et al.*, 2012). Inhibition of Hsp90 with XL888 leads to proteolytic degradation of these proteins in multiple melanoma models. Pre-clinical data has shown that such inhibitors can effectively block ERK1/2 signaling in RAF-inhibitor resistant cell lines (Paraiso *et al.*, 2012) and in combination with vemurafenib, can delay the emergence of resistance in xenograft models (Smyth *et al.*, 2014).

Whether XL888 will be hampered by the same limitations as 17-AAG remains to be seen. This first report of phase I results provides some optimism regarding the ability of XL888 to successful destabilize Hsp90 client proteins. Following 24 weeks of vemurafenib and

XL888 treatment (35mg, 45mg, 90mg, and 135mg PO BIW; for four cohorts, respectively) the authors report an overall reduction in the number of hyperproliferative/neoplastic skin lesions. Notably, the cohort treated with the highest concentration of XL888 experienced 2 verruca vulgaris lesions among six patients and no squamous cell carcinomas, keratocanthomas, or new primary melanomas. In mutant NRAS/wild-type BRAF mutant cell lines, XL888 is capable of reversing the paradoxical activation of ERK1/2 induced by vemurafenib at concentrations higher than 100 nM. Mechanistically, Phadke *et al.* show that treatment with 300 nM XL888 reduces expression of CRAF, a critical mediator of the paradox effect (Gibney *et al.*, 2013; Poulikakos *et al.*, 2010).

Inhibition of the paradox effect is undoubtedly important in the context of RAF inhibition. In addition to requiring an increased number of follow-up procedures to remove cutaneous lesions, the activation of ERK1/2 signaling can also lead to other non-cutaneous lesions that are difficult to identify (Gibney *et al.*, 2013). FDA approved RAF and MEK inhibitor combination therapies now provide this benefit, and it remains to be seen whether XL888 therapy achieves this aim in a statistically significant fashion. Perhaps more critically, these findings point to the possibility that the reduction in CRAF levels could represent a biomarker for successful Hsp90 therapy in conjunction with RAF inhibition. Despite the common use of increased Hsp70 expression as a surrogate for successful Hsp90 inhibition, patients receiving Hsp90 inhibiton often demonstrate varied expression levels compared to Hsp70 (Catalanotti *et al.*, 2012), and, as observed with 17-AAG, client protein, destabilization and treatment effects may not correlate with Hsp70 induction (Solit *et al.*, 2008). As investigation continues with XL888, validation of such a biomarker may provide a more robust measure of clinically relevant Hsp90 inhibition and favorable patient response.

Ultimately, additional data are needed to understand whether XL888 is eliciting the desired effect on Hsp90 client proteins. Pre-clinical data point to the possibility that XL888 can inhibit the diverse modes of resistance encountered with RAF inhibition and that combination therapy with vemurafenib can delay the time to relapse. Further testing of XL888 efficacy will come in the form of pre/post-treatment biopsies that measure directly the effects of Hsp90 inhibition on client protein expression and ERK1/2 pathway activation. This phase I trial was insufficiently powered to demonstrate changes in hyperproliferative lesions that are statistically useful. However, the promising results presented in this article suggest that there is indeed an inhibitory effect. Quantifying these lesions will remain a focus in an upcoming phase II clinical trial testing XL888 in conjunction with combined RAF and MEK inhibitors. Only with this added clinical data will XL888 be spared the fate of 17-AAG and the other first-generation Hsp90 inhibitors.

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Clinical Relevance

- The RAF inhibitors paradoxically cause hyperplastic lesions in melanoma patients.
- Treatment with Hsp90 inhibitors may block this event and reduce its frequency.
- Reduction of paradoxical signaling may serve as a biomarker for successful Hsp90 inhibition.