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Heat shock protein 90 inhibition limits the emergence of tamoxifen resistance.

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Background: A major limitation to the efficacy of hormonal therapies in the management of advanced breast cancers is the frequent development of acquired resistance. The mechanisms underlying such resistance are complex, but likely involve alterations in Estrogen Receptor (ER)-cofactor associations at Estrogen Responsive Elements (EREs) on the DNA and/or enhanced ER phosphorylation through growth factor signaling pathways. Indeed, altered cofactor association in some breast cancers may eventually lead to stimulation of growth by Selective Estrogen Response Modifiers (SERMs) such as tamoxifen (Tam). Because resistance poses such a prominent problem in the use of SERMs, we have examined inhibition of the molecular chaperone heat shock protein 90 (Hsp90) as an alternative approach to targeting ER function, one that could be used in combination with Tam to prevent resistance and provide more durable disease control.

Materials and Methods: Using MCF-7 breast cancer cells, disruption of ER function by ansamycin-based Hsp90 inhibitors (geldanamycin, 17AAG) and a highly potent and well-tolerated next-generation Hsp90 inhibitor (STA-9090) was monitored in the presence and absence of Tam. ER protein levels, localization, transcriptional activating activity and DNA binding were measured using standard techniques. Small molecules and lentiviral-mediated RNAi knockdown were used to determine the impact of Hsp90 compromise on the emergence of Tam-resistant clones in colony-forming assays.

Results: In the absence of Tam, Hsp90 inhibitors markedly deplete cellular ER levels by stimulating its proteasome-mediated degradation. Depletion is inhibited in cells exposed to Tam and the related antagonist, raloxifene, but not the pure antiestrogen fulvestrant. In detergent-soluble fractions, Hsp90 inhibition induces expected alterations in composition of multi-protein ER-chaperone complexes despite the presence of Tam. Using chromatin immunoprecipitation, however, we found that Tam causes prolonged association of ER with the DNA in stalled transcriptional complexes, thereby retaining the protein in the nucleus and diminishing its destabilization by Hsp90 inhibitors. Nevertheless, Hsp90 inhibition still appears to enhance the activity of Tam in cell culture,

probably by depleting a pool of residual ER that is not tightly associated with promoter elements in the presence of Tam. The net effect of combining Tam with Hsp90 inhibition, whether achieved by genetic knockdown or by concentrations of Hsp90 inhibitor that have no overt anticancer activity alone, is to dramatically limit the emergence of Tam resistant clones in cell culture. Experiments in estrogen-supplemented mice bearing MCF-7 xenografts are underway to see whether a similar effect can be achieved in vivo.

Discussion: Hsp90 inhibition decreases the survival of hormone-dependent breast cancer cells and prevents the emergence of hormone resistance in cell culture. If confirmed by ongoing animal studies, these findings provide a strong pre-clinical rationale for evaluating combined Tamoxifen-Hsp90 inhibitor treatment in patients.