A novel pharmacodynamic assay to measure Hsp90 target occupancy by the small molecule inhibitors IPI-504 and IPI-493 in tumors

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Abstract

Heat shock protein 90 (Hsp90) is a protein chaperone important for the stabilization and folding of many oncogenic proteins, and is an attractive target for cancer therapy. Infinity is developing both IPI-504 as well as IPI-493 Hsp90 inhibitors. The most advanced compound, IPI-504, has recently entered a phase II clinical trial. In preclinical animal models, IPI-504 and IPI-493 are rapidly cleared from the circulation and normal tissues but accumulate in tumor tissue. Standard measurements used to monitor the biological activity of Hsp90 inhibitors in the clinic therefore may not represent an accurate assessment of the level of target inhibition in tumor tissue. To address this issue, a pharmacodynamic activity assay was developed for Hsp90 that can accurately quantitate Hsp90 inhibition in tumor samples. Kinetic measurements demonstrate both IPI-504 and IPI-493 exhibit slow Hsp90 off rates, an effect that is exaggerated at low temperatures (35°C-4°C). This property made it feasible to develop an assay that measures the fraction of Hsp90 target protein that is occupied (inhibited) with small molecule Hsp90 inhibitors. The Hsp90 occupancy assay utilizes external binding sites for Hsp90 and can be used to directly measure Hsp90 inhibition in cells and tumor tissue after treatment with these inhibitors. We have developed a novel Hsp90 pharmacodynamic activity assay that directly measures Hsp90 occupancy in the clinic. The Hsp90 occupancy assay was validated with purified Hsp90 protein and with various cancer cell lines. We observed a good correlation between % Hsp90 occupancy and cell growth inhibition values. In conclusion, we developed a novel pharmacodynamic assay which measures the occupancy of Hsp90 by small molecule Hsp90 inhibitors that can be used to directly measure Hsp90 inhibition in tumors from patients treated with IPI-504 or IPI-493.

Off Rate is Slower Than On Rate at 4°C

• To develop an assay that measures the in-vivo occupancy of Hsp90 in the cytosol of cultured cells and xenograft tumors after drug treatment.
• To determine the percent of total Hsp90 that is occupied by IPI-504 in cultured cancer cells and xenograft tumor samples after drug treatment.
• To determine the percent Hsp90 occupancy by IPI-504 with each tumor activity in drug treated cultured cells and xenograft models.

Hsp90 Pharmacodynamic Activity Assay

Hsp90 Occupancy Determined by Titrating Open Binding Sites

Hsp90 Occupancy Validation in Xenografts

Correlation between Hsp90 Occupancy and Efficacy in A Xenograft Model

Summary

• We have developed a novel Hsp90 pharmacodynamic activity assay that directly measures Hsp90 occupancy in cells and tumor tissue after treatment with the Hsp90 inhibitor IPI-504.
• Because the dissociation rate of 17‐AAG from purified Hsp90 and/or Hsp90 in cancer cell lysates is slow (1/2h) when measured at 4°C, the maintenance of a stable Hsp90:drug complex can be preserved.
• The Hsp90 occupancy assay was validated with purified Hsp90 protein and with various cancer cell lines. We observed a good correlation between % Hsp90 occupancy and efficacy in xenograft models treated with IPI-504.
• This method may allow us to explore the relationship between Hsp90 occupancy and sensitization of other Hsp90 oncogenic client protein expression levels. Data is representative of n=2.