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

Abstract #1710

Bonnie Tillotson, John Coco, Nigel Whitebread, Kip West, Kelly Slocum, Brian Thomas, Jie Ge, Janid Ali, Emmanuel Normant, Jennifer Hoyt, John MacDougall, Jens Sydor, Vito Palombella, Julian Adams and Christian Fritz

Infinity Pharmaceuticals Inc., 780 Memorial Drive, Cambridge, Massachusetts, USA

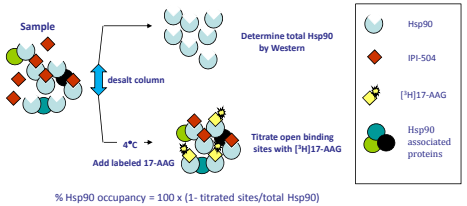
Abstract

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 iv (IPI-504) as well as oral (IPI-493) Hsp90 inhibitors. The most advanced compound, IPI-504, has recently entered a phase III clinical trial. In pre-clinical 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 that IPI-504 and IPI-493 exhibit slow Hsp90 off rates, an effect that is exaggerated at low temperatures ($t_{1/2}$ 4°C =24h). 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 method was first validated using purified Hsp90 protein that had been pre-incubated with varying molar ratios of IPI-504 to artificially create different levels of occupancy. Next, the method was applied to cultured cancer cell lines exposed to increasing concentrations of IPI-504. Tissue culture experiments demonstrated a good correlation between the occupancy of Hsp90 and growth inhibition by IPI-504, confirming that the Hsp90 inhibitor indeed affects cell growth through the inhibition of Hsp90. Finally, this assay was applied to xenograft tumor samples from mice treated with different doses of IPI-504 and IPI-493. Importantly, these xenograft studies demonstrated that Hsp90 occupancy was better correlated with Hsp90 client protein degradation and efficacy than either plasma or tumor PK. 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.

Objectives

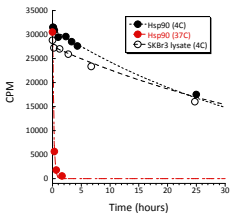
- To develop an assay that measures the in-vivo occupancy of Hsp90 by the Hsp90 inhibitors IPI-504 and IPI-493.
- To determine the percent of total Hsp90 that is occupied by IPI-504 in cultured cancer cells and xenograft tumor samples after drug treatment.
- To correlate the percent Hsp90 occupancy by IPI-504 with anti-tumor activity in drug treated cultured cells and xenograft models.

Hsp90 Pharmacodynamic Activity Assay



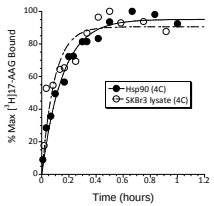
Schematic 1. Representation of the Hsp90 pharmacodynamic activity assay (Hsp90 occupancy assay). % Hsp90 occupancy is calculated from a ratio of open binding sites and total Hsp90.

Off Rate is Slower Than On Rate at 4°C



1a.

Figure 1a. The dissociation of [3H]17-AAG from purified Hsp90 and SKBr3 lysates is temperature dependent with a slow off rate at 4°C. The dissociation of [3H]17-AAG from purified Hsp90 was measured at both 4°C and 37°C and data was fit to a monoexponential decay equation. The dissociation $t_{1/2}$ at 37°C was 8min. At 4°C, the $t_{1/2}$ was considerably longer (28hr). Similarly, the dissociation rate of [3H]17-AAG from Hsp90 in SKBr3 lysates at 4°C was slow with a $t_{1/2}$ of 36hr .



1b.

Figure 1b. The association of [3H]17-AAG to Hsp90 and SKBr3 cancer lysates is faster when measured at @ 4°C. The rate of [3H]17-AAG association at concentration of 1uM to purified Hsp90 and Hsp90 in SKBr3 lysates was measured and data fit to a first order rate equation yielding $t_{1/2}$: 6min for purified Hsp90 and $t_{1/2}$= 4min for SKBr3 lysate. [3H]17-AAG binding to purified Hsp90 or to Hsp90 in cancer cell lysates reached equilibrium by 30 – 40 minutes at 4°C. Because the k_{off} is slow relative to the k_{on} at 4°C, unoccupied sites can be saturated with [3H]17-AAG without any significant dissociation of prebound 17-AAG. Therefore, the fraction of drug-bound Hsp90 in a treated tumor sample can be empirically determined.

Hsp90 Occupancy Determined by Titrating Open Binding Sites

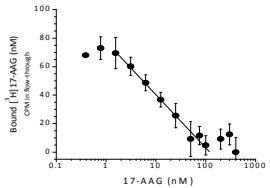


Figure 2. The fraction of purified Hsp90 that is occupied with 17-AAG can be quantified. Recombinant human Hsp90β protein (100nM) was preloaded with increasing amounts of 17-AAG to create different levels of occupancy at 4°C followed by addition of excess [3H]17-AAG. A dose dependent increase in 17-AAG results in a corresponding decrease in the amount of [3H]17-AAG bound to Hsp90 with binding spanning two orders of magnitude.

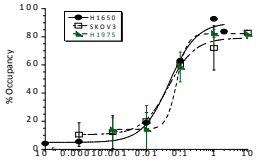


Figure 3. Hsp90 occupancy can be measured in cultured cancer cell lines pretreated with unlabeled 17-AAG. H1650, H1975 and SKOV3 cells were incubated with increasing amounts of unlabeled 17-AAG. Hsp90 occupancy levels were determined by titrating the unoccupied Hsp90 binding sites with [3H]17-AAG and measuring the total Hsp90 by quantitative western. Increasing 17-AAG concentrations corresponds to an increase in the measurable level of Hsp90 occupancy (inhibition) where 80-90% of intracellular Hsp90 is occupied with drug at the two highest 17-AAG concentrations (1-10uM).

	EC_{50} (nM)	
Cell line	**Hsp90 occupancy**	**cytotoxicity**
H1650	30 +/- 15	37
SKOV3	44.5 +/- 6.5	52
H197S	74 +/- 33	51
RS4.11	644	2000

Table 1. Hsp90 occupancy correlates with cell growth inhibition in four cancer cell lines. Comparisons of cell growth inhibition EC_{50}'s with percent Hsp90 occupancy in each cell line suggest that a 50% reduction in growth inhibition occurs when 50% of Hsp90 is occupied with 17-AAG.

Hsp90 Occupancy Validation in Xenografts

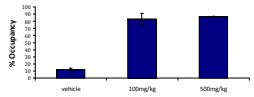


Figure 4. Hsp90 occupancy by IPI-504 in a NSCLC H1650 xenograft model. Single administration of IPI-504 i.p. at 100mg/kg and 500mg/kg produced comparable degree of Hsp90 occupancy in tumor samples from xenograft models of NSCLC.

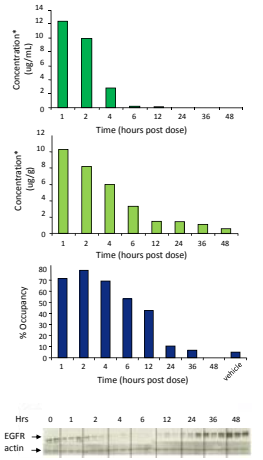


Figure 5. Hsp90 occupancy with IPI-504 dosing correlates with EGFR degradation and tumor PK. H1650 tumor bearing mice were treated with a single dose of 100mg/kg intravenous IPI-504. Tumors and blood plasma were collected at designated time points post dose. Drug levels (IPI-504, 17-AAG and 17-AG)* were quantified by LC/MS/MS. 70-80% Hsp90 occupancy was achieved up to 4hrs post dose with concurrent EGFR degradation up to 6 hrs. A drop in Hsp90 occupancy between 12 and 24 hrs is followed by recovery of client protein expression levels. Data is representative of n=2.

Correlation between Hsp90 Occupancy and Efficacy In a Xenograft Model

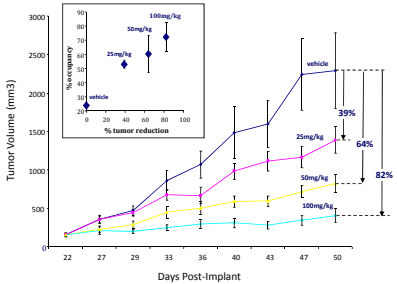


Figure 6. Hsp90 occupancy correlates with anti-tumor activity in H1650 xenograft models administered oral IPI-504. H1650 tumor bearing mice were treated with 25, 50 or 100mg/kg IPI-504 (PO, QDO) for 28 days. A separate group of H1650 tumor bearing mice were treated with a single dose of 25, 50 or 100mg/kg IPI-504 (PO) and sacrificed 2hrs post dose. A dose-dependent increase in efficacy correlates with an increase in % Hsp90 occupancy in IPI-504 treated H1650 xenograft tumors.

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 (>24hr) 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 cell growth inhibition values.
- The Hsp90 occupancy assay has allowed us to assess the relationship between Hsp90 occupancy level, tumor PD and efficacy in xenograft models treated with IPI-504.
- This method may allow us to explore the relationship between Hsp90 occupancy and sensitivities of other Hsp90 oncogenic client proteins post treatment with IPI-504 or IPI-493, an oral Hsp90 inhibitor in phase 1 clinical trials.
- The Hsp90 occupancy assay can be used to measure the extent of Hsp90 inhibition by IPI-504 and IPI-493 in patient tumor samples.