

The Hsp90 inhibitor IPI-504 inhibits proliferation and tumor growth of HER2 overexpressing breast cancer cells with acquired trastuzumab resistance

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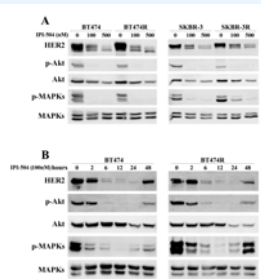
Heat shock protein 90 (Hsp90) is a conserved molecular chaperone mediating the maturation and stability of several cancer-associated proteins. Hsp90 is an emerging target in cancer, and high Hsp90 expression has been linked to decreased survival in breast cancer. IPI-504 is a novel inhibitor of Hsp90 which is currently in clinical trials in gastrointestinal stromal tumors (GIST, Phase III) and NSCLC (Phase II). The aim of this study was to assess the anti-tumor effects of IPI-504 in trastuzumab resistant, HER2 positive breast cancer cells.

We developed HER2 positive breast cancer cell lines (BT-474 and SKBR-3) resistant to trastuzumab by culturing cells in the presence of increasing concentrations of trastuzumab for more than 18 months. These cells are refractory to the antiproliferative activity of trastuzumab at doses up to 250nM in the culture medium.

IPI-504 inhibits the growth of parental (BT-474 and SKBR3) as well as trastuzumab resistant cell lines. By western blot analysis we show that IPI-504 is equally efficient in decreasing the levels of HER2, Akt, p-Akt and p-MAPKs in both parental and resistant cells. Similarly, inhibition of proliferation with accumulation in G0-G1 phase of the cell cycle in both cell types is achieved by comparable concentrations of IPI-504. *In vivo*, the growth of xenografts derived from parental BT-474 cells was, as expected, inhibited by both trastuzumab and IPI-504. In contrast, xenografts derived from trastuzumab resistant BT-474 cells responded poorly to trastuzumab but were still sensitive to IPI-504. Interestingly, the combination of trastuzumab and IPI-504 was found to be more effective than either agent alone in trastuzumab resistant tumors. In trastuzumab sensitive tumors, HER2 downregulation was effectively achieved with either trastuzumab or IPI-504, whereas in trastuzumab resistant tumors only the combination of both compounds significantly decreased the levels of HER2.

In a time course experiment using trastuzumab resistant xenografts we found that the levels of HER2, p-Akt and p-MAPK were significantly decreased after 12 hours of a single dose of IPI-504, reaching minimum levels at 24 hours. At 48 hours, both p-Akt and p-MAPK recovered, whereas HER2 levels were still low. Taken together, these results suggest that the inhibition of Hsp90 by IPI-504 can represent a valid therapeutic option in trastuzumab resistant breast cancer.

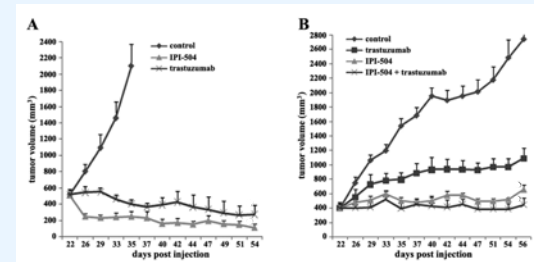
IPI-504 inhibits HER2, Akt and MAPKs pathways



Treatment with either 100 or 500nM IPI-504 for 24 hours (A) results in marked HER2 downregulation. Incubation with IPI-504 potently suppresses both Akt and MAPKs phosphorylation and decreases total levels of Akt in a dose-dependent manner.

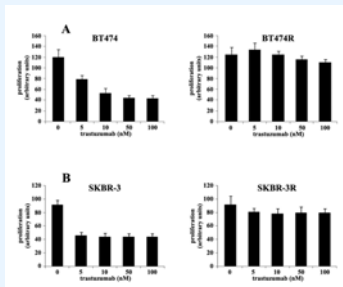
Time course experiments (B) reveal that exposure to 100nM IPI-504 for 48 hours results in a resurgence of HER2 expression. Some recovery of p-Akt and p-MAPKs expression is also observed.

IPI-504 antitumor activity *in vivo*



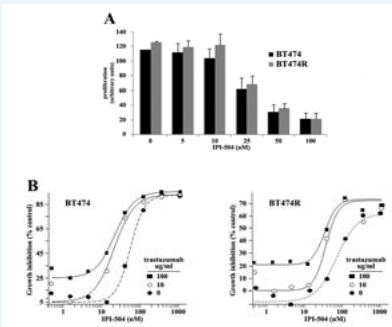
IPI-504 (100mg/Kg thrice weekly) and trastuzumab (10mg/Kg thrice weekly) independently induce tumor regression of BT474-derived xenografts (A). Tumors derived from BT474R cells respond poorly to trastuzumab but are still sensitive to IPI-504 (B). Interestingly, the combination of IPI-504 with trastuzumab in BT474R xenografts results in a greater growth inhibition than either agent alone.

Establishment of trastuzumab resistant cells



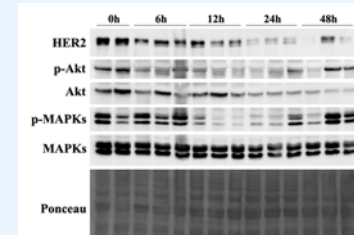
Trastuzumab resistance was induced in BT474 (A) and SKBR-3 (B) HER2 positive breast cancer cell lines by culturing parental cells with increasing concentrations of trastuzumab for greater than 18 months. Trastuzumab concentrations up to 10-fold greater than the IC₅₀ that inhibits parental cell proliferation do not affect BT474 resistant (BT474R) and SKBR-3 resistant (SKBR-3R) cell growth.

Antiproliferative activity of IPI-504 in resistant cells



Treatment with IPI-504 for 5 days produces similar antiproliferative activity in both parental and trastuzumab resistant cells (A). In both BT474 and BT474R cells, the combination of IPI-504 and trastuzumab results in superior inhibition of cell proliferation in a 3D culture model (B).

IPI-504 pharmacodynamics in tumors



Levels of HER2, p-Akt and p-MAPKs significantly decrease 12 hours after a single dose of 100mg/kg IPI-504. At 48 hours, both p-Akt and p-MAPKs recover, whereas HER2 and total Akt levels remain suppressed.

Conclusions

Our results suggest that IPI-504-mediated inhibition of Hsp90 could be a valid therapeutic strategy in HER2 positive breast cancer patients that have progressed from trastuzumab therapy. IPI-504 in combination with trastuzumab is being investigated in a clinical trial of patients with metastatic breast cancer.