Hsp90 Inhibition Results in a Significant Delay in Tumor Progression in a Model of Emerging EGFR TKI Resistance in Non-Small Cell Lung Cancer

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Abstract

Heat shock protein 90 (Hsp90) plays a role in regulating the stability of key cancer-causing proteins through its role as a protein chaperone. Proteins chaperoned by Hsp90, known as client proteins, include cancer-causing forms of ALK, BCR-ABL, EGFR, FLT3 and HER2. Infinity is developing two drug candidates in its Hsp90 chaperone inhibitor program: IPI-504 (retaspimycin hydrochloride), an intravenously-administered small molecule, and IPI-493, which is administered orally.

EGFR tyrosine kinase inhibitors (TKIs) are an effective treatment for lung cancer patients with activating mutations in EGFR. After a dramatic initial response, however, most patients become resistant to drug treatment and progress. In about half of these cases, resistance is due to a second point mutation in EGFR (T790M). It is believed that in at least some of these cases, the TKI resistance mutations are pre-existing and that treatment with TKIs selects for the resistant cells.

In an effort to model the emergence of resistance to TKIs from pre-existing mutations, we developed a novel in vivo model, in which gefitinib administration initially leads to tumor regression followed by rebound of tumor growth and outgrowth of drug resistant cells containing the T790M mutation. We show that in this model, administration of IPI-493 alone or IPI-493 following gefitinib resulted in tumor growth inhibition of 61% and 77%, respectively, when compared with gefitinib administration alone. Administration of IPI-493 alone also resulted in a delay in time to tumor progression with ~40% of animals still on study 45 days following tumor implant; all animals treated with either vehicle or gefitinib had been removed due to tumor progression. Interestingly, administration of IPI-493 following gefitinib resulted in an even more impressive delay in time to progression, with >60% of animals still on study on day 65 post-implant.

These results suggest that further studies with Hsp90 inhibitors in EGFR mutant NSCLC patients who have been pre-treated with aTKI are warranted.

Materials & Methods

Cell lines: Non-small cell lung cancer (NSCLC) cell lines, NCI-H1975 and NCI-H1650, were purchased from ATCC and cultured in RPMI 1840 supplemented with 10% fetal bovine serum. NCI-H1975 harbors an activating mutation in EGFR, L858R, and an additional "gatekeeper" mutation, T790M, which confers resistance to EGFR TKIs, erlotinib and gefitinib. NCI-H1650 harbors an activating deletion of EGFR in exon 19, E746-A750, which confers sensitivity to EGFR TKIs.

***In vivo* studies**: For assessment of compound activity in vivo NCI-H1650 and NCI-H1975 were implanted sub-cutaneously into the right rear flanks of male athymic mice (Taconic) and grown as xenografts. NCI-H1650 were implanted at a concentration of $1x10^7$ cells per animal. NCI-H1975 were implanted at a concentration of $2x10^6$ cells per animal. For the xenograft model of EGFR TKI resistance, $9.99x10^6$ NCI-H1650 (99.9%) cells were mixed with $1x10^4$ (0.1%) NCI-H1975 cells prior to implant and a total concentration of $1x10^7$ cells was implanted per animal. All compounds were administered by oral gavage at a volume of 10ml/kg. IPI-504 was administered every other day (QOD); IPI-493 was administered either QOD or three times weekly (TIW); Gefitinib was administered daily (QD). Tumor volumes were calculated twice weekly using the formula: $(width^2 X length)/2$.

Immunohistochemistry For studies involving the EGFR TKI resistant model, a small number of animals were sacrificed at the end of treatment for histopathology analysis of tumors. Tumors were formalin fixed and paraffin embedded for immunohistochemistry analysis using DelE746-A7570 and L858R EGFR mutant specific antibodies (Cell Signaling). Immunohistochemistry was performed according to manufacturer's protocol.

IPI-493 is a novel formulation of the primary active metabolite of IPI-504 & 17-AAG

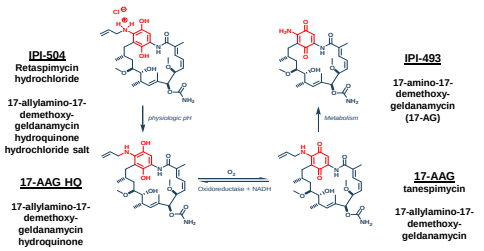

Figure 1 Metabolism of IPI-504 to IPI-493

EGFR TKI sensitive and resistant models are both sensitive to Hsp90 inhibition

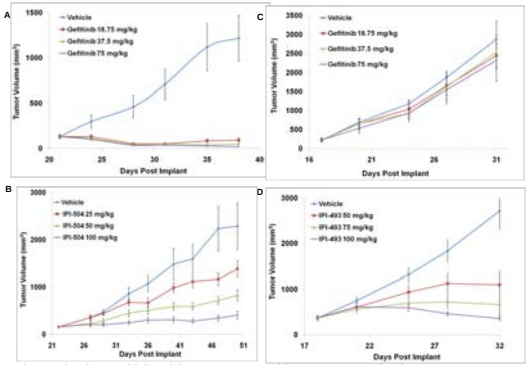

Figure 2 (A,B) - Sensitivity of the EGFR TKI-sensitive NCI-H1650 or (C,D) - EGFR TKI-resistant NCI-H1975 xenograft models to increasing concentrations of gefitinib, IPI-504 or IPI-493. Gefitnib was administered PO at 18.75, 37.5 or 75 mg/kg QD, IPI-504 was administered PO at 25, 50 or 100 mg/kg QOD and IPI-493 was administered PO at 50, 75 or 100 mg/kg QOD.

Optimization of model of emerging EGFR TKI resistance

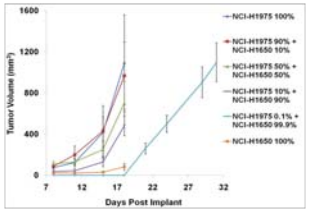

Figure 3 In vivo growth kinetics of varying ratios of NCI-H1975 mixed with NCI-H1650 cells

In the optimized model, administration of an EGFR TKI leads to initial tumor regression followed by emergence of resistant cells and tumor rebound

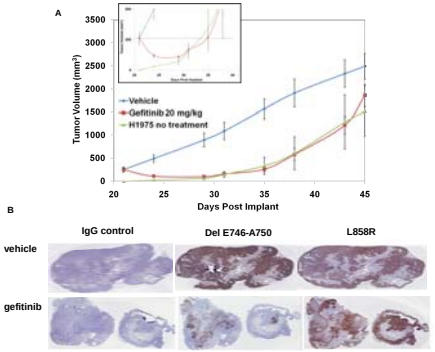

Figure 4 (A) – Activity of gefitinib in the optimized mixed xenograft model (0.1% NCI-H1975, 99.9% NCI-H1650). Gefitinib was administered PO at 20 mg/kg QD and growth kinetics of gefitinib-treated arm was compared with a corresponding untreated arm of NCI-H1975. (B) – Immunohistochemistry of mixed xenograft using EGFR mutant specific antibodies. Tumors were harvested when they reached $3000mm^3$ (day 37 post-implant for vehicle arm and day 46 post-implant for gefitinib arm).

Administration of IPI-493 significantly delays the emergence of EGFR TKI resistant cells

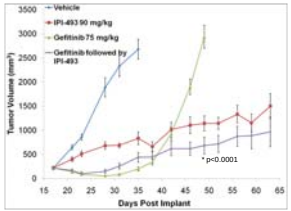

Figure 5 Activity of IPI-493, gefitinib or IPI-493 following gefitinib in the model of emerging EGFR TKI resistance. Gefitinib was administered PO at 75 mg/kg QD; IPI-493 was administered PO at 90 mg/kg TIW.

Administration of IPI-493 following an EGFR TKI significantly delays time to tumor progression

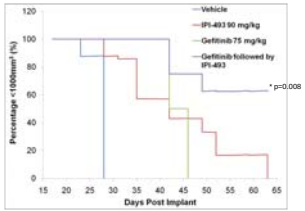

Figure 6 Percentage of animals per arm with tumors < $1000mm^3$. Gefitinib was administered PO at 75 mg/kg QD; IPI-493 was administered PO at 90 mg/kg TIW.

Conclusions

- We have developed a novel *in vivo* model of emerging EGFR TKI-resistance in which administration of the EGFR TKI, gefitinib, results in initial tumor regression followed by the emergence an of EGFR TKI resistant population of cells and rapid tumor growth rebound.
- Following emergence of EGFR TKI-resistance administration of the Hsp90 inhibitor IPI-493 results in a significant delay in the outgrowth of resistant cells and delay in time to tumor progression.
- Taken together, these results suggest that further studies with Hsp90 inhibitors in EGFR mutant NSCLC patients who have been pre-treated with an EGFR TKI are warranted.