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-related proteins through its role as a member of the Hsp90 chaperone program. It is therefore a highly attractive target for cancer therapy. However, resistance to Hsp90 inhibitors has been a major clinical roadblock to the development of these agents for cancer treatments. In this study, we have developed a novel in vivo model of emerging EGFR TKI resistance, in which gefitinib administration initially leads to tumor regression followed by emergence of resistant cells and tumor rebound. We show that 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 leads to initial tumor regression followed by emergence of resistant cells and tumor rebound. We demonstrate 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 alone.

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 1640 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-A757, which confers sensitivity to EGFR TKIs.

In vivo studies: For assessment of compound activity in vivo, NCI-H1650 and NCI-H1975 were implanted subcutaneously 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. For the xenograft model of EGFR TKI resistance, 99.9% NCI-H1650 (99.9%) cells were mixed with 0.1% NCI-H1975 cells prior to implant and a total concentration of 1x10^7 cells were implanted per animal. Tumors were harvested when they reached 3000 mm^3. All compounds were administered by oral gavage, 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.

Immunochemistry: For studies involving the EGFR TKI resistant model, a small number of mice were sacrificed at the end of treatment for histopathology analysis of tumors. Tumors were formalin fixed and paraffin embedded for immunohistochemistry analysis using CellTIDE-EGFR assay. Immunohistochemistry was performed according to manufacturer's protocol.

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 of resistant cells and rapid tumor growth rebound.

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

Figure 1 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.

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