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**Title:** Addition of the HIF-1 $\alpha$  inhibitor PX-478 enhances the therapeutic efficacy of EGFR inhibitors in an orthotopic human lung adenocarcinoma model

**Introduction:** Multiple studies demonstrate that EGFR signaling confers a survival advantage under hypoxic conditions by inducing VEGF expression through HIF-1 $\alpha$  dependent and independent mechanisms. However, clinically EGFR inhibition alone is not always effective in NSCLC tumors particularly for those tumors that express a mutant K-Ras gene. We therefore studied the effects of PX-478, a small molecule inhibitor of HIF-1 $\alpha$ , in combination with the EGFR inhibitors erlotinib and cetuximab in human lung adenocarcinomas that carry an activating K-Ras mutation growing orthotopically in mice.

**Methods:** NCI-H441 human lung adenocarcinoma cells ( $1 \times 10^6$ ), were injected into the left lungs of nude mice. Mice were randomized (10/group) 20 days after injection, when lung tumors were visible in a subset of 4 mice, to treatment with vehicle, PX-478 (20 mg/kg/day for 5 days), erlotinib (50mg/kg/day), cetuximab (1 $\mu$ g/mouse, every third day) or any combination of the drugs. The experiment was terminated when control mice became moribund 63 days after injection. Mice were sacrificed and assessed for lung tumor burden, pleural effusion and metastasis. Lung tumors and adjacent normal tissues were collected for immunohistochemical analyses.

**Results:** Treatment of mice with either cetuximab or PX-478 as monotherapy reduced the median primary lung tumor volume by 44% ( $p=0.006$ ) and 77% ( $p<0.0001$ ), respectively, whilst treatment with erlotinib was only marginally effective in this model. The combination of PX-478 with erlotinib or cetuximab was significantly superior to monotherapy with a reduction of lung tumor volume by 95% ( $p<0.0001$ ) and 90% ( $p<0.0001$ ) respectively, as compared to control. PX-478 monotherapy reduced the incidence of mediastinal metastasis by 80% ( $p=0.0001$ ) while EGFR inhibition had no significant effect. Combined therapy with PX-478 and erlotinib or cetuximab further reduced metastasis. Trimodality therapy completely prevented mediastinal metastasis and reduced tumor volume by 97% ( $p<0.0001$ ). Immuno-histochemical studies of lung tumors are now being completed to assess tumor and endothelial cell proliferation and apoptosis, microvessel density, and the expression of EGFR and HIF-1 $\alpha$ .

**Conclusions:** We have shown that the inclusion of the HIF-1 $\alpha$  inhibitor PX-478 enhances the therapeutic efficacy of EGFR inhibitors in a human lung adenocarcinoma harboring a K-Ras mutation. These data suggest that HIF-1 $\alpha$  antagonism could be an effective strategy to overcome the relative resistance to EGFR inhibition that has been observed in K-Ras mutated NSCLC tumors in the clinic. Our data provide the basis for a combined modality treatment approach with these two agents for lung cancer.