37. A novel technique to preclinically assess the ability of targeted therapies to inhibit both primary and metastatic non-small cell lung carcinoma

Erin Trachet, Lauren Kucharczyk and Scott Wise; Labcorp Early Development Laboratories Inc., Ann Arbor, MI

Introduction

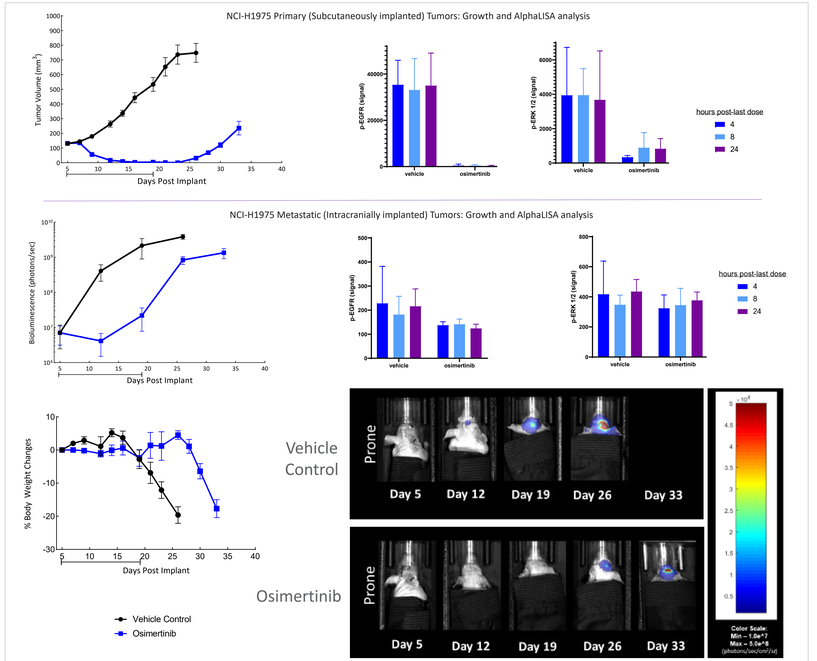
- Clinically, lung cancer often metastasizes to the brain, significantly reducing life expectancy. Many patients with non-small cell lung cancer (NSCLC) already have brain metastasis when they are first diagnosed. Currently there are no targeted therapies specific for brain metastases, and the blood-brain barrier can pose a physiologic impediment to many cytotoxic drugs and antibody-based therapies.
- Xenograft models for metastatic brain disease via a direct intracranial implant coupled with a subcutaneous “primary” tumor effectively allow evaluation of the response to treatment at both locations.
- NCI-H1975-Luc and PC-9-Luc-mCh-puro are both human non-small cell lung carcinoma cell lines that harbor unique mutational EGFR T790M status.
- Osimertinib, an approved first-line treatment for EGFR+ NSCLC patients, was investigated in these tumor models.

Methods

- NCI-H1975-Luc and PC-9-Luc-mCh-puro; human non-small cell lung carcinoma cell lines obtained from ATCC. In separate studies, each cell line was implanted subcutaneously (SC) in the right axilla the “primary tumor” and intracranially (IC) the “metastatic tumor” into female HSD:Athymic nude-*Foxn1*^{nu} mice. Intracranial implants were performed under isoflurane using a stereotaxic frame.
- Once tumors were established at both implant sites oral (PO) treatment with Osimertinib (Selleck Chemicals) was initiated at 25mg/kg once a day for 14 consecutive days.
- Subcutaneous tumor progression was monitored by digital caliper. Intracranial metastatic tumor burden was tracked by bioluminescence imaging (BLI) using an IVIS® Spectrum (Caliper Life Sciences, now part of Perkin Elmer).
- All animal work was performed in an AAALAC-accredited facility, in alignment with applicable animal welfare regulations and with predetermined humane euthanasia criteria on all studies.
- Subcutaneous tumors and metastatic brain tumors were harvested at three time points: 4, 8 and 24 hrs after the third treatment. Primary tumors and metastatic brain tumor samples were processed and analyzed for total and phospho-EGFR and total and phospho-ERK1/2 using AlphaLISA™ SureFire Ultra Detection (Perkin Elmer).
- AlphaLISA assay was performed following manufacturer’s recommendation. Samples were analyzed on a BioTek® Instruments Synergy 2 reader equipped with a 635 nm dichroic mirror and 680/30 and 570/100 nm excitation and emission filters, respectively.

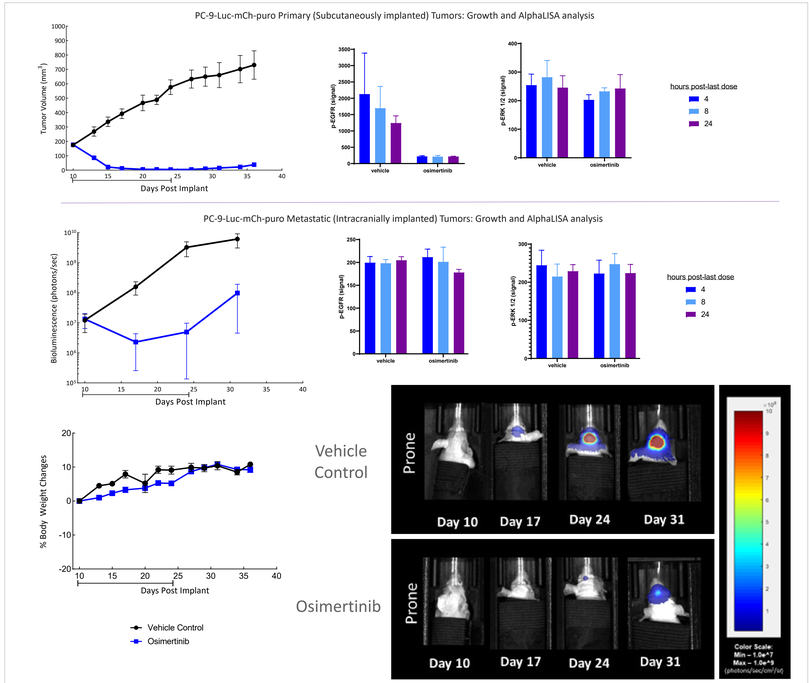
Cell Line	Implant Location	Tumor Doubling Time (days)	Time to Treatment Start (days)
NCI-H1975-Luc	SC	7.4	5 (130mm^3)
NCI-H1975-Luc	IC	2.4	5 (7.10E+06 p/s)
PC-9-Luc-mCh-puro	SC	13.0	10 (175mm^3)
PC-9-Luc-mCh-puro	IC	2.2	10 (1.21E+06 p/s)

Table 1. Tumor growth by location for both cell lines.



Treatment	Dosage Level, Route & Schedule	Body Weight Change	Primary (SC) Tumor PRs (%)	Primary (SC) Tumor CRs (%)	Metastatic (IC) Tumor PRs (%)	Metastatic (IC) Tumor CRs (%)
Vehicle Control	0.2mL/20, PO, QDx14	-19.6	0%	0%	0%	0%
Osimertinib	25mg/kg, PO, QDx14	-6.4	0%	100%	50%	100%

Figure 1. NCI-H1975-Luc tumor growth kinetics with response to Osimertinib treatment for both subcutaneous and intracranial implant. AlphaLISA for total and phospho EGFR and ERK1/2 from both subcutaneous and intracranial tumors.



Treatment	Dosage Level, Route & Schedule	Body Weight Change	Primary (SC) Tumor PRs (%)	Primary (SC) Tumor CRs (%)	Metastatic (IC) Tumor PRs (%)	Metastatic (IC) Tumor CRs (%)
Vehicle Control	0.2mL/20, PO, QDx14	10.8	0%	0%	10%	0%
Osimertinib	25mg/kg, PO, QDx14	10.8	0%	100%	40%	60%

Figure 2. PC-9-Luc-mCh-puro tumor growth kinetics with response to Osimertinib treatment for both subcutaneous and intracranial implant. AlphaLISA for total and phospho EGFR and ERK1/2 from both subcutaneous and intracranial tumors.

Results and Conclusions

- Disease progression for both the NCI-H1975-Luc and PC-9-Luc-mCh-puro at both primary (SC) and metastatic (IC) implant sites was reproduceable with minimal variability. However, in the NCI-H1975-Luc study, there were several mice that presented clinical signs and body weight loss indicative of progressive intracranial tumor growth.
- As expected, treatment with Osimertinib at 25mg/kg orally for 14 days was highly efficacious against both cell lines and both tumor locations, resulting in stable metastatic disease in the brain and tumor regression of the primary subcutaneous tumor.
- In the NCI-H1975-Luc model, both the primary and metastatic brain tumors had measurable pEGFR and pERK1/2. In the primary (SC) tumor, these levels were much higher than their metastatic counterpart and in the face of Osimertinib treatment resulted in substantial decreases in expression levels across all three time points. At the metastatic site (IC) following Osimertinib treatment the expression of pEGFR and pERK1/2 was slightly reduced, indicating that the treatment was able to pass through the blood-brain barrier and reach the tumor.
- In the PC-9-Luc-mCh-puro model, both the primary and metastatic brain tumors had measurable pEGFR and pERK1/2. In the primary (SC) tumor following treatment with Osimertinib, only the pEGFR expression was highly suppressed and the pERK1/2 levels were only slightly inhibited regardless of the time point. Interestingly, the expression of pEGFR and pERK1/2 were relatively unchanged in the metastatic brain tumor, even though there was an inhibition of tumor growth based on the longitudinal BLI analysis.

DOWNLOAD THIS POSTER

AACR Annual Meeting, April 14-19, 2023, Orlando, FL

labcorp Drug Development