Validated Calu-6 Xenograft Model:
Subcutaneous Xenograft Tumor Model

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Lung Cancer: Early Detection and Advanced Therapeutics

Lung cancer is a malignant tumor that originates in the tissues of the lungs, typically arising from epithelial cells lining the airways. It is one of the most prevalent and deadly cancers globally, with a high rate of metastasis to other organs, making it difficult to treat effectively. The two main types of lung cancer are non-small cell lung cancer (NSCLC), which accounts for approximately 85% of cases, and small cell lung cancer (SCLC), which is more aggressive and tends to spread rapidly. Early-stage lung cancer is often asymptomatic, leading to delayed diagnosis and a poor prognosis for many patients. Due to this, ongoing research into the molecular mechanisms underlying lung cancer is crucial for improving early detection, treatment efficacy, and patient outcomes. Current treatments for lung cancer include surgery, chemotherapy, radiation, and increasingly, targeted therapies and immunotherapy, which offer hope for personalized treatment options. Xenografts in lung cancer research involve implanting human lung cancer cells or tumor tissue into immunocompromised mice to study tumor growth, drug responses, and disease progression in a biologically relevant model.

Calu-6 Cell Line

The Calu-6 cell line is an epithelial cell line derived from the lung tissue of a 61-year-old Caucasian female diagnosed with anaplastic carcinoma. This cell line has become a valuable model for lung cancer research, particularly in studying the molecular mechanisms underlying lung tumor progression and therapeutic resistance. Due to its origin from a non-small cell lung cancer (NSCLC) tumor, Calu-6 cells retain many of the characteristics of primary lung epithelial cells, making them suitable for various *in vitro* and *in vivo* experiments. The Calu-6 cell line is widely used to test novel cancer therapies, including chemotherapy, radiation, and targeted treatments, as well as to explore the effects of immune therapies. Additionally, Calu-6 cells are particularly useful in the development of inhaled therapeutics, as they mimic the lung's epithelial barriers and are employed in testing the safety and efficacy of aerosolized drugs. Their robust growth and adaptability to culture conditions allow them to be utilized in long-term studies and drug resistance testing.

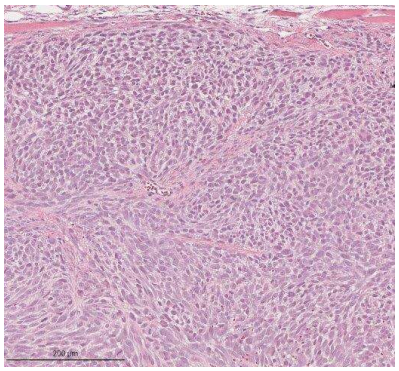

Figure 1. Tumor Histology. H&E stained section of subcutaneously-implanted Calu-6 tumor (Altogen Labs).

Altogen Labs Validated Calu-6 Xenograft Model

At Altogen labs, in the preclinical xenograft study utilizing the Calu-6 cell line, cells are first cultured under conditions that promote exponential growth prior to being harvested for injection. To prepare for injection, Calu-6 cells are trypsinized, and cell viability is assessed using the trypan blue exclusion test, ensuring a minimum of 99% viable cells. The cell suspension is then adjusted to the required density before being injected. Athymic BALB/C (nu/nu) mice, aged 11 to 12 weeks, receive a single subcutaneous injection of one million cells in a volume of 100-150 µL, mixed with Matrigel, into a single hind leg. Tumor growth is monitored by palpating the injection sites three times a week until tum ors are established, and tumor size is measured using digital calipers until reaching an average size of 100-120 mm³. Following tumor establishment, animals are randomly assigned to treatment groups, and the compound of interest is

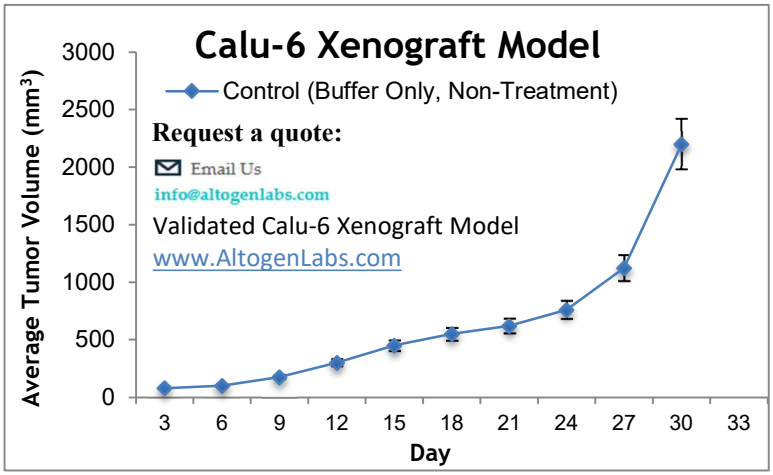

Figure 2. Tumor growth of subcutaneous xenograft Calu-6 cells *in vivo*, tumor volume, mean +/- SEM (Altogen Labs).

administered according to the pre-established treatment schedule. Tumors are measured daily, and the animals' body weights are recorded up to three times per week. Mice are euthanized when the tumors reach a predetermined size, typically around 2,000 mm³. A necropsy is performed at the termination of the experiment, with tumors excised, weighed, and documented using digital imaging. Tissues are then collected for further analysis, with options for stabilization in RNAlater reagent, snap freezing in liquid nitrogen, or preparation for histological analysis.

Subcutaneous Calu-6 Lung Cancer Xenograft Model

Subcutaneous Calu-6 xenografts are a widely used model in preclinical cancer research to study lung cancer progression and treatment efficacy. In a subcutaneous model, Calu-6 human lung cancer cells are implanted under the skin of immunocompromised mice, to establish tumors that mimic human lung cancer. This approach provides a convenient and reproducible method for evaluating the growth of lung cancer in a controlled *in vivo* environment. The model is particularly valuable for testing the effectiveness of various therapeutic strategies, including chemotherapy, radiation, targeted therapies, and immunotherapies. Subcutaneous xenografts allow for easy monitoring of tumor growth through palpation and caliper measurement, providing real-time data on tumor progression. Additionally, this model is used for assessing the pharmacokinetics, toxicity, and survival outcomes of potential treatments.

Case Study: Response to Cediranib in Calu-3 and Calu-6 NSCLC Xenografts

In a study conducted by Jiang Y *et al*., published by *Lung Cancer* journal, researchers investigated the acute vascular response to the VEGFR tyrosine kinase inhibitor cediranib in non-small-cell lung cancer (NSCLC) xenografts with distinct tumor stromal architectures. Two xenograft models were employed: Calu-3 (representing the stromal vessel phenotype) and Calu-6 (representing the tumor vessel phenotype). The results demonstrated that cediranib markedly reduced tumor perfusion and induced hypoxia in Calu-3 xenografts but had minimal effects on Calu-6 tumors. These differences highlight the sensitivity of stromal vessel phenotypes to cediranib treatment compared to tumor vessel phenotypes. Moreover, Calu-3 xenografts exhibited significant tumor regression following treatment, whereas Calu-6 tumors only showed a trend toward growth inhibition. This study emphasizes the potential of tumor stromal architecture as a predictor of response to VEGFR TKI therapy, with Calu-6 serving as a critical model for investigating the less responsive tumor vessel phenotype.

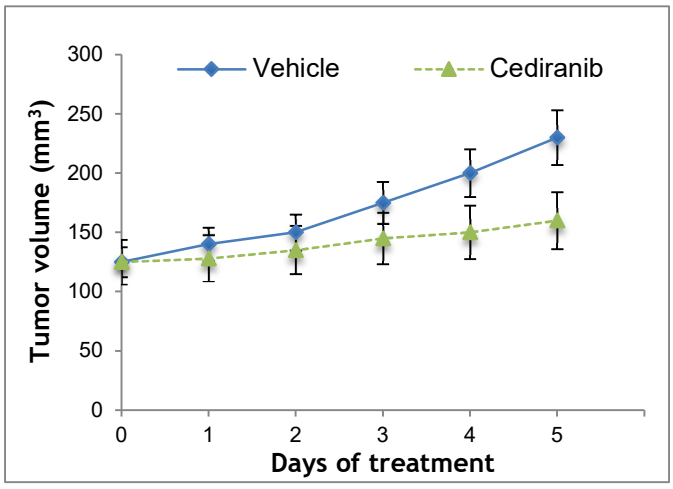

Figure 3. Cediranib (6 mg/kg) effectively inhibited Calu-6 xenograft tumor growth.

Additional Case Study: Efficacy of Motesanib in Calu-6 NSCLC Xenografts

In another study done by Coxon A *et al*., published in *Molecular Cancer* journal, researchers explored the efficacy of motesanib, a VEGF receptor antagonist, in inhibiting tumor growth in five human non-small-cell lung cancer (NSCLC) xenograft models, including the Calu-6 model. Motesanib alone demonstrated dose-dependent tumor growth inhibition across all models, but Calu-6 tumors were notably resistant, requiring the highest tested dose to achieve significant inhibition. This resistance highlights the unique biology of Calu-6, which represents an aggressive NSCLC subtype with reduced sensitivity to single-agent angiogenesis inhibitors. However, when combined with standard chemotherapy agents like cisplatin, motesanib significantly enhanced tumor growth suppression in Calu-6 and other models, suggesting a synergistic effect. The study attributed motesanib's antitumor activity to its antiangiogenic mechanisms, targeting tumor vasculature rather than tumor cells directly.

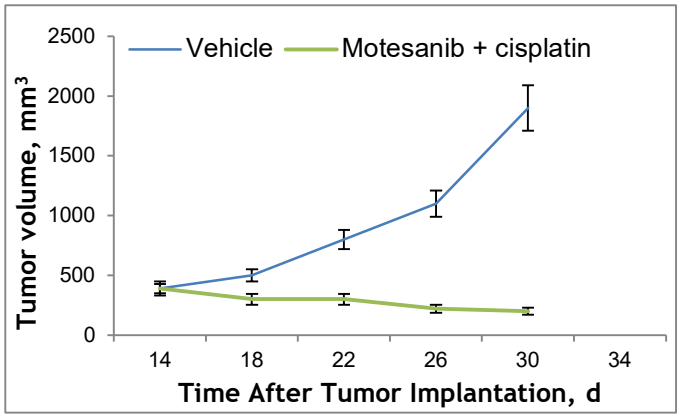

Figure 4. Combined use of motesanib (75 mg/kg) and cisplatin (5 mg/kg), suppressed Calu-6 xenograft tumor growth.

Oncogene Characteristics of Calu-6

The Calu-6 cell line is a valuable model for studying the oncogenic characteristics of lung cancer, particularly in relation to key oncogenes such as KRAS. KRAS mutations, commonly associated with non-small cell lung cancer (NSCLC), are present in Calu-6 cells and contribute to the cell line's aggressive tumorigenic behavior, including enhanced cell proliferation, survival, and resistance to apoptosis. These characteristics make the Calu-6 cell line an ideal platform for investigating the molecular pathways driving lung cancer and evaluating potential therapeutic targets. Additionally, Calu-6 allows for the assessment of the effects of oncogene-targeted therapies, providing critical insights into treatment efficacy and resistance mechanisms. By studying the oncogenic properties of Calu-6, researchers can gain a deeper understanding of the molecular underpinnings of lung cancer and explore novel strategies for targeted treatment.

The Calu-6 xenograft model offers several research options, at Altogen Labs, these include tumor growth delay (TGD) and tumor growth inhibition (TGI) assessments, which can be evaluated based on latency. Calu-6 also provides flexibility in dosing frequency and duration, as well as various dosing routes, such as intravenous, intratracheal, continuous infusion, intraperitoneal, intratumoral, oral gavage, topical, intramuscular, subcutaneous, and intranasal administration. Cutting-edge techniques, like micro-injection and pump-controlled IV injection, can also be utilized. Additionally, tumor pathology and immunohistochemistry can be performed to assess tumor characteristics, and alternative cell engraftment sites, such as orthotopic transplantation, tail vein injection, left ventricular injection for metastasis studies, mammary fat pad injection, and intraperitoneal injection, can be employed. The model supports ADME, safety toxicology, genotoxicity, and blood chemistry analysis. Toxicity and survival can also be evaluated, with the option of implementing a broad health observation program. A positive control group using known chemotherapy drugs can be treated by intramuscular injection daily throughout the study duration. For advanced imaging, fluorescence-based whole-body imaging can be incorporated into the study design.

References:

Calu-6 Xenograft Model. https://altogenlabs.com/xenograft-models/lung-cancer-xenograft/calu-6-xenograft-model/

Coxon A, Ziegler B, Kaufman S, Xu M, Wang H, Weishuhn D, Schmidt J, Sweet H, Starnes C, Saffran D, Polverino A. Antitumor activity of motesanib alone and in combination with cisplatin or docetaxel in multiple human non-small-cell lung cancer xenograft models. *Mol Cancer.* 2012 Sep 19;11:70. doi: 10.1186/1476-4598-11-70. PMID: 22992329; PMCID: PMC3515409.

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Keywords: Calu-6, xenograft, in vivo, cancer, preclinical, research, *in vivo* pharmacology

Other Available Validated Altogen Labs Xenograft Models:

A549 Xenograft Model: https://altogenlabs.com/xenograft-models/lung-cancer-xenograft/a549-xenograft-model/

Calu-3 Xenograft Model: https://altogenlabs.com/xenograft-models/lung-cancer-xenograft/calu-3-xenograft-model/

NCI-H460 Xenograft Model: https://altogenlabs.com/xenograft-models/lung-cancer-xenograft/h460-xenograft-model/

NCI-H1975 Xenograft Model: https://altogenlabs.com/xenograft-models/lung-cancer-xenograft/nci-h1975-xenograft-model/

NCI-H226 Xenograft Model: https://altogenlabs.com/xenograft-models/lung-cancer-xenograft/nci-h226-xenograft-model/

NCI-H1155 Xenograft Model: https://altogenlabs.com/xenograft-models/lung-cancer-xenograft/h1155-xenograft-model/