Validated H460 Xenograft Model:
Subcutaneous And Orthotopic Xenograft Tumor Model

By Altogen Labs, 11200 Menchaca Road, Suite 203, Austin, TX 78748
Phone: (512) 433-6177 | Email: info@altogenlabs.com

Advancing Lung Cancer Research Through Xenograft Models

Lung cancer is one of the leading causes of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) accounting for the majority of cases. Large cell lung carcinoma (LCLC) is a subtype of NSCLC characterized by undifferentiated tumor cells that do not fit into the more common classifications of adenocarcinoma or squamous cell carcinoma. LCLC is often diagnosed at advanced stages due to its aggressive nature and lack of early symptoms, contributing to poor patient prognosis. Xenografts, in which human cancer cells are transplanted into immunocompromised mice, play a crucial role in cancer research by providing a model to study tumor progression and evaluate new therapeutic strategies. These models are particularly valuable for LCLC, as they allow researchers to investigate the tumor's response to potential treatments in a controlled *in vivo* environment. Xenografts of LCLC enable the exploration of molecular targets, the development of personalized treatments, and the assessment of drug efficacy. By utilizing these models, researchers can better understand the pathophysiology of LCLC and identify novel therapeutic interventions that may improve patient outcomes. Moreover, xenografts offer insights into tumor heterogeneity and the mechanisms behind metastasis, which are critical challenges in the treatment of advanced lung cancer.

NCI-H460 Cell Line

The NCI-H460 cell line is an epithelial-derived line established in 1982 from a male patient diagnosed with large cell lung carcinoma (LCLC). It is a well-characterized, tumorigenic cell line, capable of forming tumors when implanted into immunocompromised nude mice, making it a valuable model for studying lung cancer biology and potential therapies. The cells express p53 mRNA, which is indicative of its involvement in the regulation of cell cycle and apoptosis, though p53 mutations or dysfunctions are often observed in cancer. Notably, the H460 line does not express neurofilament triplet protein, a marker typically associated with neuronal differentiation, but it does exhibit positive staining for vimentin and keratin. The H460 cell line does not demonstrate significant structural DNA abnormalities, which further supports its use as a consistent model for studying the molecular mechanisms of large cell lung carcinoma. As a result, it is frequently employed in drug testing, molecular biology studies, and understanding the pathophysiology of lung cancer, particularly in evaluating the effectiveness of novel therapeutic agents.

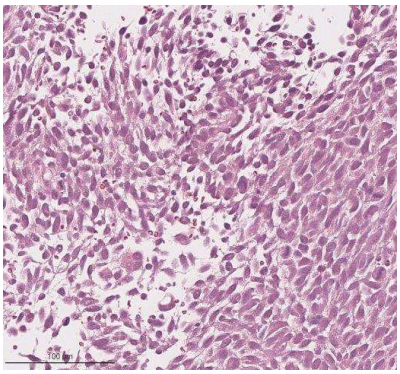

Figure 1. Tumor Histology. H&E stained section of subcutaneously-implanted H460 tumor (Altogen Labs).

Altogen Labs Validated H460 Xenograft Model

At Altogen Labs, the preclinical xenograft study begins by maintaining H460 cell cultures in exponential growth prior to collection. Cells are harvested through trypsinization, and their count and viability are assessed using trypan blue exclusion, requiring at least 98% viability. The cell suspension is then adjusted to the appropriate density for inoculation. A suspension of one million H460 cells, combined with Matrigel solution (0.1–0.2 mL total volume), is subcutaneously injected into the flank of the hind leg of each mouse, using either NOD/SCID or athymic BALB/C mice aged 11–12 weeks. Tumor establishment at the injection sites is monitored, with tumor sizes measured using digital calipers until they reach an average volume of 80–120 mm³. Following randomization into treatment

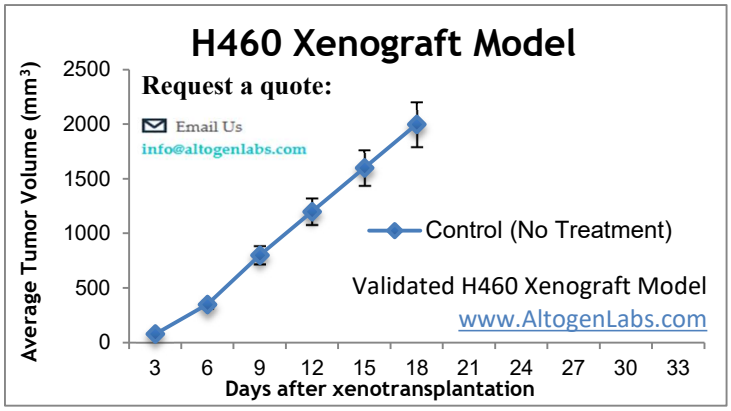

Figure 2. Tumor growth of subcutaneous xenograft H460 cells *in vivo*, tumor volume, mean +/- SEM (Altogen Labs).

cohorts, test compounds are administered according to the treatment schedule. Tumor measurements are recorded daily, and mouse weights are monitored three times per week. Once tumors reach a predetermined size limit (e.g., 2,000 mm³), the mice are euthanized, and a necropsy is conducted. Tumors are excised, weighed, and digitally imaged. Additional tissues are collected through standard gross necropsy procedures. Tumors and tissues are then processed for downstream analyses, including snap freezing, stabilization in RNA-Later reagent, histology preparation, or nucleic acid isolation for genetic studies.

Case Study: Targeting NCI-H460 Cells by Inducing G2/M Arrest via Ras/ERK Pathway and p53-p21 Activation

In a study administered by Lv C, *et al.*, published by *Marine Drugs* journal, researchers examined the effects of asperolide A, a marine-derived tetranorditerpenoid, on the proliferation of NCI-H460 lung carcinoma cells. The compound induced G2/M phase cell cycle arrest by stabilizing the p53-p21 pathway, a mechanism that inhibits cell cycle progression. This arrest was regulated by the Ras/Raf/MEK/ERK signaling pathway, suggesting that ERK activation plays a critical role in the process. *In vitro*, asperolide A significantly reduced cyclin B1 and CDC2 levels while promoting the activation of p53 and its downstream target, p21, leading to cell cycle disruption. Dominant-negative mutations in Ras blocked these effects, confirming the involvement of the Ras-mediated signaling cascade. *In vivo*, asperolide A inhibited tumor growth in NCI-H460 xenografts with lower toxicity compared to cisplatin. These findings highlight the therapeutic potential of asperolide A in targeting NSCLC through cell cycle modulation and reduced side effects.

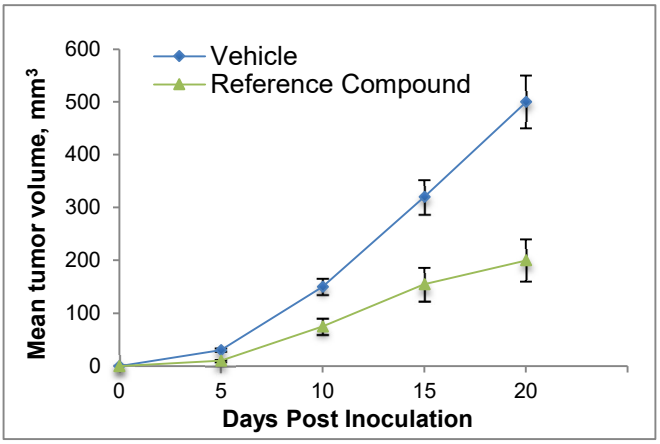

Figure 3. Tumor growth in the H460 xenograft model was significantly suppressed following treatment with the reference compound (5 mg/kg).

H460 Handling Procedure and Subculturing Protocol

To ensure optimal viability of the H460 cells, researchers thaw the vial and initiated culture immediately upon receipt. In cases where continued storage was required, the frozen culture was stored in liquid nitrogen vapor phase rather than at -70°C, as the latter resulted in a loss of viability. Thawing was performed using gentle agitation in a 37°C water bath for approximately two minutes, with the O-ring and cap kept out of the water to prevent contamination. Once thawed, the vial was decontaminated with 70% ethanol, and all subsequent procedures were carried out under strict aseptic conditions. The contents of the vial were transferred to a 75 cm² tissue culture flask, diluted with the recommended complete culture medium, and incubated at 37°C in a 5% CO2 atmosphere. Researchers ensure that the growth medium reaches the appropriate pH of 7.0 to 7.6 before adding the thawed cells by pre-incubating the culture vessel for at least 15 minutes. If desired, the cryoprotective agent could be removed by centrifuging the cell suspension at 125 x g for 5 to 10 minutes, followed by resuspension in fresh growth medium at the recommended dilution ratio.

In the preclinical subculture protocol, researchers use a 75 cm² flask, with volumes of reagents adjusted proportionally for culture vessels of different sizes. Corning T-75 flasks are recommended for the subculturing process. First, the culture medium is removed and discarded. The cell layer is then briefly rinsed with a 0.25% (w/v) Trypsin-0.53 mM EDTA solution to eliminate serum traces containing trypsin inhibitor. A 2.0 to 3.0 ml volume of Trypsin-EDTA solution was added to the flask, and the cells were observed under an inverted microscope until the cell layer dispersed, typically within 5 to 15 minutes. During this process, researchers recommend to be careful not to agitate the flask to prevent cell clumping; if necessary, cells that were difficult to detach were incubated at 37°C to aid in dispersion. Following detachment, 6.0 to 8.0 ml of complete growth medium was added, and the cell suspension was aspirated by gentle pipetting. Aliquots of the suspension were then transferred to new culture vessels, which were incubated at 37°C. A sub-cultivation ratio ranging from 1:3 to 1:8 was maintained, and medium renewal was performed twice per week to ensure optimal culture conditions.

Subcutaneous H460 Lung Cancer Xenograft Model

The subcutaneous H460 lung cancer xenograft model involves the implantation of H460 cells under the skin of immunocompromised mice, allowing for the *in vivo* study of tumor growth and therapeutic responses. This model represents key characteristics of large cell lung carcinoma (LCLC), and provides a reliable platform for evaluating the efficacy of various anticancer therapies. With consistent tumor formation and predictable growth kinetics, it is widely used in preclinical research to assess tumor growth inhibition, tumor growth delay, and combination treatment strategies. The

subcutaneous location enables easy monitoring of tumor volume and facilitates downstream analyses, such as histology and molecular profiling.

Orthotopic H460 Xenograft Model

H460 cells are also used in orthotopic lung cancer models to better replicate the tumor's native microenvironment. These models involve implanting H460 cells directly into the lungs of immunocompromised mice, allowing for the evaluation of tumor growth, progression, and response to therapies. Advanced imaging techniques, such as bioluminescence, are employed to monitor tumor development and metastasis in real time. Orthotopic models have an advantage over traditional subcutaneous models, as they more accurately mimic the tumor in a human, including the potential for metastasis and interactions within the lung microenvironment.

H460 as a Preclinical Model for Lung Cancer Therapy

The H460 lung cancer cell line exhibits strong oncogenic properties, largely due to its side population (SP), which is enriched with stem-like cancer cells. These SP cells demonstrate high tumorigenicity, self-renewal, and significant proliferative capacity, forming anchorage-independent spheres, a hallmark of cancer stem cells. They preferentially express ABCG2, a multidrug resistance protein, and SMO, a key mediator of the Hedgehog (HH) signaling pathway, which regulates cell cycle progression and self-renewal. Additionally, H460 cells are known to exhibit high metabolic activity and resistance to hypoxic conditions, which are hallmarks of aggressive tumors. These cells do not present significant structural DNA abnormalities but have been reported to exhibit altered signaling pathways, such as those involving EGFR, KRAS, or PI3K, which are implicated in cell proliferation and survival. Due to their robust growth characteristics, metastatic potential, and responsiveness to therapeutic agents, H460 cells serve as a reliable preclinical model for evaluating the efficacy of novel cancer therapies and understanding the mechanisms underlying lung cancer oncogenesis.

The H460 xenograft model offers various experimental options to study tumor biology and therapeutic interventions. These are offered at Altogen Labs and can include assessments of tumor growth delay (TGD or latency) and tumor growth inhibition (TGI). Dosing regimens can be customized with flexibility in frequency, duration, and administration route, such as intravenous, intratracheal, continuous infusion, intraperitoneal, intratumoral, oral gavage, topical, intramuscular, subcutaneous, intranasal, and advanced methods like micro-injection or pump-controlled IV injection. Tumor immunohistochemistry is available for detailed analysis, along with alternative cell engraftment sites, including orthotopic transplantation, tail vein or left ventricular injections for metastasis studies, and injection into the mammary fat pad or peritoneal cavity. Toxicity and survival studies can also be performed, with the option of a comprehensive health observation program. Additional analyses include gross necropsies, histopathology, and imaging studies using fluorescence-based whole-body imaging. A positive control group employing known chemotherapy agents is often included to validate experimental outcomes. These diverse options enable comprehensive evaluation of therapeutic efficacy and tumor behavior in the H460 xenograft model.

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