Validated H1993 Xenograft Model:

Subcutaneous, Orthotopic, And Metastatic Xenograft Tumor Model

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

Non-Small Cell Lung Cancer Research and The Role of Xenograft Models in Preclinical Studies

Lung cancer represents one of the leading causes of cancer-related mortality globally and is primarily classified into two major histological subtypes: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), with NSCLC comprising approximately 85% of cases. NSCLC is further subdivided into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, each exhibiting distinct molecular and clinical features that influence treatment strategies. Xenografts, which involve the implantation of human tumor tissue or cells into immunodeficient animal models, serve as valuable tools for studying cancer biology and evaluating therapeutic interventions. These models are often utilized to investigate the pathophysiology of NSCLC and test potential treatments *in vivo*. By closely monitoring tumor growth and response to therapy in xenograft models, researchers can identify promising therapeutic candidates and better predict clinical outcomes. Xenografts play a critical role in preclinical cancer research, providing insights that bridge laboratory-based studies and clinical trial development.

NCI-H1993 Cell Line

The NCI-H1993 (H1993) cell line is an epithelial-derived culture established from a 47-year-old white female patient diagnosed with stage 3A non-small cell lung cancer (NSCLC), specifically adenocarcinoma. This cell line is characterized by its epithelial morphology and provides a valuable *in vitro* model for studying the molecular mechanisms underlying NSCLC progression. As a representation of advanced-stage lung cancer, H1993 cells exhibit key genetic and phenotypic features that make them a useful tool for drug screening, therapeutic research, and the exploration of resistance mechanisms. The cell line has also been employed in studies focused on identifying novel molecular targets for treatment and evaluating the efficacy of potential anticancer compounds. Additionally, the use of H1993 cells can contribute to investigations into the tumor microenvironment and the interactions between cancer cells and stromal components.

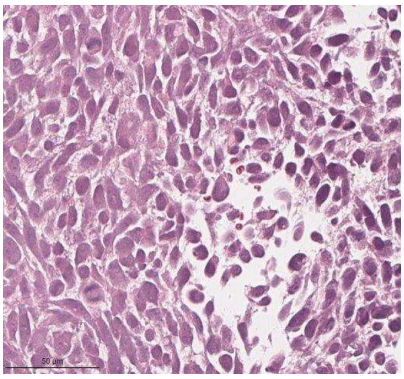

Figure 1. Tumor Histology. H&E stained section of subcutaneously-implanted H1993 tumor (Altogen Labs).

Altogen Labs Validated H1993 Xenograft Model

H1993 cells are harvested during the exponential growth phase via trypsinization, centrifuged, and resuspended in sterile serum-free medium supplemented with 50% Matrigel at a concentration of 50 × 10^6 cells/mL. A 100 µL suspension containing 5 × 10^6 cells is subcutaneously implanted into the hind-flank region of immunocompromised mice, with one tumor established per mouse. Tumors are allowed to grow to a volume of 50–200 mm^3 prior to the administration of the investigational agent. Tumor growth is monitored biweekly using digital calipers, and mice are randomized into control and treatment groups to evaluate therapeutic efficacy. Throughout the study, tumor volume, body weight, and general health are systematically recorded. Treatment response is assessed by measuring tumor size reduction, performing imaging analyses, and conducting post-mortem histological evaluations to investigate tumor architecture, cell viability, and molecular markers associated with therapy.

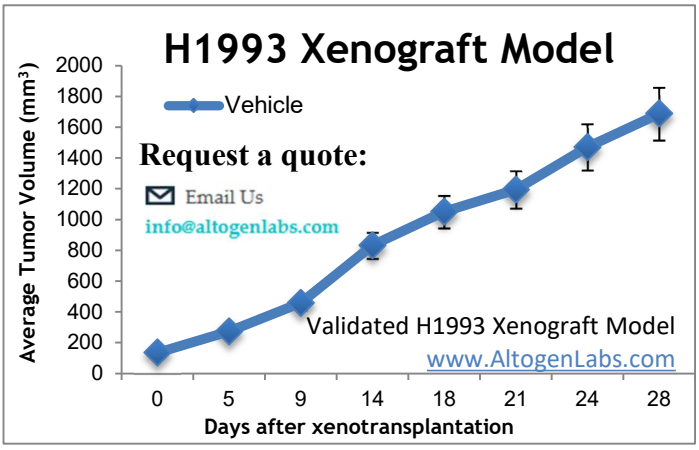

Figure 2. NCI-H1993 adenocarcinoma xenografted in immunocompromised mice, mean values +/- SEM.

Case Study: Antitumor Mechanisms of Gambogic Acid in MET-Amplified NSCLC

A study done by Li D, *et al.*, published by the *Oncology Letters* journal discussed the antitumor mechanisms of gambogic acid (GA) using NCI-H1993 xenograft models of non-small cell lung cancer (NSCLC), which harbor a MET gene amplification. The researchers administered varying doses of GA to mice and observed a dose-dependent inhibition of tumor growth without significant toxicity. GA was found to downregulate the expression of phosphorylated MET (p-MET) and its downstream signaling molecules, p-AKT and p-ERK, as shown through western blot analysis. Immunohistochemical staining revealed reduced Ki-67 expression, indicating inhibited tumor cell proliferation. From this study, researchers suggest GA's potential as a therapeutic agent targeting MET-amplified NSCLC, emphasizing its ability to modulate the MET signaling pathway effectively.

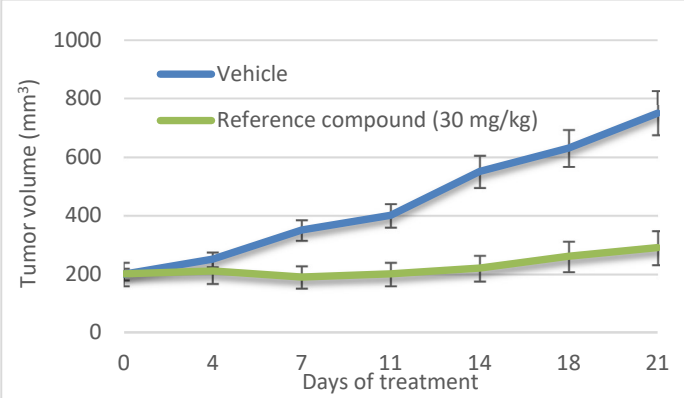

Figure 3. Treatment with gambogic acid (30 mg/kg) led to a reduction in H1993 xenograft tumor size.

Additional Case Study: Dexamethasone-Induced Growth Arrest in NCI-H1993 Cells

Another study conducted by Huffman KE, *et al.*, published by *Frontiers in Oncology* journal focuses on the role of H1993 cells in determining therapeutic vulnerabilities in non-small cell lung cancer (NSCLC). The glucocorticoid receptor (GR) agonist dexamethasone (DEX) was shown to inhibit the growth of H1993 cells by inducing G1/S cell cycle arrest. Essentially, DEX was shown to inhibit tumor growth as effectively as cisplatin in H1993 xenografts. When used in combination, DEX and cisplatin exhibited superior anti-tumor activity compared to either agent alone, suggesting a synergistic or additive effect. This combination did not compromise the efficacy of cisplatin, making it a promising approach for targeting LKB1-mutant NSCLC. Furthermore, DEX-treated NCI-H1993 xenografts demonstrated markers of senescence and reduced tumor cell migration, emphasizing its therapeutic potential. These findings identify glucocorticoid receptor (GR) agonists, such as DEX, as a novel precision medicine approach for LKB1-mutant NSCLCs.

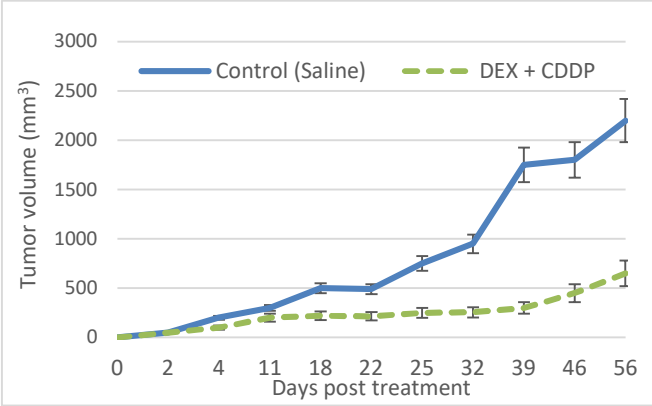

Figure 4. Co-treatment with dexamethasone (2 mg/kg) and cisplatin (5 mg/kg) resulted in a significant inhibition in tumor growth in H1993 xenograft models.

Key Mutations and Oncogenic Traits in H1993 NSCLC Cells

H1993 is a human lung cancer cell line that exhibits oncogenic characteristics commonly observed in non-small cell lung cancer (NSCLC). It is known to harbor mutations in key genes such as KRAS and TP53, which are critical drivers of tumorigenesis. The cells demonstrate enhanced proliferation, resistance to apoptosis, and the ability to invade surrounding tissues, all of which contribute to their aggressive behavior. Additionally, MET gene dysregulation, through overexpression or mutations, plays a significant role in driving cell proliferation, migration, and invasiveness, further supporting the tumor's growth and metastatic potential. H1993 cells also exhibit increased angiogenesis, providing the tumor with the necessary blood supply to support rapid growth. They undergo epithelial-to-mesenchymal transition (EMT), a process associated with metastasis, further enhancing their ability to spread to distant sites. These characteristics make H1993 a useful model for studying lung cancer biology and testing potential therapeutic strategies.

Preclinical Insights From the H1993 Subcutaneous Xenograft Model

The subcutaneous H1993 xenograft model involves the implantation of H1993 tumor cells, derived from human lung adenocarcinoma with MET gene amplification, into immunocompromised mice to study non-small cell lung cancer (NSCLC). These models are established by injecting the tumor cells subcutaneously into the immunocompromised mice, allowing for reproducible monitoring of tumor growth and therapeutic responses. This model provides a robust platform for evaluating the efficacy of MET-targeted therapies and investigating the molecular mechanisms underlying tumor progression and drug resistance. The subcutaneous location enables straightforward, non-invasive measurement of tumor volume, making it suitable for longitudinal studies on treatment efficacy. Frequently utilized in preclinical research, the NCI-

H1993 xenograft model supports the development of novel therapies while complementing more complex orthotopic and metastatic models for translational cancer studies.

Metastatic H1993 Models: Advancing NSCLC Research and Therapy

Metastatic H1993 models are valuable tools for studying the dissemination of non-small cell lung cancer (NSCLC) with MET amplification, providing insights into the mechanisms of tumor invasion and organ-specific metastasis. These models are typically established by injecting H1993 cells intravenously or orthotopically, allowing tumor cells to circulate and colonize distant organs such as the lungs, liver, or bones. Metastatic models closely mimic the progression of advanced-stage lung cancer and are instrumental in evaluating therapies that target metastatic spread, including anti-MET agents, immunotherapies, and anti-angiogenic drugs. Researchers often monitor metastatic lesions through advanced imaging techniques, such as bioluminescence or fluorescence imaging, to track tumor burden in real-time. Histological analyses of metastatic sites provide additional insights into cellular and molecular changes induced by treatment. These models are particularly useful for assessing combination therapies and studying biomarkers associated with metastatic progression, offering translational relevance for clinical applications.

Studying Lung Cancer in Orthotopic H1993 Models

Orthotopic H1993 models involve the implantation of H1993 cells directly into the lungs of immunocompromised mice, creating a biologically relevant microenvironment to study non-small cell lung cancer (NSCLC). These models more accurately recapitulate the tumor's growth, interactions with the lung microenvironment, and potential for spontaneous metastasis compared to subcutaneous models. Orthotopic models are ideal for evaluating MET-targeted therapies, as the H1993 cell line is highly dependent on MET signaling for proliferation and survival. Tumor progression can be monitored using advanced imaging techniques, enabling longitudinal studies of therapeutic efficacy. Histological and molecular analyses of primary lung tumors and metastatic lesions provide insights into treatment-induced effects on tumor architecture, angiogenesis, and signaling pathways. These models are particularly valuable for studying drug resistance and testing novel combination therapies in a setting that closely resembles human lung cancer biology. The orthotopic H1993 model serves as a powerful tool for preclinical research, bridging the gap between *in vitro* studies and clinical applications.

H1993 Subculturing Protocols and Handling Procedure

To ensure optimal viability, researchers thawed the vial and initiated culture as soon as the H1993 cells were received. In cases where continued storage was necessary, they ensured that the frozen culture was stored in liquid nitrogen vapor phase, as storage at -70°C would result in a loss of viability. The vial was thawed quickly by gentle agitation in a 37°C water bath, with the O-ring and cap kept out of the water to prevent contamination. Thawing was completed in approximately 2 minutes. After thawing, the vial was removed from the water bath and decontaminated by dipping or spraying with 70% ethanol, and all subsequent procedures were conducted under strict aseptic conditions. The contents of the vial were transferred into a centrifuge tube containing 9.0 mL of complete growth medium and centrifuged at approximately 125 x g for 5 to 7 minutes. The resulting cell pellet was resuspended in the recommended complete growth medium and dispensed into a 25 cm² or 75 cm² culture flask. To avoid excessive alkalinity, researchers ensured that the medium in the culture vessel was equilibrated in the incubator for at least 15 minutes before the cells were added, allowing the medium to reach a pH of 7.0 to 7.6. The cultures were then incubated at 37°C in a suitable incubator with a 5% CO2 atmosphere, as recommended for the specific medium used.

For the H1993 protocol, the volumes are based on 75 cm² flasks, and the amount of dissociation medium is adjusted accordingly for culture vessels of different sizes. Researchers begin by removing and discarding the culture medium, then briefly rinse the cell layer with calcium- and magnesium-free Dulbecco's phosphate-buffered saline (D-PBS) or a 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove any traces of serum containing trypsin inhibitor. Next, 2.0 to 3.0 mL of Trypsin-EDTA solution is added to the flask, and cells are observed under an inverted microscope until the cell layer disperses, typically within 5 to 15 minutes. To prevent cell clumping, it is important not to agitate the flask by shaking or tapping it while waiting for the cells to detach. If detachment is slow, the flask may be placed at 37°C to facilitate dispersal. Once the cells have detached, 2.0 to 3.0 mL of complete growth medium is added, and the cells are aspirated gently by pipetting. The cell pellet is then resuspended in fresh growth medium and aliquots of the suspension are transferred to new culture vessels. Finally, the cultures are incubated at 37°C, with a sub-cultivation ratio of 1:2 to 1:6.

The H1993 xenograft model provides a robust platform for evaluating therapeutic agents targeting non-small cell lung cancer (NSCLC) with MET gene amplification in preclinical studies. At Altogen Labs, key experimental endpoints include Tumor Growth Delay (TGD) and Tumor Growth Inhibition (TGI), offer critical insights into the efficacy of investigational

therapies. The H1993 model supports flexible dosing regimens, including intravenous, intraperitoneal, intratumoral, and oral gavage administration, allowing for the design of tailored treatment protocols. For metastasis studies, alternative methods such as orthotopic implantation or tail vein injections can be employed to mimic advanced disease stages. Comprehensive evaluations include tumor immunohistochemistry, survival analysis, gross necropsy, and detailed histopathology, enabling a thorough assessment of therapeutic responses. The H1993 model is particularly effective for investigating the efficacy of MET-targeted therapies and combination treatments, as well as exploring the molecular mechanisms underlying tumor growth and resistance in NSCLC.

References:

Li D, Yang H, Li R, Wang Y, Wang W, Li D, Ma S, Zhang X. Antitumor activity of gambogic acid on NCI-H1993 xenografts via MET signaling pathway downregulation. Oncol Lett. 2015 Nov;10(5):2802-2806. doi: 10.3892/ol.2015.3719. Epub 2015 Sep 17. Erratum in: *Oncol Lett*. 2021 Mar;21(3):216. doi: 10.3892/ol.2021.12477. PMID: 26722245; PMCID: PMC4665713.

Hu R, Huffman KE, Chu M, Zhang Y, Minna JD, Yu Y. Quantitative Secretomic Analysis Identifies Extracellular Protein Factors That Modulate the Metastatic Phenotype of Non-Small Cell Lung Cancer. *J Proteome* Res. 2016 Feb 5;15(2):477-86. doi: 10.1021/acs.jproteome.5b00819. Epub 2016 Jan 25. PMID: 26736068; PMCID: PMC5001500.

Huffman KE, Li LS, Carstens R, Park H, Girard L, Avila K, Wei S, Kollipara R, Timmons B, Sudderth J, Bendris N, Kim J, Villalobos P, Fujimoto J, Schmid S, Deberardinis RJ, Wistuba I, Heymach J, Kittler R, Akbay EA, Posner B, Wang Y, Lam S, Kliewer SA, Mangelsdorf DJ, Minna JD. Glucocorticoid mediated inhibition of LKB1 mutant non-small cell lung cancers. *Front Oncol.* 2023 Mar 23;13:1025443. doi: 10.3389/fonc.2023.1025443. PMID: 37035141; PMCID: PMC10078807.

NCI-H1993 [H1993]. https://www.atcc.org/products/crl-5909

Keywords: NCI-H1993, H1993, xenograft, *in vivo*, cancer, preclinical, research, *in vivo* pharmacology, orthotopic, metastatic

Other Available Altogen Labs Validated 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/

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

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

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

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

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