Validated U118 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 GBM Treatment Strategies Using U118 Xenograft Models

Glioblastoma multiforme (GBM) is an aggressive and treatment-resistant brain cancer with limited therapeutic options and poor patient survival outcomes. The development of effective treatments has been hindered by the tumor's genetic heterogeneity, invasive growth, and resistance to standard therapies such as temozolomide. Xenograft models have become essential in glioblastoma research, providing biologically relevant systems for studying tumor progression and evaluating novel therapeutics *in vivo*. The U118 glioblastoma cell line, derived from human astrocytoma, has been widely used in preclinical studies due to its mesenchymal phenotype, loss of PTEN, mutated p53, and activation of the PI3K/AKT pathway. These characteristics mirror key features of aggressive GBM subtypes. While U118 has contributed to studies of single-agent drug responses, its application in xenograft models, particularly for assessing combination therapies and epigenetic interventions, remains underutilized. This research seeks to expand the utility of U118 by exploring its response to combined PI3K/mTOR and HDAC inhibition, using both *in vitro* assays and orthotopic xenograft models to better understand therapeutic vulnerabilities in mesenchymal GBM.

U118 Cell Line

The U118 glioblastoma cell line, derived from human astrocytoma, is widely used as a preclinical model to investigate the molecular biology and therapeutic responses of glioblastoma multiforme (GBM). It is defined by the loss of PTEN expression, a mutant p53 genotype, and constitutive activation of the PI3K/AKT signaling pathway, all of which are features commonly associated with GBM progression and resistance to therapy. U118 cells display a mesenchymal phenotype and express markers such as CD44 and YKL-40, which are linked to increased invasiveness and poor clinical outcomes. Although the cell line shows responsiveness to some chemotherapeutic agents, it exhibits relative resistance to temozolomide (TMZ), reflecting the treatment challenges faced in clinical settings. While U118 is frequently employed in monotherapy screening and standard *in vitro* assays, it remains underutilized in studies exploring combination therapies or epigenetic modulation. Moreover, its application in orthotopic xenograft modeling and investigations of tumor microenvironment interactions is limited. These gaps underscore the need for further research to clarify the value of U118 in modeling drug resistance and testing novel therapeutic approaches.

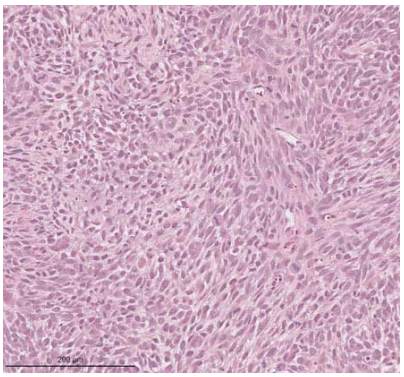

Figure 1. Tumor Histology. H&E stained section of a subcutaneously-implanted U118 tumor (Altogen Labs).

Altogen Labs Validated U118 Xenograft Model

U118 glioblastoma cells are cultured under aseptic conditions and maintained in the exponential growth phase to ensure optimal viability for *in vivo* implantation. Prior to injection, cells are enzymatically dissociated using trypsin, and viability is confirmed to be at least 98% using a trypan blue exclusion assay. The cell suspension is then adjusted to the desired density, and each mouse receives a single subcutaneous injection of 1 × 10^6 U118 cells suspended in 100 µL of a Matrigel mixture. Injections are administered into the right flank under sterile conditions. Tumor establishment is monitored by palpation and caliper measurement three times per week until tumors reach a volume of approximately 50-150 mm^3.

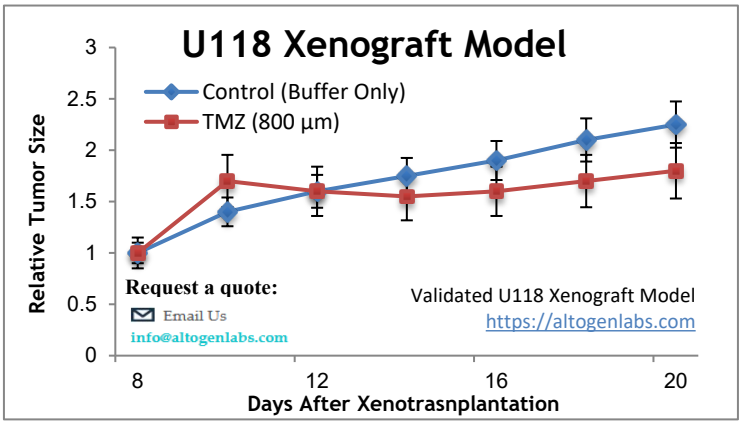

Figure 2. Growth of U118 glioblastoma tumors in immunocompromised mice following xenotransplantation, mean values +/- SEM (Altogen Labs).

Once tumors are established, animals are randomized into treatment groups, and administration of test compounds proceeds according to a predetermined dosing regimen. Tumor volumes are measured daily using digital calipers, and body weights are recorded three times per week throughout the study period to monitor general health and treatment-related toxicity. The study endpoint is defined by tumor growth reaching 2,000 mm^3 or earlier if required by IACUC-approved humane endpoints. At termination, a full necropsy is conducted to assess gross pathology and enable comprehensive tissue analysis. Tumors are excised, weighed, and documented through digital imaging to quantify treatment effects. Depending on the objectives of downstream molecular analyses, tissues are stabilized in RNAlater, snap-frozen in liquid nitrogen, or fixed in formalin for histological processing. All procedures are conducted in accordance with institutional animal care and use committee (IACUC) guidelines to ensure animal welfare and scientific reproducibility.

Subcutaneous Modeling with SNB-75 Cells

Subcutaneous xenograft transplantation using the SNB-75 glioblastoma cell line offers a robust *in vivo* model for studying tumor progression and evaluating therapeutic efficacy. SNB-75 cells, derived from human glioblastoma, exhibit epithelial morphology and key molecular alterations, including p53 pathway disruption, EGFR expression, and PTEN loss, making them relevant for investigating oncogenic signaling and treatment resistance. When injected subcutaneously into immunodeficient mice within a supportive matrix such as Matrigel, SNB-75 reliably forms tumors that can be monitored through caliper measurements. The model has demonstrated moderate tumor take rates and sensitivity to various chemotherapeutic and targeted agents. Despite its utility, the SNB-75 xenograft system remains underutilized in studies involving combination therapies or immune modulation. Expanding the application of this model through the integration of molecular profiling and advanced pharmacodynamic analyses could significantly enhance its translational value in glioblastoma research.

Modeling Mesenchymal GBM Using U118 Orthotopic Transplants

Orthotopic xenograft models provide a clinically relevant platform for studying glioblastoma multiforme (GBM) by replicating the native brain microenvironment, including tumor-stroma interactions, vascular architecture, and therapeutic barriers such as the blood-brain barrier. Although U118 glioblastoma cells have traditionally been used in subcutaneous models, they have also been successfully adapted for orthotopic transplantation via stereotactic injection into the brains of immunodeficient mice. U118 cells, which harbor PTEN loss, p53 mutation, and PI3K/AKT pathway activation, represent the mesenchymal subtype of GBM and are suitable for modeling resistant tumor phenotypes. Recent studies utilizing luciferase-tagged U118 cells (U118-Luc) have enabled real-time, noninvasive tracking of intracranial tumor growth and demonstrated the effectiveness of experimental therapeutics, including targeted fusion proteins and radiosensitizers. These findings reinforce the value of U118 orthotopic xenografts in evaluating drug efficacy within a more representative physiological context and support their broader use in translational GBM research.

Evaluating Therapeutic Response in U118 Glioblastoma Models

The U118 glioblastoma cell line offers a relevant model for investigating mesenchymal glioblastoma, especially in the context of gene-based therapies and radio-sensitization strategies. Characterized by PTEN deletion and p53 mutation, U118 cells exhibit aberrant activation of the PI3K/AKT pathway and are notably resistant to conventional treatments. When evaluated *in vitro*, U118 cells demonstrate sensitivity to certain targeted agents that inhibit pathways involved in proliferation and angiogenesis, such as MET and VEGFR2. Treatment results in reduced cell viability, induction of senescence, and suppression of key proliferative regulators including cyclin D1, CDK4, and phosphorylated Rb. Senescence is further supported by increased β-galactosidase activity and upregulation of p16. Notably, the combination of targeted therapy with ionizing radiation significantly enhances cell death, indicating a synergistic effect and potential for overcoming intrinsic radio-resistance.

Orthotopic xenograft models using luciferase-tagged U118 cells allow real-time monitoring of tumor progression within the brain and enable precise assessment of therapeutic impact. *In vivo* results show that effective gene delivery to intracranial tumors can reduce tumor proliferation, suppress angiogenesis, and increase apoptotic activity. These effects are corroborated by decreased expression of Ki67 and CD31, as well as increased TUNEL-positive cells within tumor sections. Despite the promising biological outcomes, limitations such as small sample sizes and the exclusive use of a single cell line underscore the need for broader validation across glioblastoma subtypes. Nonetheless, the U118 model remains instrumental in dissecting molecular mechanisms of treatment response and in preclinical testing of multimodal therapeutic strategies targeting resistant forms of GBM.

Case Study: SELK Identified as a Critical Target of miR-181 in U118 Cells

A study, conducted by Xu CH, *et* al., published in the *American Journal of Translational Research*, explores the tumor-suppressive role of microRNA-181 (miR-181) in glioblastoma, with a specific focus on the U118 cell line. U118 cells, which display aggressive growth behavior and notable resistance to chemotherapy, were found to express significantly lower levels of miR-181 compared to normal astrocytes. Overexpression of miR-181 led to marked reductions in proliferation and colony formation, accompanied by enhanced apoptosis confirmed through nuclear fragmentation and shifts in Bax and Bcl-2 expression. miR-181 also improved the sensitivity of U118 cells to temozolomide, the frontline chemotherapeutic for glioblastoma, and significantly reduced invasive capacity. Bioinformatic prediction, luciferase reporter assays, and protein analysis identified Selenoprotein K (SELK) as a direct target of miR-181. Functional assays demonstrated that silencing SELK mimicked the effects of miR-181 overexpression, while forced SELK expression reversed these effects, implicating the miR-181-SELK axis in regulating glioblastoma cell behavior.

The methodology employed by the authors was comprehensive and well-controlled, incorporating qRT-PCR, WST-1 viability assays, invasion assays, and western blot analysis in both U118 and U87 glioma cells. While the study offers compelling evidence for the tumor-suppressive function of miR-181, it is limited by the exclusive use of *in vitro* systems and relatively small sample sizes. Nevertheless, the findings present miR-181 as a promising molecular candidate for glioblastoma therapy and underscore the importance of SELK as a novel regulatory target. Published in a peer-reviewed journal and supported by the Natural Science Foundation of Jiangxi, China, this research contributes meaningfully to our understanding of noncoding RNA-mediated regulation in glioblastoma and warrants further investigation *in vivo*, particularly through the use of orthotopic xenograft models to assess translational relevance.

Additional Case Study: U118 Glioblastoma Models Reveal Microenvironment-Driven Changes

A study by Creighton *et al.*, published in *Genome Biology* journal, presents a comprehensive comparison of gene expression profiles between cultured U118 glioblastoma cells and their subcutaneous xenograft tumors in immunodeficient mice. Using Affymetrix microarrays, the researchers identified 368 genes with significant expression differences between *in vitro* and *in vivo* conditions ($p < 0.01$, fold change greater than 2). Among these, 112 genes were upregulated in xenografts and 256 in cultured cells. U118 xenografts showed increased expression of genes related to extracellular matrix remodeling, adhesion, cytokine activity, and angiogenesis, including COL1A1, COL1A2, COL5A2, PDGFRB, and ELN. In contrast, cultured U118 cells expressed higher levels of genes linked to cell cycle progression, DNA replication, and metabolic activity, reflecting a more proliferative state in standard culture environments.

The study's strength lies in its use of biological replicates, statistical rigor, and functional annotation to validate its findings. Principal component analysis confirmed that both *in vitro* and *in vivo* U118 profiles clustered with brain tumor datasets, supporting the model's lineage fidelity. Interestingly, even when implanted in the same host environment, U118 and A549 cells exhibited distinct extracellular matrix expression patterns, suggesting cell-type-specific adaptations. Mouse RNA contamination was ruled out through control analyses, strengthening the conclusion that gene expression changes originated from human tumor cells. These findings emphasize the importance of xenograft models for capturing context-dependent gene regulation that is absent *in vitro*. The U118 xenograft model is particularly valuable for studying glioblastoma-associated processes such as extracellular matrix remodeling and angiogenesis.

Bio-printed U118 Model Mimics Glioma Angiogenesis

Coaxial extrusion bioprinting has been applied to develop a three-dimensional glioblastoma model using U118 glioma cells and human endothelial cells to study tumor-induced angiogenesis. In this system, U118 cells were placed in the outer shell of hydrogel microfibers, while HUVECs occupied the inner core, creating a structure that closely replicates the tumor microenvironment. U118 cells showed strong proliferation and significantly elevated secretion of VEGFA and bFGF in the presence of HUVECs, promoting endothelial cell sprouting, chemotaxis, and tubule formation. Gene and protein expression analyses confirmed increased levels of angiogenic markers such as CD31 and VEGFR2 in the HUVEC population. These results demonstrate that U118 cells stimulate angiogenesis both through paracrine signaling and possibly through more direct mechanisms.

Additional evidence suggests that U118 cells may contribute to neovascularization through endothelial differentiation or cell fusion. *In vivo* implantation of U118-loaded microfibers resulted in tumor formation with histological features resembling human glioblastoma, including vascular structures containing cells that coexpressed glioma and endothelial markers like GFAP and vWF. This bioprinted model offers a physiologically relevant platform to study tumor-vascular interactions in a spatially defined environment. While the subcutaneous setting limits full recapitulation of brain-specific cues and immune interactions, the approach provides valuable insight into the role of glioma cells in vascular remodeling.

U118 Resistance to TMZ Linked to Survival Pathway Activation

The U118 glioblastoma cell line provides a valuable model for studying resistance to temozolomide (TMZ), a standard chemotherapeutic agent used in glioblastoma treatment. Exposure of U118 cells to increasing concentrations of TMZ resulted in a clear reduction in proliferation, particularly at doses above 100 micromolar. Despite this growth inhibition, apoptosis levels remained low, and no significant cell cycle arrest in G1 or G2 phases was observed. Chromatin condensation and nuclear abnormalities confirmed limited apoptotic activity, while a marked increase in LC3 expression suggested that autophagy may serve as a compensatory survival mechanism. These results support the hypothesis that U118 cells evade TMZ-induced death by activating non-apoptotic pathways and maintaining survival signaling.

Further analysis revealed that U118 cells retained basal activation of both the PI3K/Akt and ERK1/2 MAPK pathways during TMZ treatment. Pharmacologic inhibition of these pathways using wortmannin, rapamycin, and U-0126, individually and in combination with TMZ, resulted in significant increases in apoptotic cell death. The most pronounced effect occurred when all three inhibitors were used in conjunction with TMZ, yielding a 71 percent apoptotic rate. This synergistic effect highlights the critical role of these signaling pathways in maintaining glioma cell survival. The methods employed, including flow cytometry, Western blotting, and proliferation assays, were robust and well controlled, although limited to *in vitro* conditions. These findings emphasize the need for integrated therapeutic approaches that target multiple survival mechanisms and warrant further investigation in orthotopic models to better replicate the brain tumor microenvironment.

Reversing Temozolomide Resistance in U118 with Metabolic Inhibition

The U118 glioblastoma cell line, characterized by high MGMT expression and resistance to temozolomide (TMZ), serves as a valuable model for investigating mechanisms of chemoresistance and combination therapy strategies. In this context, inhibition of phosphoglycerate dehydrogenase (PHGDH) using a selective small molecule was shown to significantly enhance the cytotoxic effects of TMZ in U118 cells. Combination treatment reduced cell viability, increased apoptosis, and lowered the IC50 of TMZ. Upregulation of cleaved caspase-3 and PARP indicated enhanced apoptotic signaling, while elevated reactive oxygen species (ROS) levels suggested that oxidative damage contributed to the therapeutic synergy. The cytotoxic effect was attenuated by the ROS scavenger NAC, confirming a ROS-dependent mechanism of action.

Further analysis revealed that PHGDH inhibition suppressed MGMT expression without altering promoter methylation, suggesting an epigenetic or signaling-based mechanism. Downregulation of Wnt/β-catenin signaling components, including β-catenin, LEF1, and TCF1/7, as well as downstream targets c-Myc and Cyclin D1, supports a model in which metabolic disruption alters gene regulation critical for glioblastoma survival. In subcutaneous U118 xenograft models, the combination therapy significantly reduced tumor growth without observable toxicity, highlighting the therapeutic potential of targeting serine biosynthesis in chemoresistant glioma. These findings underscore the value of U118 as a platform for evaluating metabolic and signaling-based interventions and warrant further investigation in orthotopic models to replicate brain-specific microenvironmental influences.

U118 Glioblastoma Cells Respond to PP2A Reactivation

The U118 glioblastoma cell line exhibits elevated expression of endogenous PP2A inhibitor proteins, such as PME-1, CIP2A, and SET, despite lacking mutations in core PP2A components, indicating that non-genetic suppression contributes to its tumorigenic signaling. Treatment with the small molecule activator NZ-8-061 led to a dose-dependent decrease in U118 cell viability and colony formation, a response mirrored across other glioblastoma lines with diverse mutation profiles and kinase inhibitor resistance. The specificity of this effect was confirmed using TRC-766, a structurally similar but biologically inactive compound, which failed to reduce viability. U118 cells also responded to a second activator, DBK-1154, which demonstrated greater brain penetration and more potent induction of apoptosis. Compared to traditional kinase inhibitors, which often showed limited efficacy, these PP2A activators produced consistent and robust reductions in viability, positioning U118 as a relevant model for studying phosphatase-driven therapeutic responses in glioblastoma.

The U118 glioblastoma cell line is a widely utilized *in vitro* and *in vivo* model in neuro-oncology research at Altogen Labs. Originating from human glioblastoma multiforme (GBM), U118 cells harbor genetic alterations characteristic of high-grade gliomas, including loss-of-function mutations in the TP53 tumor suppressor gene and aberrations in PTEN, a key regulator of the PI3K/AKT signaling pathway. These features contribute to unchecked cell proliferation, therapeutic resistance, and tumor progression, making U118 a clinically relevant system for studying glioma biology. U118 cells exhibit a mesenchymal-like phenotype and are known for their high motility and invasiveness, which closely mimic the diffuse infiltrative behavior of glioblastoma in patients. Additionally, U118 cells display resistance to several standard-of-care chemotherapeutics, making them a valuable model for testing novel agents that can overcome inherent drug resistance mechanisms.

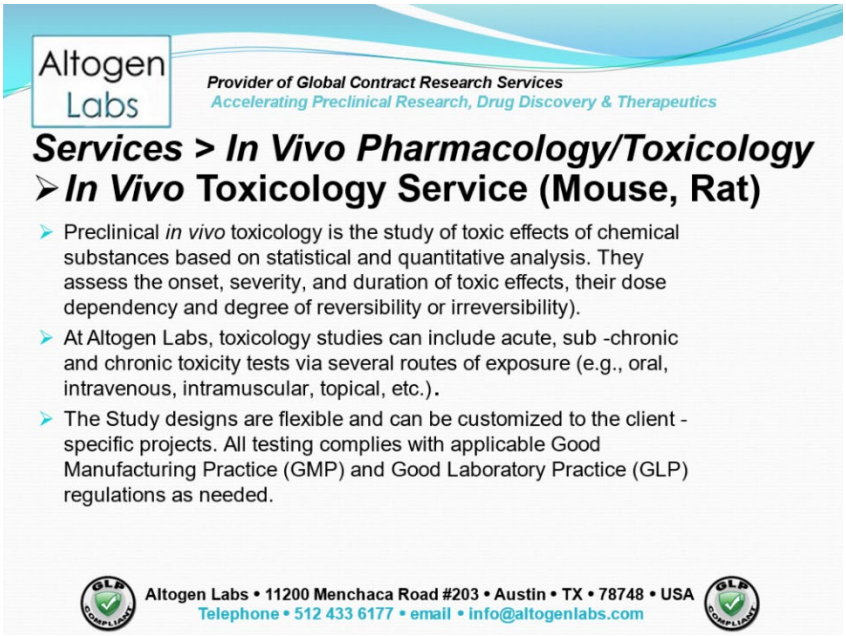

Figure 3. *In vivo* xenograft modeling services offered by Altogen Labs, including U118 glioblastoma studies, with customizable protocols tailored to client-specific research goals.

At Altogen Labs, U118 cells are routinely employed in xenograft models through subcutaneous and orthotopic implantation into immunodeficient mouse strains. These models provide a reliable platform for evaluating tumor growth dynamics, drug resistance mechanisms, and therapeutic responses under physiologically relevant conditions. All *in vivo* work involving U118 xenografts is conducted in GLP-compliant, IACUC-regulated animal facilities. Mice are monitored daily for tumor growth, behavior, and general health status. Study deliverables include full experimental protocols, health and welfare documentation, raw data, and statistical analyses. Available study endpoints include tumor growth inhibition (TGI), tumor growth delay (TGD), and survival analysis. Additional customization options encompass various dosing regimens, immunohistochemical analysis, histopathology, and blood chemistry profiling. Advanced molecular assays, such as RNA and protein extraction, qPCR, Western blotting, and gene expression profiling, are available to support mechanistic studies. Optional imaging endpoints include MRI, fluorescence-based imaging, and bioluminescent monitoring, enabling longitudinal assessment of tumor progression and therapeutic response. U118 xenograft studies can also incorporate positive control agents, administered intramuscularly or systemically at defined dosages, and lipid metabolism assays for evaluating metabolic reprogramming in GBM.

Figure 4. Comprehensive *in vivo* toxicology testing services provided by Altogen Labs, including acute, sub-chronic, chronic toxicity, pharmacokinetics, and immunotoxicology evaluations.

References:

Carmo A, Carvalheiro H, Crespo I, Nunes I, Lopes MC. Effect of temozolomide on the U-118 glioma cell line. *Oncol Lett.* 2011 Nov;2(6):1165-1170. doi: 10.3892/ol.2011.406. Epub 2011 Sep 2. PMID: 22848283; PMCID: PMC3406512.

Creighton C, Kuick R, Misek DE, Rickman DS, Brichory FM, Rouillard JM, Omenn GS, Hanash S. Profiling of pathway-specific changes in gene expression following growth of human cancer cell lines transplanted into mice. *Genome Biol.* 2003;4(7):R46. doi: 10.1186/gb-2003-4-7-r46. Epub 2003 Jun 23. PMID: 12844362; PMCID: PMC193633.

Jin L, Kiang KM, Cheng SY, Leung GK. Pharmacological inhibition of serine synthesis enhances temozolomide efficacy by decreasing O^6-methylguanine DNA methyltransferase (MGMT) expression and reactive oxygen species (ROS)-mediated DNA damage in glioblastoma. *Lab Invest.* 2022 Feb;102(2):194-203. doi: 10.1038/s41374-021-00666-7. Epub 2021 Oct 8. PMID: 34625658.

Wang X, Li X, Zhang Y, Long X, Zhang H, Xu T, Niu C. Coaxially Bioprinted Cell-Laden Tubular-Like Structure for Studying Glioma Angiogenesis. *Front Bioeng Biotechnol*. 2021 Oct 1;9:761861. doi: 10.3389/fbioe.2021.761861. PMID: 34660561; PMCID: PMC8517394.

Xu CH, Xiao LM, Zeng EM, Chen LK, Zheng SY, Li DH, Liu Y. MicroRNA-181 inhibits the proliferation, drug sensitivity and invasion of human glioma cells by targeting Selenoprotein K (SELK). *Am J Transl Res.* 2019 Oct 15;11(10):6632-6640. Erratum in: Am J Transl Res. 2021 Aug 15;13(8):9914-9917. PMID: 31737213; PMCID: PMC6834513.

Zhang, J., Li, T., Liu, L. *et al.* A novel PH1/pE27HGFK1 nanoparticles for orthotopic glioblastoma therapy. *Cancer Nano* 15, 29 (2024). https://doi.org/10.1186/s12645-024-00267-1

Keywords: U118, xenograft, *in vivo*, cancer, preclinical, glioblastoma, brain, research, *in vivo* pharmacology, brain cancer, glioblastoma multiforme, CDX, PDX, orthotopic

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/

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