Validated COLO205 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 Colorectal Cancer Research Through Xenograft Modeling

Colorectal cancer (CRC) is a leading cause of cancer-related mortality globally, characterized by substantial molecular heterogeneity that complicates treatment and contributes to poor outcomes in advanced stages. While significant progress has been made in identifying key oncogenic drivers such as APC, KRAS, TP53, and BRAF, effective translation of this knowledge into durable therapeutic interventions remains a challenge. Xenograft models, particularly cell line-derived xenografts (CDXs), are essential in bridging the gap between *in vitro* studies and clinical application by enabling the investigation of tumor behavior, therapeutic response, and mechanisms of resistance in a biologically relevant setting. Despite their utility, traditional xenografts often lack tumor microenvironmental complexity and immune interactions, limiting their capacity to model the full spectrum of disease progression. This research seeks to address these limitations by leveraging the COLO205 xenograft model to investigate long-term therapeutic adaptation and metabolic reprogramming in BRAF-mutant CRC. By integrating molecular and metabolic analyses within a dynamic *in vivo* framework, this study aims to elucidate resistance mechanisms and identify predictive biomarkers, ultimately contributing to the development of more precise and effective therapeutic strategies for colorectal cancer.

COLO205 Cell Line

The COLO205 cell line, established from the ascitic fluid of a patient with colorectal adenocarcinoma, serves as a widely utilized *in vitro* model for investigating the molecular and pharmacological characteristics of colorectal cancer. This epithelial cell line exhibits a microsatellite-stable genotype and harbors a homozygous BRAF^V600E mutation, while remaining wild-type for KRAS and TP53, thereby representing a genetically defined subset of colorectal tumors with distinct clinical behavior. COLO205 has been extensively employed in studies evaluating MAPK pathway inhibitors, particularly BRAF-targeted therapies, which demonstrate initial efficacy but are frequently undermined by adaptive resistance through EGFR-mediated signaling feedback. Combination regimens incorporating BRAF and EGFR inhibitors have shown synergistic cytotoxicity, although resistance mechanisms such as alternative receptor tyrosine kinase activation and PI3K/AKT pathway engagement continue to pose therapeutic challenges. Additionally, COLO205 exhibits a pronounced apoptotic response to targeted agents, rendering it a valuable model for dissecting programmed cell death mechanisms.

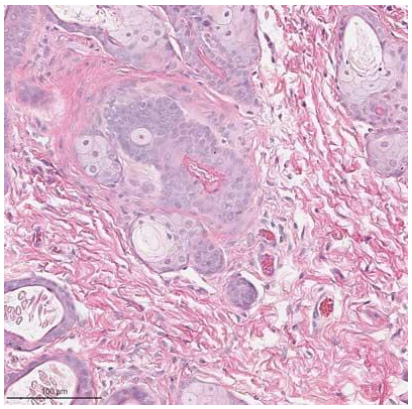

Figure 1. Tumor Histology. H&E stained section of a subcutaneously-implanted COLO205 tumor (Altogen Labs).

Altogen Labs Validated COLO205 Xenograft Model

COLO205 cells are maintained under aseptic conditions and cultured during their exponential growth phase prior to injection. Cells are harvested using trypsinization, and viability is confirmed using the trypan blue exclusion assay with a minimum acceptance threshold of 98 percent. The cell suspension is then diluted to the appropriate density for implantation. Each athymic BALB/c (nu/nu) mouse, 10 weeks of age, receives a single subcutaneous injection of 1 × 10^6 COLO205 cells suspended in 100 microliters of Matrigel into the flank of a hind leg. Injection sites are monitored three times per week until tumors are established, with digital caliper measurements used to track tumor growth.

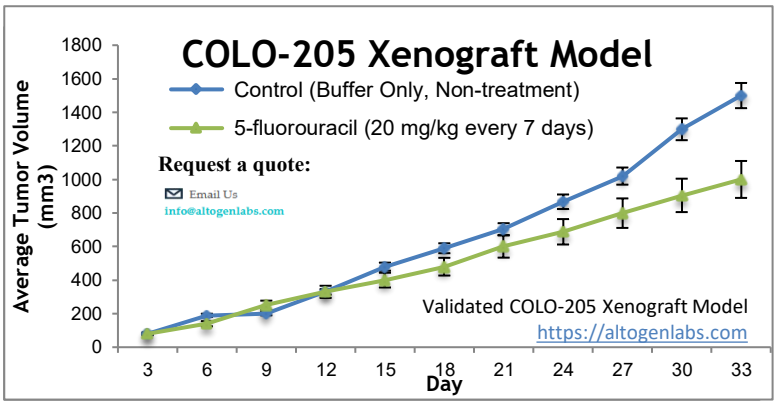

Figure 2. Tumor growth inhibition in the COLO-205 colon cancer xenograft model. Mean tumor volumes ± SEM are shown for treated and control groups. Study performed using the validated COLO-205 model (Altogen Labs).

Once tumors reach an average volume of 50 to 150 mm³, animals are randomized into treatment cohorts. Compounds of interest are administered in accordance with the study protocol, and tumor measurements along with body weights are recorded up to three times weekly throughout the treatment period. When tumors approach 2,000 mm² or reach a pre-specified size limit, animals are humanely euthanized. A full necropsy is performed, and tissues are collected as specified in the experimental termination criteria. Tumors are excised, weighed, and imaged digitally. Collected samples may be flash-frozen in liquid nitrogen, preserved in RNAlater, or processed for histological evaluation, depending on downstream analytical requirements. All procedures are conducted in compliance with the Guide for the Care and Use of Laboratory Animals and under protocols approved by the Institutional Animal Care and Use Committee (IACUC). This xenograft model offers a validated platform for evaluating the *in vivo* efficacy of chemotherapeutic agents, molecular inhibitors, and targeted therapies against colorectal cancer, and supports a wide range of translational studies.

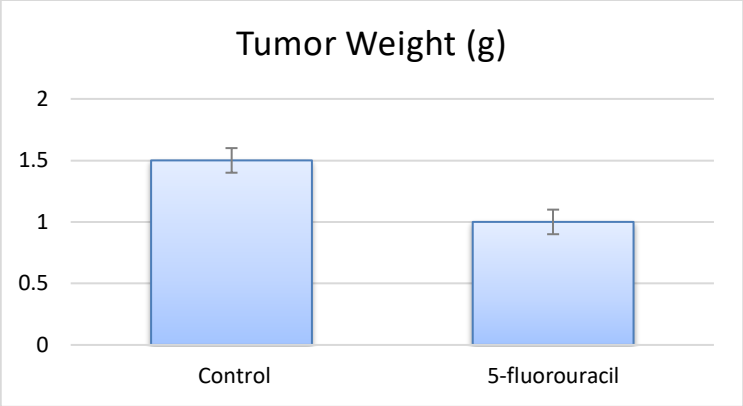

Figure 3. Final tumor weight of COLO205 xenografts in control (buffer only) and 5-fluorouracil-treated (20 mg/kg weekly) mice. Data represent mean values ± SEM, showing reduced tumor burden following treatment. Xenograft model and *in vivo* study conducted by Altogen Labs.

Subcutaneous Xenografts in Colorectal Cancer Research

Subcutaneous xenograft transplantation using the COLO205 cell line is a widely employed preclinical approach for modeling human colorectal cancer, offering a reproducible and accessible system for evaluating tumor growth and therapeutic response. Derived from a patient with advanced colorectal adenocarcinoma, COLO205 cells harbor the BRAF^V600E mutation and have been instrumental in studies investigating targeted inhibition of the MAPK pathway. Typically injected into the flank of immunocompromised BALB/c nude mice in a Matrigel suspension, COLO205 cells form measurable tumors within two weeks, enabling longitudinal assessment of drug efficacy. This model has demonstrated sensitivity to BRAF and EGFR inhibitors, particularly in combination, and has been used to characterize mechanisms of acquired resistance involving PI3K/AKT and MET signaling. Recent investigations have leveraged COLO205 xenografts to explore gene expression dynamics, apoptosis regulation, and metabolic adaptation under therapeutic stress, revealing exploitable vulnerabilities. Although limited by its lack of immune components and non-orthotopic tumor growth, the COLO205 subcutaneous model remains a critical platform for translational colorectal cancer research, supporting the development and refinement of targeted treatment strategies.

***In vivo* Characterization of COLO205 in Orthotopic Transplantation**

Orthotopic xenograft transplantation using COLO205 cells provides a biologically relevant model for studying colorectal cancer by replicating the tumor's native anatomical environment and local microenvironmental interactions. This approach surpasses the limitations of subcutaneous models by enabling investigation of tumor progression, invasion, and early metastatic events within the colon. Studies employing COLO205 cells engineered to express luciferase and GFP have demonstrated successful engraftment into the submucosa of the mouse cecum, allowing for non-invasive monitoring of tumor growth and regional lymphatic spread. While distant metastasis to organs such as the liver and lungs is infrequent in this model, the localized tumor development closely mimics early-stage clinical disease. Orthotopic models using COLO205 have proven valuable in assessing therapeutic responses under more clinically relevant conditions, particularly in relation to drug penetration, stromal interactions, and local tissue architecture. Although the use of immunodeficient hosts restricts the study of immune-based therapies, this model facilitates in-depth analysis of tumor-intrinsic features, epithelial-mesenchymal transition, and resistance mechanisms. As such, COLO205 orthotopic xenografts offer a critical platform for advancing translational research and preclinical evaluation of novel treatments in colorectal cancer.

Case Study: Enhanced Apoptotic Response in COLO205 with Targeted TRAIL Therapy

In a study published by *Cell Death and Disease* journal, Siegemund et al. present a detailed evaluation of a dimerized TRAIL fusion protein (DbaEGFR-scTRAIL) designed to target EGFR-positive colorectal cancer, using the COLO205 cell line as a model system. Compared to its monovalent and non-targeted counterparts, DbaEGFR-scTRAIL demonstrated

significantly enhanced apoptotic activity, with approximately 100-fold greater potency than scTRAIL and a tenfold increase over scFvaEGFR-scTRAIL at sub-nanomolar concentrations. *In vivo* treatment of COLO205 xenografts resulted in substantial tumor regression and prolonged survival, with most treated animals showing undetectable tumor volumes. The observed apoptosis was confirmed to be TRAIL-dependent through caspase inhibition assays, and targeting specificity was validated by cetuximab competition studies. These findings provide strong evidence that combining antigen-directed delivery with controlled ligand oligomerization can significantly improve the therapeutic effect in colorectal cancer models.

The study employed a rigorous experimental framework, incorporating relevant controls, apoptosis sensitizers, and multiple quantitative assays. The COLO205 cell line, which expresses EGFR and is sensitive to TRAIL signaling, proved to be a highly suitable model for assessing targeted pro-apoptotic therapies. Despite the strengths of the study design, the exclusive use of immunodeficient mice and a single tumor type limits the generalizability of the findings. Moreover, the potential for off-target effects in normal EGFR-expressing tissues warrants further investigation. Nevertheless, the results underscore the utility of COLO205 xenografts in preclinical oncology and support further exploration of dimerized TRAIL fusion proteins. Future studies should incorporate immune-competent models and long-term assessments of therapeutic safety and efficacy.

Additional Case Study: Targeting Mitosis in Colorectal Cancer Using Cmpd-A and COLO205 Models

In another study published by *PLoS One* journal, Ohashi et al. describe the discovery and characterization of Compound-A (Cmpd-A), a novel time-dependent small-molecule inhibitor of the mitotic kinesin motor protein CENP-E. Using the COLO205 colorectal cancer cell line, the study demonstrates that Cmpd-A effectively induces chromosome misalignment, activates the spindle assembly checkpoint (SAC), and results in mitotic arrest followed by apoptosis. *In vivo* pharmacokinetic and pharmacodynamic analysis showed that intraperitoneal administration of Cmpd-A in COLO205 xenograft-bearing mice caused a transient increase in phosphohistone H3 (pHH3), a mitotic marker, peaking at 8 hours and declining after 24 hours. This temporal profile informed a refined dosing schedule that achieved substantial antitumor efficacy with no observed toxicity. Mice receiving three doses over 24 hours showed an 89 percent reduction in tumor growth by day 8, without significant weight loss or systemic side effects.

The findings indicate several key patterns. First, COLO205 was highly sensitive to Cmpd-A, both *in vitro* and *in vivo*, confirming its suitability for studying mitotic disruption in colorectal cancer. Second, the antiproliferative effects were tightly correlated with SAC activation, particularly involving BubR1 localization and caspase-3/7 activation. Third, although CENP-E mRNA levels varied across cell lines, sensitivity to Cmpd-A was not predicted by expression level alone, indicating that additional cellular factors modulate responsiveness. The study's use of synchronized cell cycle assays, RNA interference, FACS, and xenograft modeling provides a comprehensive framework for mechanistic and functional validation. However, limitations include the use of immunodeficient mice, which precludes assessment of immune-mediated effects, and a focus on a limited number of responsive and resistant cell lines, which narrows broader clinical extrapolation. Methodologically, the study is well designed, with controlled comparisons between treated and untreated groups, as well as between SAC-intact and SAC-impaired conditions. The COLO205 xenograft model stands out for its reproducibility, high take rate, and suitability for dynamic PK/PD modeling. Nonetheless, future research should investigate the utility of pHH3 as a reliable biomarker across tumor types, identify additional markers predictive of Cmpd-A sensitivity, and assess the drug's efficacy in immune-competent and orthotopic colorectal cancer models. The clear suppression of tumor growth in COLO205 xenografts positions this model as a valuable platform for evaluating mitosis-targeting agents and contributes meaningfully to the development of next-generation non-tubulin-based anticancer therapeutics.

Targeting VEGF-A and Ang-2 Suppresses Tumor Growth and Metastasis

The COLO205 colorectal cancer xenograft model provides a robust platform for evaluating angiogenesis-targeting therapies and has demonstrated high sensitivity to dual inhibition of vascular endothelial growth factor A (VEGF-A) and angiopoietin-2 (Ang-2). A tetravalent bispecific antibody targeting both ligands achieved an 87 percent reduction in tumor growth, outperforming VEGF-A inhibition alone, which resulted in 66 percent tumor growth inhibition, and Ang-2 inhibition alone, which produced a 47 percent reduction. This combined approach remained effective even after the development of resistance to VEGF-targeted therapy, leading to a 60 percent decrease in tumor volume compared to continued VEGF-A monotherapy. The bispecific antibody also significantly suppressed lung metastasis by 66 percent. Histological examination revealed extensive tumor cell death and near-complete inhibition of microvessel formation in treated tumors, confirming its anti-angiogenic and anti-metastatic effects. These findings highlight the potential of COLO205 xenografts to model therapeutic resistance and validate the strategy of targeting multiple angiogenic pathways to enhance treatment efficacy.

Patterns in the data consistently show superior outcomes with dual-targeting approaches across key measures such as tumor regression, metastasis suppression, and vascular density reduction. The COLO205 model is particularly suited for this type of evaluation due to its reproducibility, human VEGF dependency, and capacity to reflect adaptive mechanisms such as Ang-2 upregulation following VEGF-A inhibition. These characteristics provide insights into how tumors may circumvent single-pathway blockade and underscore the importance of simultaneous pathway targeting. Methodologically, the model supports reliable measurements of tumor volume, histological changes, and metastatic burden. However, the use of immunodeficient hosts limits evaluation of immune-related effects, and lack of cross-reactivity with host VEGF may lead to an underestimation of effects on the tumor microenvironment.

Dual Treatment Suppresses Drug Resistance and Metastasis in COLO205

The COLO205 colorectal cancer cell line serves as an effective *in vivo* platform for evaluating synergistic drug responses, particularly in the context of adjuvant chemotherapy. Treatment with a combination of 5-fluorouracil (5-FU) and Tanshinone IIA (Tan-IIA), a diterpene quinone derived from *Salvia miltiorrhiza*, resulted in substantial tumor volume reduction compared to 5-FU monotherapy or vehicle controls. Over a four-week treatment period in SCID mouse xenograft models, the combined therapy reduced average tumor volumes by over 50 percent relative to 5-FU alone, with no significant adverse effects on animal weight. Western blot analyses revealed that the combination therapy led to downregulation of several key proteins associated with drug resistance, angiogenesis, autophagy, and metastasis. These include P-glycoprotein (P-gp), VEGF, LC3-II, MMP-7, and NF-κB p65, all of which were expressed at significantly lower levels in tumors treated with 5-FU plus Tan-IIA than in those treated with 5-FU alone.

The expression patterns of these biomarkers suggest a coordinated suppression of pathways that normally promote therapeutic resistance and tumor progression. P-gp downregulation implies reduced efflux of chemotherapeutic agents, enhancing intracellular 5-FU efficacy. The observed decrease in LC3-II, a marker of autophagy, is particularly significant, as autophagy can allow tumor cells to survive cytotoxic stress. Inhibition of VEGF and MMP-7 indicates suppression of angiogenesis and metastatic potential, while the reduction of NF-κB p65 suggests diminished inflammatory and survival signaling. These findings align with prior evidence that inhibition of NF-κB enhances 5-FU sensitivity. The methodology employed in this study, including rigorous protein quantification and appropriate controls, supports the reliability of the results. However, limitations include the use of immunodeficient mice and a narrow treatment window, which may not fully reflect clinical complexity. Still, COLO205 proves to be a powerful model for evaluating combination therapies, especially those targeting drug resistance and metastatic signaling. Further studies should explore the long-term durability of these effects, integration with immune-competent systems, and potential for clinical translation in human colorectal cancer treatment.

Targeting Oncogenic Signaling in COLO205 Through FRα-Mediated TP53 Activation

COLO205, a colorectal cancer cell line expressing wild-type TP53 and folate receptor alpha (FRα), provides a valuable model for investigating nutrient-regulated oncogenic signaling. Exposure to folic acid (FA) significantly reduces COLO205 proliferation through a defined signaling cascade involving FRα, c-SRC, ERK1/2, and NFκB. This pathway culminates in the upregulation of TP53 and its downstream targets CDKN1A (p21) and CDKN1B (p27), resulting in G0/G1 cell cycle arrest. The anti-proliferative effect was confirmed both *in vitro* and *in vivo*, where FA treatment led to substantial tumor regression and increased lifespan in xenograft-bearing mice. Inhibition of any pathway component or TP53 itself abolished the effects, confirming the pathway's specificity. Chromatin immunoprecipitation further supported NFκB binding to the TP53 promoter, establishing transcriptional activation as a central mechanism. Additionally, angiogenesis was impaired, as indicated by reduced von Willebrand factor expression in treated tumors.

The signaling cascade activated by FA was shown to be tightly regulated and time-dependent, with a transient increase in TP53 and its targets, likely due to feedback through MDM2-mediated degradation. Despite this narrow activation window, longer FA exposure restored elevated protein levels, supporting its sustained regulatory role. The COLO205 model's expression of FRα and responsiveness to FA make it an ideal system for studying folate-targeted therapies. Methodologically, the research was well controlled, using pharmacologic inhibitors, gene knockdown, and multiple validation assays. Future studies should investigate FA's effects in immune-competent systems, assess therapeutic synergy with existing chemotherapies, and further explore the regulation of CDKN1B by TP53. These findings support COLO205's relevance in modeling FA-responsive signaling and underscore the broader therapeutic potential of targeting folate-regulated pathways in colorectal cancer.

Altogen Labs offers a comprehensive portfolio of preclinical research services utilizing over 90 standardized cell line-derived xenograft (CDX) models and more than 30 patient-derived xenograft (PDX) models. For studies investigating the functional role of specific proteins or gene products in tumor development and progression, Altogen Labs provides custom generation of overexpression cell lines and RNA interference (RNAi) models, enabling long-term silencing or ectopic expression of oncogenes, tumor suppressors, or other regulatory targets. Gene expression profiling is supported through quantitative RT-PCR for mRNA analysis and high-throughput protein expression analysis using the WES platform by ProteinSimple. These tools are integral to mechanistic studies of drug response, biomarker validation, and pathway interrogation. Researchers benefit from the ability to incorporate molecular data alongside phenotypic readouts, increasing the translational relevance of their findings. The integration of genetic engineering with *in vivo* modeling ensures a flexible and highly customizable experimental workflow.

Figure 4. *In vivo* xenograft services for the COLO205 model at Altogen Labs, including tumor growth studies, immunohistochemistry, toxicity, histopathology, and imaging.

All *in vivo* studies are conducted in accordance with IACUC guidelines and Good Laboratory Practice (GLP) standards. Animals are acclimated and sorted by body mass prior to study initiation, and are monitored daily for tumor progression and clinical health status. Clients receive comprehensive study documentation, including detailed methodology, results, statistical analyses, and raw datasets. For the COLO205 xenograft model specifically, available study endpoints include tumor growth delay (TGD), tumor growth inhibition (TGI), survival, and toxicity assessments. Multiple dosing routes are supported, including intravenous, intraperitoneal, oral, and micro-injection-based methods. Additional capabilities include orthotopic and metastatic engraftment techniques, immunohistochemistry, histopathology, blood chemistry analysis, lipid and metabolic profiling, and collection of tissue for downstream molecular analysis. A positive control group treated with cyclophosphamide at 50 mg/kg by intramuscular injection is available to establish comparative efficacy. Altogen Labs' extensive *in vivo* infrastructure ensures that COLO205 xenograft studies are executed with scientific rigor, operational precision, and support for oncology research.

Figure 5. Range of *in vivo* toxicology and pharmacology services offered by Altogen Labs, including acute and chronic toxicity studies, pharmacokinetics, immunotoxicity, and absorption analysis.

References:

Kuo, CT., Chang, C. & Lee, WS. Folic acid inhibits COLO-205 colon cancer cell proliferation through activating the FRα/c-SRC/ERK1/2/NFκB/TP53 pathway: *in vitro* and *in vivo* studies. *Sci Rep* 5, 11187 (2015). https://doi.org/10.1038/srep11187

Ohashi A, Ohori M, Iwai K, Nambu T, Miyamoto M, Kawamoto T, Okaniwa M. A Novel Time-Dependent CENP-E Inhibitor with Potent Antitumor Activity. *PLoS One*. 2015 Dec 9;10(12):e0144675. doi: 10.1371/journal.pone.0144675. PMID: 26649895; PMCID: PMC4674098.

Scheuer W, Thomas M, Hanke P, Sam J, Osl F, Weininger D, Baehner M, Seeber S, Kettenberger H, Schanzer J, Brinkmann U, Weidner KM, Regula J, Klein C. Anti-tumoral, anti-angiogenic and anti-metastatic efficacy of a tetravalent bispecific antibody (TAvi6) targeting VEGF-A and angiopoietin-2. *MAbs*. 2016;8(3):562-73. doi: 10.1080/19420862.2016.1147640. PMID: 26864324; PMCID: PMC4966847.

Siegemund M, Pollak N, Seifert O, Wahl K, Hanak K, Vogel A, Nussler AK, Göttsch D, Münkel S, Bantel H, Kontermann RE, Pfizenmaier K. Superior antitumoral activity of dimerized targeted single-chain TRAIL fusion proteins under retention of tumor selectivity. *Cell Death Dis.* 2012 Apr 12;3(4):e295. doi: 10.1038/cddis.2012.29. PMID: 22495350; PMCID: PMC3358007.

Su CC. Tanshinone IIA potentiates the efficacy of 5-FU in Colo205 colon cancer cells *in vivo* through downregulation of P-gp and LC3-II. *Exp Ther Med*. 2012 Mar;3(3):555-559. doi: 10.3892/etm.2011.441. Epub 2011 Dec 28. PMID: 22969929; PMCID: PMC3438563.

Keywords: COLO205, colon, xenograft, *in vivo*, cancer, preclinical, research, *in vivo* pharmacology, colon cancer, colorectal, 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/