Validated LN229 Xenograft Model:

Subcutaneous, Orthotopic, And Metastatic Xenograft Tumor Model

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Advancing Brain Cancer Research Through Xenograft Models

Brain cancer, particularly glioblastoma multiforme (GBM), is one of the most aggressive and lethal types of brain tumors, with poor prognosis and limited treatment options. GBM is characterized by rapid growth, resistance to conventional therapies, and high recurrence rates, making it a major challenge in both clinical and research settings. Despite advances in surgical techniques, radiation, and chemotherapy, the survival rate for patients remains dismal, underscoring the need for more effective therapeutic strategies. Xenograft models, where human tumor tissues are implanted into immunocompromised mice, provide critical insights into tumor biology and the efficacy of novel treatments. These models mimic human cancer more accurately than traditional cell line studies, allowing researchers to study tumor microenvironment interactions, metastatic behavior, and drug responses in a clinically relevant context. Additionally, patient-derived xenografts (PDX) offer a closer representation of individual patient tumors, further improving the translational potential of preclinical studies. Brain cancer xenograft models have become indispensable for evaluating experimental therapies, including targeted therapies, immunotherapies, and novel drug combinations.

LN229 Cell Line

The LN229 cell line is a widely used model in cancer research, particularly for studying glioblastoma, a highly aggressive form of brain cancer. Isolated in 1979 from the right frontal parieto-occipital cortex of a 60-year-old female patient with glioblastoma, LN229 exhibits an epithelial-like morphology. This cell has also become valuable in neuroscience and oncology research. It retains many characteristics of the original tumor, including genetic mutations and phenotypic features, making it an ideal model for investigating glioblastoma biology. Researchers use LN229 cells to study tumor growth, invasiveness, and response to various therapeutic agents. Additionally, this cell line serves as a model for understanding the molecular mechanisms of glioblastoma progression and resistance to treatment. It is also employed in preclinical studies to evaluate novel therapies and drug candidates aimed at targeting glioblastoma. LN229 cells are also utilized to explore the effects of the tumor microenvironment and the role of glial cells in tumor progression. Their ability to form tumor xenografts in animal models makes them an essential resource for evaluating potential therapeutic interventions and understanding glioblastoma's complex biology *in vivo*.

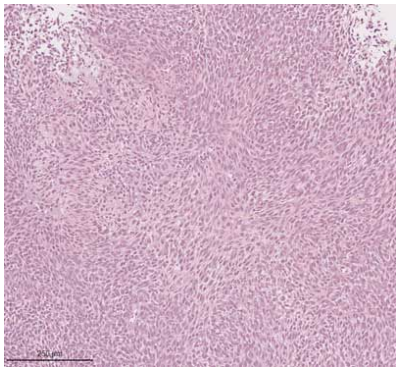

Figure 1. Tumor Histology. H&E stained section of a subcutaneously-implanted LN229 tumor (Altogen Labs).

Altogen Labs Validated LN229 Xenograft Model

Altogen Labs offers xenotransplantation services using LN-229 glioblastoma cells to investigate tumor formation and progression. The LN-229 cell line is used to create the Cell Line Derived Xenograft (CDX) LN229 xenograft mouse model, which is instrumental in studying the effects of various therapeutic approaches. This model is utilized for evaluating targeted therapies, such as S1P-induced apoptosis (e.g., SK1-I) and inhibition of AKT signaling, as well as for testing tumor growth suppression with chemotherapies like temozolomide or cisplatin. For basic study design, LN-229 cells are cultured to an exponential growth phase and collected for inoculation, with cell viability confirmed via trypan blue exclusion, ensuring a minimum of 99% viability.

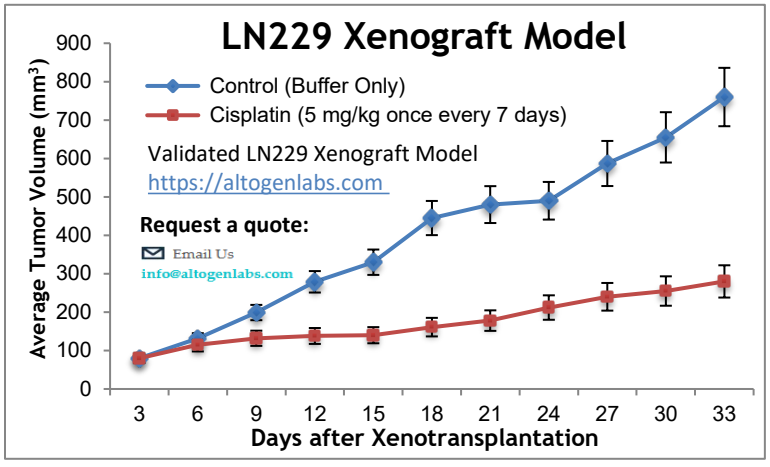

Figure 2. LN229 brain cancer xenografted in immunocompromised mice, mean values +/- SEM (Altogen Labs).

The cell suspension is adjusted so that 100 µL of a 50% Matrigel solution with the LN229 cell suspension contains one million viable cells. The cells are then inoculated subcutaneously (s.c.) into the hind leg of each mouse. The mice used for xenotransplantation are of the NOD.CB17-Prkdc or athymic BALB/C strain, aged 9-11 weeks at the time of inoculation. The cell suspension is then adjusted to a concentration that allows for the inoculation of one million cells in 100 µL of a 50% Matrigel solution per mouse. The xenotransplantation is conducted in NOD.CB17-Prkdc or athymic BALB/C mice, aged 9-11 weeks. Tumors are monitored with digital calipers, with an initial tumor size of 80-120 mm³ required to begin the study. Mice are randomized into cohorts, and test compounds are administered according to the specified schedule. Tumor growth, as well as mouse weight, are recorded until the tumors reach predetermined size limits. Upon necropsy, tumors are excised, weighed, and digital images are captured. Tissues can be collected for downstream analysis, including RNA-later preservation, snap freezing, nucleic acid isolation, or histological processing.

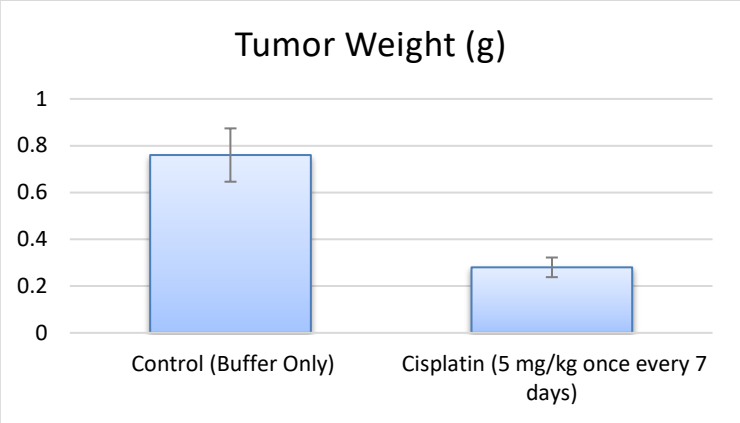

Figure 3. Tumor weight of LN229 cells in control, buffer only mice and cisplatin treated mice at end of the study (Altogen Labs).

Understanding Tumor Microenvironment in Glioblastoma Growth

Glioblastoma multiforme (GBM) is an aggressive brain cancer known for its invasive growth into surrounding brain tissue. The LN229 glioblastoma cell line is a valuable model for studying this invasive behavior, as it more closely mimics human GBM than other commonly used cell lines like U87. Unlike U87 cells, which form well-defined tumor margins, LN229 cells exhibit diffuse growth and migrate extensively along blood vessels, demonstrating their high invasive potential. This migration is influenced by interactions with the tumor microenvironment, particularly through the CXCR4-STAT3 signaling axis. LN229 cells respond strongly to CXCL12, a chemokine secreted by brain endothelial cells, which enhances their infiltration into brain tissue. Moreover, LN229 tumors recruit and polarize microglia and macrophages into an immunosuppressive M2-like state, supporting further tumor progression. These findings highlight LN229 as a crucial part for studying glioblastoma invasion and potential therapeutic targets that could disrupt tumor spread.

A Natural Compound's Potential in Glioblastoma Treatment

Glioblastoma is an aggressive brain tumor with limited treatment options, making the search for new therapies essential. One promising approach involves Prodigiosin, a natural compound known for its ability to disrupt cancer cell growth. This compound works by interfering with a key signaling pathway that cancer cells use to survive and multiply. Specifically, it reduces the levels of KIAA1524, a protein that normally blocks an enzyme called PP2A. When PP2A is reactivated, it stops the activation of Akt, a molecule that promotes tumor growth. As a result, cancer cells experience increased levels of tumor-suppressing proteins like p53 and p21, leading to cell cycle arrest and halted proliferation. In laboratory models, Prodigiosin has shown significant effects in reducing glioblastoma tumor size and preventing further spread. Additionally, it disrupts a transcription factor called ELK1, further limiting the expression of KIAA1524. By targeting these critical pathways, Prodigiosin offers a potential new strategy for combating glioblastoma and improving patient outcomes.

Evaluating Therapeutic Efficacy with the Subcutaneous LN229 Glioblastoma Model

The subcutaneous LN229 model is a commonly used xenograft model for studying glioblastoma, where LN229 cells are implanted beneath the skin of immunodeficient mice. This model offers a straightforward method for tumor growth monitoring, as tumors can be easily palpated and measured externally. While it does not fully replicate the tumor microenvironment of the brain, the subcutaneous LN229 model is valuable for initial screening of potential therapeutic agents and for studying basic tumor biology. The tumors grow in a more accessible location, allowing for consistent monitoring of tumor size and treatment responses. Researchers use this model to evaluate the efficacy of various therapies, including chemotherapy, targeted treatments, and immunotherapies, by measuring tumor growth inhibition or regression. The subcutaneous LN229 model can also be used to assess systemic effects, including toxicity, and to track survival and overall health of the animals during treatment.

Investigating Glioblastoma Therapy with the Orthotopic LN229 Model

The orthotopic LN229 model is a widely used *in vivo* system for studying glioblastoma, where LN229 cells are implanted directly into the brain, mimicking the natural tumor environment. This model allows researchers to observe tumor growth, invasiveness, and interactions with the surrounding brain tissue, providing valuable insights into glioblastoma's biology and pathology. By implanting the cells into the brain, the orthotopic model preserves the complex tumor microenvironment, including blood-brain barrier interactions and immune responses. The model is often utilized to evaluate the efficacy of novel therapeutic agents, such as targeted therapies and immunotherapies, in a setting that closely resembles clinical conditions. Additionally, the orthotopic LN229 model is employed to study tumor progression, including invasion into neighboring tissues and resistance to treatment, which is a key challenge in glioblastoma therapy. Researchers can also assess the effects of genetic modifications, such as overexpression or silencing of specific genes, to investigate the molecular drivers of glioblastoma progression. This model has proven to be a powerful tool for preclinical testing and the development of new therapeutic strategies.

Understanding Glioblastoma Metastasis with the LN229 Model

The metastatic LN229 model is an advanced preclinical tool used to study the spread of glioblastoma beyond its primary site. In this model, LN229 cells are typically injected into the bloodstream, often via the tail vein, to investigate the potential for metastatic dissemination to distant organs. Unlike primary brain tumors, metastatic models provide insights into how glioblastoma cells invade other tissues, including the lungs, liver, and bones, mimicking the complex biology of metastatic spread in human disease. Researchers utilize this model to understand the molecular mechanisms underlying glioblastoma metastasis, including the role of tumor microenvironment interactions and cell adhesion processes. The metastatic LN229 model is also instrumental in evaluating the effectiveness of novel therapies aimed at preventing or treating metastasis. By tracking tumor growth in distant organs, researchers can assess the therapeutic potential of drugs that target metastatic pathways. This model allows for a more comprehensive understanding of glioblastoma progression, providing a critical platform for evaluating combination therapies that target both primary and metastatic tumors.

Case Study: Biguanides as Potential Therapeutic Agents for LN229 Glioma Cells

Glioblastoma is an aggressive brain cancer with poor treatment outcomes, making it essential to research and explore novel therapeutic approaches. A study by Wang Y, *et al*., published by *OncoTargets and Therapy* journal, investigated the effects of biguanides, specifically phenformin (Phen) and metformin (Met), on the LN229 glioma cell line. Both drugs significantly inhibited LN229 cell proliferation, induced cell cycle arrest, and promoted cell death through mitochondrial reactive oxygen species (ROS) imbalance. *In vitro* experiments demonstrated that Phen and Met reduced LN229 migration and colony formation, likely through changes in E-cadherin and vimentin expression. *In vivo*, treatment with Phen and Met effectively suppressed LN229 tumor growth and metastasis in a mouse xenograft model. The study also revealed that the antitumor effects of these drugs were ROS-dependent, as the ROS inhibitor NAC successfully rescued LN229 cells from death. Interestingly, while both drugs activated AMPK, an AMPK inhibitor did not reverse their cytotoxic effects, suggesting an AMPK-independent mechanism of action. These findings highlight Phen and Met as promising adjunct therapies for glioblastoma treatment, particularly for targeting highly invasive LN229 cells.

Targeting Acid Adaptation in LN229 Glioblastoma Cells

Glioblastoma is an aggressive brain cancer with complex cellular adaptations that support tumor survival and progression. The LN229 glioblastoma cell line has been instrumental in studying how extracellular acidity influences tumor behavior. Changes in proton concentrations in the tumor microenvironment affect the structure of surface lipids, such as cholesterol and GM3 glycosphingolipid, which in turn regulate cell survival, migration, and proliferation. At lower pH levels, LN229 cells undergo significant lipid remodeling, forming protective clusters that enhance resistance to harsh conditions. These adaptations promote oncogenic survival by preventing membrane degradation and supporting metabolic flexibility. Additionally, targeting key surface lipids in LN229 cells, such as GM3, with specific antibodies can mimic low pH conditions and induce differentiation or programmed cell death, providing a potential therapeutic strategy. The study of LN229 oncogenes in these acidic environments offers valuable insight into how glioblastoma cells evade treatment and continue to grow. Understanding these mechanisms could lead to new approaches that exploit tumor vulnerabilities and improve glioblastoma therapies.

The LN229 cell line, derived from a human glioblastoma, is frequently used in preclinical research to study glioblastoma biology and to evaluate new treatments through the LN-229 xenograft model. This model plays a crucial role in determining the safety and effectiveness of potential therapies before clinical trials in humans. A xenograft involves transplanting cells or tissue from one species into another, and in the case of glioblastoma, this typically involves injecting human-derived tumor cells into immunodeficient animal models. Xenotransplantation is widely used to assess drug efficacy against specific cancer types and to model tumor behaviors in a controlled, reproducible setting. These studies are complex, requiring careful selection of animal models, tumorigenic cell lines, dosing regimens, and detailed analyses of tumor growth, histology, and molecular expression markers (mRNA and protein levels). The LN229 xenograft model enables researchers to study how glioblastoma cells interact with their microenvironment, evaluate tumor progression, and assess therapeutic interventions in a setting that mirrors human disease progression.

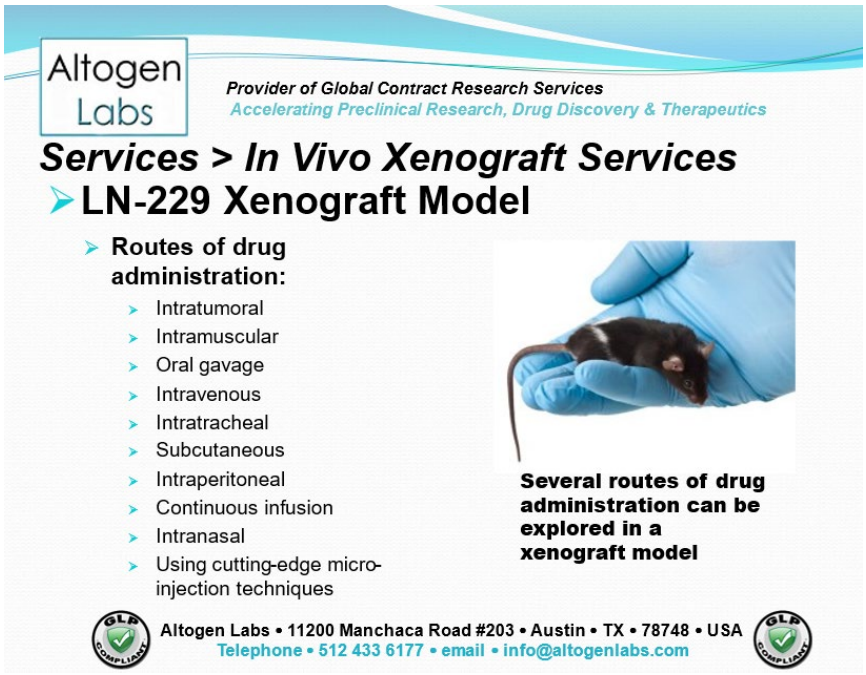

Figure 4. Available *in vivo* xenograft services at Altogen Labs for LN229 (Altogen Labs).

At Altogen Labs, we offer a range of services utilizing over 90 standard Cell Line Derived Xenograft (CDX) models and 30+ PDX models, providing an expansive platform for researchers to test new therapies. Our xenograft models allow researchers to explore the role of proteins or gene products in tumor growth regulation through the use of genetically engineered cell lines that overexpress proteins or exhibit long-term gene silencing via RNAi. We also provide quantitative gene expression analysis (qPCR) and protein expression analysis using the WES system (ProteinSimple), allowing for in-depth molecular insights into tumor behavior. Animal handling at Altogen Labs is conducted according to IACUC-regulated and GLP-compliant procedures, ensuring that all studies meet the highest ethical and scientific standards. Tumor studies are initiated when the tumor size reaches a specified volume (50-100 mm^3), and the animals are monitored closely for tumor development and clinical signs. In addition to tumor growth studies, services include histology, protein/RNA isolation, gene expression analysis, and imaging studies, as well as toxicity assessments and necropsies. Flexible dosing schedules, a variety of dosing routes, and alternative engraftment sites such as orthotopic transplantation and tail vein injection further enhance the ability to investigate glioblastoma therapies. Our comprehensive suite of services ensures that researchers have the necessary tools to evaluate both primary and metastatic tumor behavior, making the LN229 xenograft model an invaluable resource for preclinical glioblastoma research.

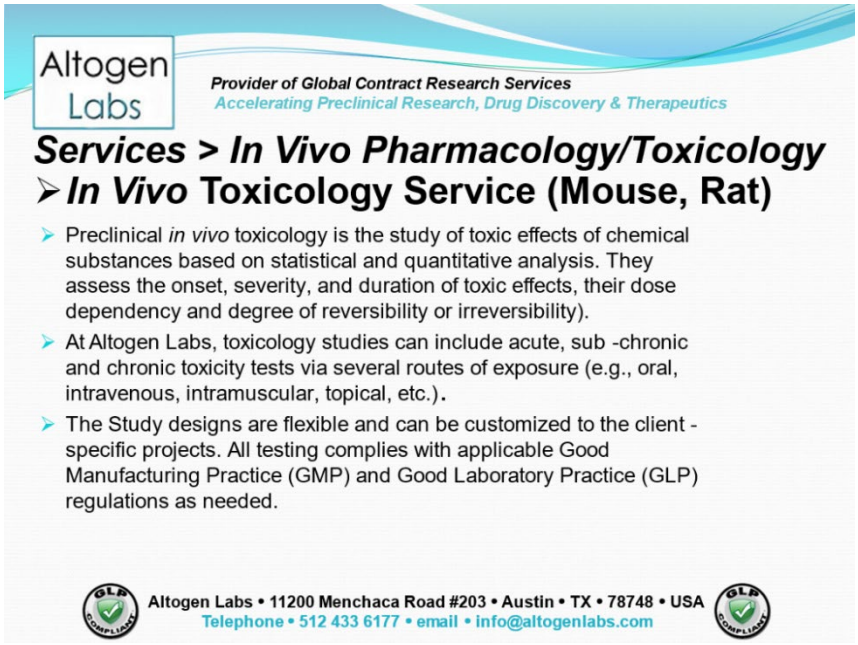

Figure 5. *In vivo* toxicology services provided by Altogen Labs (Altogen Labs).

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Keywords: LN229, brain cancer, xenograft, brain, *in vivo*, cancer, preclinical, research, *in vivo* pharmacology, orthotopic, metastatic, PDX, CDX

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/

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