Validated LL/2 Allograft Model:
Subcutaneous And Orthotopic Allograft Tumor Model

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Syngeneic Models and Allografts in Lung Cancer Research

Lung cancer is the leading cause of cancer-related mortality worldwide, characterized by its aggressive nature, high metastatic potential, and resistance to therapy. Preclinical cancer research relies heavily on murine models to investigate lung cancer biology, evaluate novel therapeutic strategies, and study the tumor microenvironment. Specifically, syngeneic mouse models offer a physiologically relevant system for studying tumor progression and immune interactions. In addition to the utility of murine models; allograft models, where tumor cells from one genetically similar mouse are transplanted into another, offer valuable insights into cancer progression and treatment responses. These models allow researchers to investigate various aspects of tumor biology, such as immune response, tumor growth dynamics, and the impact of specific therapies, while maintaining the genetic background of the host. Allografts are particularly useful for studying the interactions between tumor cells and the host immune system, making them an essential tool for evaluating immunotherapies and other treatment strategies.

LL/2 Cell Line

The LL/2 (Lewis lung carcinoma 2) cell line is a cancer model derived from a spontaneous lung tumor in a mouse of the C57BL/6J strain, which is highly susceptible to lung tumors. These cells exhibit key characteristics of lung cancer, including rapid proliferation, invasive behavior, and metastatic potential, making them a valuable tool for studying tumor progression. Additionally, LL/2 cells also express lung cancer markers such as carcinoembryonic antigen (CEA) and cytokeratin, allowing them to be valuable and useful models. They are widely used in cancer research, particularly in the study of immunotherapy, tumor microenvironment interactions, and metastasis. LL/2 cells are also able to respond to various cytokines and chemokines, allowing researchers to investigate the effects of these signaling molecules on tumor growth and survival. The well-documented metastatic capacity of LL/2 cells mean that they are frequently used to evaluate the efficacy of chemotherapeutic agents and thus, has contributed to our understanding of lung cancer biology and is a cornerstone in preclinical research.

Altogen Labs Validated LL/2 Allograft Model

At Altogen Labs, the preclinical allograft study of LL/2 begins by maintaining all tissue culture flasks aseptically to support the exponential growth of LL/2 cells. The cells are collected using trypsin-EDTA, and their viability is assessed through trypan blue exclusion. A suspension of 1 x 10^6 LL/2 cells in 100 µL of Matrigel is subcutaneously injected into C57BL/6 mice (10-12 weeks old). Tumor development at the injection site is closely monitored using digital calipers until the tumors reach a size of 50-150 mm^3. Test compounds are administered according to the established dosing schedule, and tumor measurements are recorded daily. Mouse body weights are taken three times a week. The in-life portion of the study ends once the maximum tumor size limit is reached. A necropsy is then performed as specified in the study design, and tumors are excised, weighed, and imaged. At the client's request, tissues may be fixed in 10% NBF, snap-frozen, or preserved in RNAlater for further analysis.

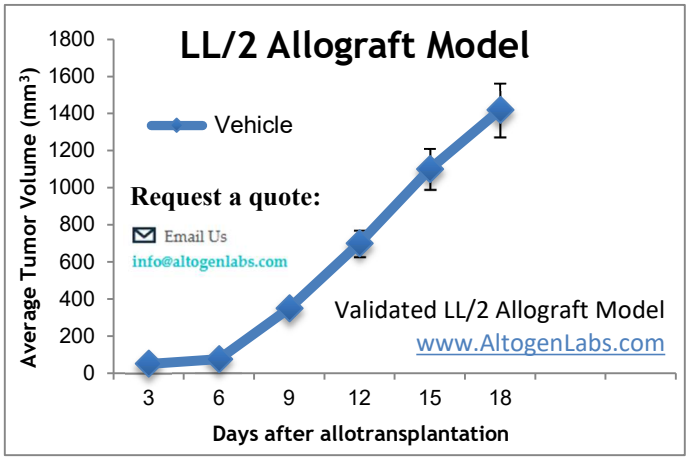

Figure 1. LL/2 tumor growth after allografted into immunocompromised mice, mean values +/- SEM.

Preclinical Applications of Subcutaneous LL/2 Lung Cancer Allograft Models

Subcutaneous LL/2 models involve the implantation of cells under the skin of immunocompromised or syngeneic mice, creating a simplified system to study tumor growth and therapeutic interventions. These models allow for easy monitoring

of tumor size and provide a controlled environment for evaluating drug efficacy, immune responses, and tumor biology. Additionally, the subcutaneous location of the tumor facilitates repeated sampling, such as biopsies, and non-invasive imaging, making it ideal for preclinical testing of new treatments. While they are useful for studying tumor growth and response to therapy, subcutaneous models do not fully replicate the complex interactions of tumor cells with their native organ microenvironment, limiting their use for studying metastasis and tissue-specific responses, but are still important to cancer research and are widely employed due to their simplicity and reproducibility in experimental settings.

LL/2 Orthotopic Model for Lung Cancer Progression and Metastasis

Implanting LL/2 tumor cells into the lungs of immunocompromised mice allows for interactions between the tumor and the surrounding lung stroma, which affects tumor growth, differentiation, and drug sensitivity. From this, LL/2 cells can metastasize to distant organs, with patterns of spread resembling those seen in human lung cancer. This makes the LL/2 orthotopic model particularly valuable for studying lung cancer progression and evaluating potential therapeutic agents in a more biologically relevant context. However, the metastasis in LL/2 models is often highly heterogeneous, with variability in the spread and development of secondary tumors

Case Study: Chemotherapy in LL/2 Lung Cancer Models

In a study conducted by Cheng, *et al.*, published by *Molecular Cancer* journal, researchers explored the role of the CXCR2 protein expression in lung cancer progression and therapy, emphasizing the function of LL/2 (Lewis lung carcinoma) cells as a key model system. LL/2 cells were used in both *in vitro* and *in vivo* models to investigate how CXCR2 influences tumor growth, neutrophil infiltration, and response to chemotherapy. The results demonstrated that CXCR2 is highly expressed in lung cancer cells, including LL/2, and promotes tumor cell proliferation, resistance to apoptosis, and epithelial-to-mesenchymal transition (EMT) through the p38/ERK MAPK signaling pathway. In mouse models, CXCR2 inhibition using SB225002 significantly reduced tumor-associated neutrophil (TAN) infiltration, suppressed tumor growth, and enhanced CD8+ T cell activation. Moreover, LL/2 cells were used to show how CXCR2 expression is upregulated following cisplatin treatment, suggesting a potential role in chemoresistance. The combination of CXCR2 inhibition with cisplatin exhibited synergistic anti-tumor effects, indicating that targeting CXCR2 in LL/2-driven lung cancer models could improve therapeutic efficacy.

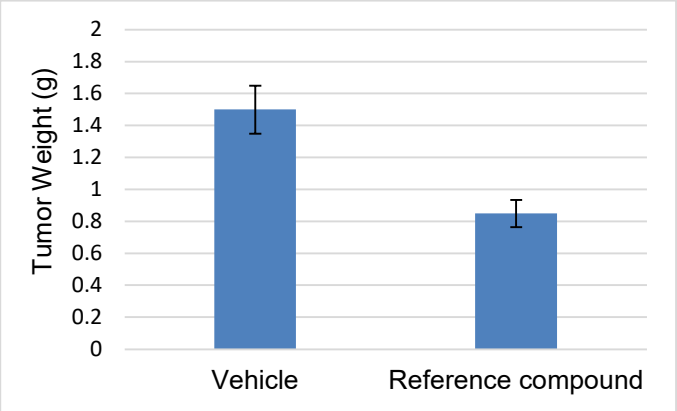

Figure 2. Treatment with reference compound, SB225002 (10 mg/kg) resulted in a reduction of the size of the LL/2 allograft tumors.

Tumorigenicity and Metastasis of LL/2 Cells in Mice

LL/2 cells are known for their high tumorigenicity, meaning they can readily form tumors when implanted into either immunocompromised or syngeneic C57BL/6 mice. While these tumors can metastasize, the cells exhibit relatively low metastatic potential in regular mice, with metastatic spread being limited compared to more aggressive cancer cell lines. Additionally, they have also been described as weakly responsive to checkpoint blockade immunotherapy, making them less effective for studying the full potential of immune checkpoint inhibitors in promoting anti-tumor immunity. These limitations suggests that additional therapies or models might be necessary to explore immune checkpoint therapy more comprehensively. Regardless, LL/2 cells are commonly used in preclinical cancer research, especially in the context of tumor growth and immunotherapy evaluation.

Genomic Insights into LL/2 Model Highlight Its Relevance for Cancer Therapy

Whole-exome sequencing of LL/2 reveals it as a hypermutated KRAS/NRAS-mutant cancer with extensive regional mutation clusters caused by chromosomal instability and frequent structural rearrangements. The LL/2 genome harbors over 20,000 somatic mutations, including 33 deleterious mutations in key cancer genes such as KRAS, NRAS, and Trp53, with biallelic deletions in Cdkn2a and Cdkn2b. This mutation profile suggests LL/2 shares molecular similarities with human lung adenocarcinoma, making it a valuable model for studying lung cancer progression and response to therapy. Additionally, LL/2 is known for its aggressive growth, high vascularization, and metastatic potential, particularly to the

lungs, lymph nodes, and liver. The model's syngeneic nature allows for immune-competent studies, enabling the evaluation of immune responses and targeted therapies.

The LL/2 allograft model offers several options for studying tumor growth and response to treatment. At Altogen Labs, these include assessments of LL/2 tumor growth delay (TGD; latency) and tumor growth inhibition (TGI). Dosing can be administered through various routes, such as intravenous, intratracheal, continuous infusion, intraperitoneal, intratumoral, oral gavage, topical, intramuscular, subcutaneous, or intranasal injection, with advanced techniques like micro-injection and pump-controlled IV injection available. Additionally, tumor immunohistochemistry can be performed to analyze LL/2 tumor characteristics. Alternative cell engraftment sites, such as orthotopic transplantation, tail vein injection, left ventricular injection for metastasis studies, and injection into the mammary fat pad or peritoneum, are also possible. Blood chemistry analysis and optional toxicity studies, including broad health observation programs, are available. Further evaluations include gross necropsies, histopathology, and imaging studies using fluorescence-based whole-body imaging. A positive control group can be included with cyclophosphamide at a dosage of 30-50 mg/kg, and lipid distribution and metabolic assays can also be performed.

Patient-Derived Tumor Organoids in Advancing Cancer Drug Testing

Organoids are three-dimensional, *in vitro* cultures derived from patient tumor samples that replicate key features of the original tumor, including genetic and phenotypic heterogeneity. Unlike traditional 2D cell cultures, organoids maintain complex tissue architecture and can be expanded efficiently from primary patient material, making them valuable tools for personalized cancer research and drug screening. Compared to xenograft and allograft models, organoids offer a faster and more scalable platform for testing therapeutic responses, though they lack interactions with tumor-associated stroma and immune cells. Advances in organoid technology have enabled the development of patient-derived tumor organoid (PDTO) biobanks, which serve as living repositories for studying cancer progression and resistance mechanisms. Organoid models have been particularly useful in high-throughput drug screening, allowing researchers to identify potential treatments based on individual tumor characteristics.

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LL/2 Xenograft Model. https://altogenlabs.com/xenograft-models/lung-cancer-xenograft/ll2-xenograft-model/

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