Validated 4T1 Allograft Model:

Subcutaneous, Orthotopic, And Metastatic Allograft Tumor Model

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

Understanding Breast Cancer with Allograft Models

Breast cancer is one of the most prevalent and aggressive forms of cancer globally, with significant impact on public health. It encompasses a heterogeneous group of tumors, characterized by variations in gene expression, metastatic potential, and response to treatment. While early-stage breast cancer is often treatable, metastatic breast cancer remains a major challenge due to its complexity and resistance to current therapies. Animal models, specifically allografts, are typically utilized for studying breast cancer biology, tumor progression, and therapeutic efficacy. Allograft models involve the transplantation of tumor tissue or cells from a donor animal into a genetically identical recipient, preserving the tumor's biological characteristics. These models allow for the study of metastasis, immune response, and drug resistance in a more physiologically relevant setting compared to *in vitro* systems. Allografts also provide an opportunity to evaluate novel therapies, including targeted treatments, immunotherapies, and combination therapies. By simulating the human disease in a controlled environment, these models offer invaluable insights into the complexities of breast cancer and facilitate the development of effective treatment strategies. In particular, allografts derived from breast cancer cells or tissues enable the testing of new drugs in a manner that closely reflects patient outcomes.

4T1 Cell Line

The 4T1 cell line is a highly tumorigenic epithelial cell line derived from a mammary gland tumor of a BALB/c mouse. It is known for its resistance to 6-thioguanine without requiring mutagen treatment, which makes it an intriguing model for studying resistance mechanisms. The 4T1 cell line exhibits robust tumor growth and metastatic spread, with patterns that closely mimic human stage IV breast cancer. Due to its aggressive nature, the 4T1 model is frequently used to study the biology of tumor progression and metastasis. In both post-operative and nonsurgical settings, the 4T1-induced tumors demonstrate spontaneous metastasis with similar kinetics, offering a reliable platform for preclinical testing of anti-cancer therapies. Additionally, the cell line's ability to spread to distant organs, including the lungs and liver, makes it ideal for research on tumor-host interactions, immune responses, and potential therapeutic interventions. The 4T1 model is widely employed in cancer research to evaluate novel drugs, immunotherapies, and treatment regimens, particularly for metastatic breast cancer.

Altogen Labs Validated 4T1 Allograft Model

At Altogen Labs, in preclinical studies, 4T1 cells are initially maintained under optimal conditions of exponential growth to ensure a sufficient number of viable cells for injection. The cells are carefully prepared for injection through trypsinization, followed by a viability check using trypan blue exclusion, which confirms that a minimum of 98% of the cells are viable. Once prepared, the cell suspension is adjusted to the appropriate density for injection. Each NOD/SCID mouse (10-12 weeks old) is then subcutaneously injected with one million viable cells in a 100-microliter volume of Matrigel-4T1 cell suspension, targeting the flank of the hind leg. After injection, the tumor injection sites are palpated three times a week to monitor the development and progression of the tumors. Tumors are measured using digital calipers to monitor growth and are tracked until they reach an average size of 50-150 mm³, at which point further analysis and treatments can proceed.

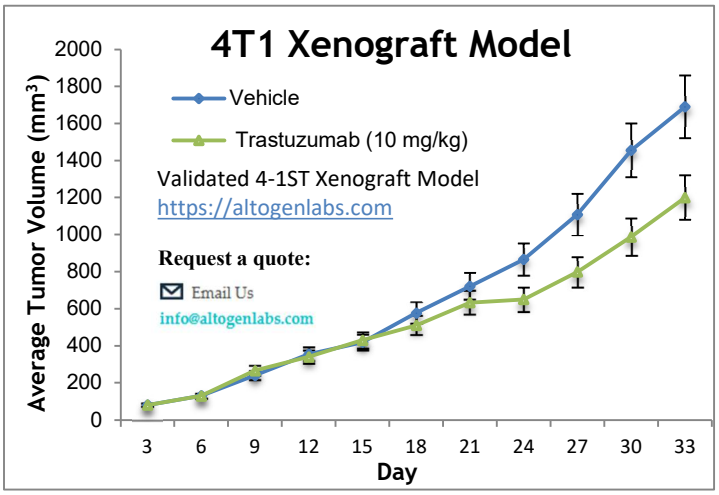

Figure 1. 4T1 breast cancer allografted in immunocompromised mice, mean values +/- SEM (Altogen Labs).

Once tumors are established, animals are randomized into different treatment cohorts to assess the efficacy of various compounds or treatments. The administration of the experimental compound is carried out according to a specific treatment schedule, and tumors are measured daily to monitor progress. Mouse weights are recorded 2-3 times weekly to assess overall health and any potential side effects from the treatments. Mice are euthanized when the tumors reach a size of 2,000 mm³ or exceed the predetermined size limit outlined by the approved IACUC protocol. After euthanasia, a necropsy is performed, and tissues are harvested for further analysis, including the excision and weighing of the tumors. Tumor growth and response to treatment are documented using digital imaging. Additionally, tissues are collected for downstream analysis, with options for snap freezing in liquid nitrogen, stabilization in RNAlater reagent, or histological preparation, allowing for a comprehensive evaluation of treatment effects on tumor biology and surrounding tissue.

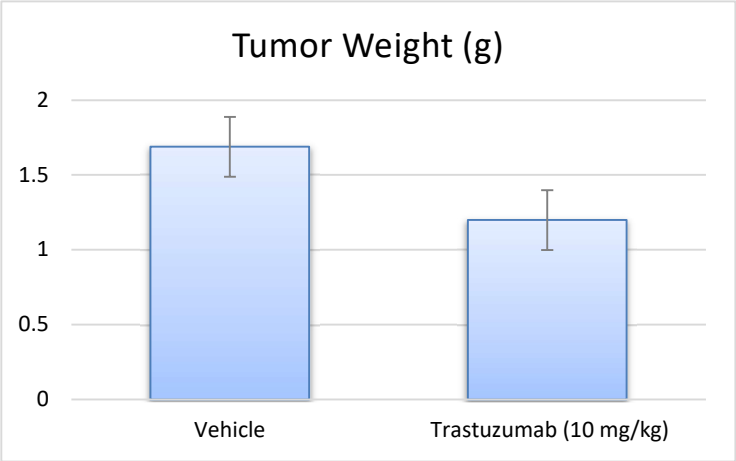

Figure 2. Tumor weight of 4T1 cells in control, buffer only mice and trastuzumab treated mice at end of the study (Altogen Labs).

Chemotherapy: Harnessing 4T1 Tumors to Evaluate Immune Checkpoint Therapy

The 4T1 murine breast cancer model plays a pivotal role in evaluating a novel hydrogel-based immunotherapy, PEIGel, designed for intratumoral drug delivery. This hydrogel integrates polyethylenimine (PEI), a polymer with inherent immune-stimulating properties, and magnesium ions to enhance immune response modulation. Research has shown that in 4T1 tumors, PEIGel demonstrated the ability to convert an immunosuppressive tumor microenvironment into an immune-responsive state by increasing PD-L1 expression and promoting M1 macrophage polarization. Encapsulation of anti-PD-L1 antibodies within PEIGel enabled prolonged release, significantly enhancing immune checkpoint blockade (ICB) therapy against 4T1 tumors. Notably, *in vivo* studies showed that PEIGel administration reduced both primary tumor growth and distant metastases while also preventing tumor relapse post-surgery. The study suggests that PEIGel not only acts as a drug carrier but also actively modulates tumor metabolism and immune response, presenting a promising strategy for localized cancer immunotherapy.

The Impact of Obesity on 4T1 Oncogenic Pathways and Immune Evasion

The 4T1 murine breast cancer model demonstrates a strong oncogenic profile that is significantly influenced by obesity, leading to enhanced tumor growth and metastasis. Research has found that high-fat diet-induced obesity promotes metabolic dysfunction, increasing levels of leptin and inflammatory cytokines that fuel tumor proliferation. 4T1 oncogenes drive aggressive behavior, with increased expression of CXCR4 and CCR9 enhancing tumor cell migration through chemokine gradients. In obese mice, 4T1 cells show a higher capacity for colonizing metastatic niches, particularly within lymph nodes and bone marrow, where immune cell infiltration is compromised. This environment favors immune evasion, as tumor-draining lymph nodes exhibit reduced CD8+ T cell presence and impaired antigen presentation by dendritic cells. Additionally, obesity disrupts bone marrow-derived cytokine signaling, further impairing hematopoiesis and immune responses against tumors. These findings highlight the interplay between metabolic alterations, immune suppression, and oncogene-driven metastatic potential in 4T1 breast cancer cells, offering insights into obesity's role in cancer progression and potential therapeutic targets.

Assessing Tumor Progression in the Subcutaneous 4T1 Model

The subcutaneous 4T1 model is one of the most widely used animal models in breast cancer research, where 4T1 tumor cells are injected into the subcutaneous space, typically on the flank of BALB/c mice. This model is ideal for studying tumor growth and testing therapeutic strategies due to its ease of administration and monitoring. Tumors in the subcutaneous 4T1 model typically grow in a predictable manner, allowing for consistent measurement of tumor size and assessment of treatment efficacy. While the subcutaneous implantation does not fully replicate the local tissue environment of primary mammary tumors, it provides valuable insight into tumor biology, immune response, and drug efficacy. This model also offers the advantage of clear, accessible tumor sites for repeated measurements and interventions. The 4T1 cells in the subcutaneous model have been shown to metastasize to distant organs such as the lungs and liver, although metastasis is less robust compared to orthotopic models. It is commonly used for preclinical testing of anti-cancer drugs, including

chemotherapy, targeted therapies, and immunotherapies, helping to evaluate their effects on tumor growth and progression. Due to its reproducibility and simplicity, the subcutaneous 4T1 model remains a cornerstone in breast cancer research.

Advancements in Preclinical Studies Using the Orthotopic 4T1 Model

The orthotopic 4T1 model involves the injection of 4T1 tumor cells into the mammary fat pad of BALB/c mice, replicating the natural site of tumor development in humans. This model closely mimics the biological characteristics of human breast cancer, including tumor growth, invasiveness, and metastasis. Tumors in this model grow in the same tissue environment as in human patients, providing a more physiologically relevant context for studying tumor biology and therapeutic responses. The 4T1 cells used in the orthotopic model exhibit spontaneous metastasis, particularly to the lungs, liver, and bones, making it an effective model for studying metastatic progression. Researchers can monitor tumor growth and metastasis in real-time using imaging techniques such as bioluminescence or magnetic resonance imaging (MRI). Additionally, this model is widely used to evaluate the efficacy of novel therapies, including chemotherapy, targeted treatments, and immunotherapies, offering insights into how these treatments might perform in clinical settings. The orthotopic 4T1 model provides researchers insight when studying the complex interactions between tumors and their microenvironment, providing a platform for preclinical testing of new breast cancer therapies.

Understanding Metastatic Progression with the 4T1 Model

The metastatic 4T1 model is a highly effective system for studying the progression of breast cancer metastasis, as 4T1 cells are known to spontaneously spread to distant organs such as the lungs, liver, and bones. In this model, 4T1 cells are typically implanted either orthotopically or subcutaneously in BALB/c mice, and the resulting tumors closely mimic the metastatic behavior seen in human breast cancer. The model is valuable for understanding the molecular mechanisms driving metastasis, as it allows researchers to study tumor cell invasion, colonization of distant tissues, and interactions with the host immune system. By monitoring the spread of the tumor cells, either through imaging technologies like bioluminescence or through histological examination, researchers can assess the efficacy of novel anti-metastatic therapies. The metastatic 4T1 model is widely used to evaluate drugs that aim to block metastasis, such as chemotherapeutic agents, targeted therapies, and immunotherapies. It has been employed to test treatments that target both primary tumor growth and secondary tumor spread, offering a comprehensive approach to studying therapeutic interventions. Given its reproducibility and relevance to human disease, the metastatic 4T1 model is crucial in advancing our understanding of metastatic breast cancer and for developing more effective treatments.

Case Study: Harnessing 4T1 Cells to Explore Oncolytic Virus-Based Breast Cancer Therapy

A study conducted by Deng H, *et al*, published by *Frontiers in Microbiology* journal, investigates the antitumor potential of the Orf virus (ORFV) in combination with a PAK4 inhibitor in breast cancer, using the murine 4T1 cell model. 4T1 cells, known for their aggressive and metastatic properties, were utilized to evaluate ORFV's oncolytic effects and its impact on the tumor microenvironment. ORFV demonstrated direct tumor-killing effects by inducing G2/M cell cycle arrest and apoptosis, while also stimulating an immune response in the tumor-bearing host. *In vivo* studies using 4T1 tumor-bearing BALB/c mice showed significant tumor growth suppression following intratumoral injection of ORFV. Further screening with a CRISPR-Cas9 kinase knockout library identified PAK4 as a key modulator of ORFV's efficacy. The study found that co-treatment with the PAK4 inhibitor PF-3758309 enhanced ORFV's tumor-suppressive effects by inhibiting cell proliferation and migration. Immunological assessments revealed that ORFV altered the tumor microenvironment by upregulating chemokines and activating T-cell responses, further contributing to its antitumor effects. These findings suggest that combining ORFV with targeted kinase inhibition presents a promising therapeutic strategy for aggressive breast cancers, with 4T1 serving as a valuable preclinical model for evaluating viral-based cancer therapies.

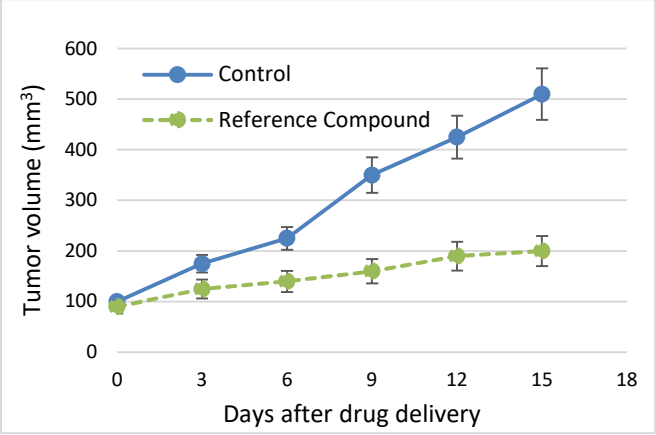

Figure 3. 4T1 tumor growth was suppressed when treated with the reference compound (10^7 pfu).

Oncogenes in 4T1 Tumors

The 4T1 murine breast cancer model harbors a distinct oncogenic landscape that mirrors human triple-negative breast cancer (TNBC). Key oncogenic mutations in Trp53 and Pik3g drive tumor growth and survival, while the absence of mutations in Brca1 and Brca2 differentiates it from hereditary breast cancer models. Highly expressed markers such as Top2a, Birc5, and Mki67 indicate robust proliferative potential, whereas elevated levels of Msln, Ect2, and Plk1 highlight its aggressive metastatic behavior. The integration of the murine mammary tumor virus (MMTV) further influences oncogene expression, particularly through Fgfr2, which is implicated in cancer cell survival and progression. Additionally, 4T1 cells exhibit mutations in Nav3, Cenpf, Muc5Ac, and Gas1, genes linked to tumorigenesis and immune evasion. With a high expression of Gpa33 and Epcam, traditionally associated with gastrointestinal malignancies, 4T1 tumors demonstrate a unique antigenic profile. This comprehensive oncogenic landscape makes 4T1 a powerful model for studying metastatic breast cancer and testing novel therapeutic interventions.

Investigating Immunotherapies Using Syngeneic Mouse Models

Syngeneic mouse models are essential in cancer research, particularly for studying the complex interactions between tumors and the immune system. These models involve implanting tumor cells into genetically identical, immunocompetent mice, ensuring that the host immune system remains fully functional and capable of mounting a natural response to tumor growth. By preserving immune system integrity, syngeneic models allow researchers to evaluate the efficacy of immunotherapies in a biologically relevant environment. These models are widely used to explore tumor immune evasion mechanisms, identify novel therapeutic targets, and assess immune checkpoint inhibitors, cancer vaccines, and other immunomodulatory treatments. Additionally, they provide valuable insights into metastatic progression, the tumor microenvironment, and the influence of various treatments on immune activation. The ability to study immune responses within an intact host system makes syngeneic mouse models highly relevant for preclinical immunotherapy research, helping to bridge the gap between *in vitro* studies and clinical applications in cancer treatment.

Altogen Labs offers a comprehensive range of laboratory services to support preclinical oncology research, including an extensive collection of over 30 standard Cell Line Derived Xenograft (CDX) models and more than 20 Patient-Derived Xenograft (PDX) models. In addition to xenograft models, Altogen Labs specializes in the development of genetically engineered cell lines that facilitate the study of specific proteins and gene products involved in tumor regulation. Researchers can utilize protein overexpression models to investigate oncogene activation and tumor suppressor function, as well as RNA interference (RNAi) cell lines designed for long-term gene silencing studies. These engineered models are valuable for elucidating cancer-related pathways and identifying potential therapeutic targets. Furthermore, Altogen Labs provides advanced gene and protein expression analysis, including quantitative mRNA expression profiling using RT-PCR and protein expression studies through the WES system (ProteinSimple). These analytical techniques enable precise molecular characterization of tumor samples, allowing researchers to better understand cancer progression, drug mechanisms, and biomarker discovery for targeted therapies.

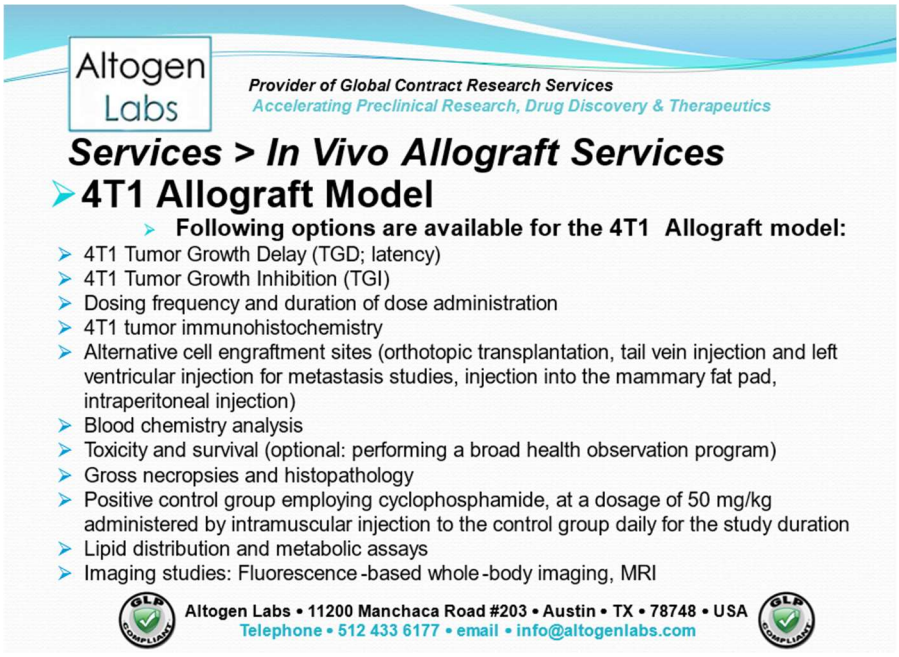

Figure 4. Available *in vivo* xenograft services at Altogen Labs for the 4T1 cell line (Altogen Labs.)

Xenograft studies are complex, requiring careful selection of the appropriate animal model, tumorigenic cell line, and method of administration, as well as dosing and tumor analysis (histology, mRNA, and protein expression). Dosing begins once the mean tumor size reaches a specific volume, with mice dosed once or twice daily for up to 28 days. Altogen Labs ensures that all animal handling and maintenance adhere to IACUC regulations and GLP compliance. A detailed report, including experimental procedures, results, and statistical analysis, is provided to clients, and additional services such as tissue collection, histology, and gene expression analysis are available. For the 4T1 xenograft model, a variety of services, including tumor growth delay, tumor growth inhibition studies, different dosing frequencies and routes, and advanced imaging studies, are available to support comprehensive cancer research.

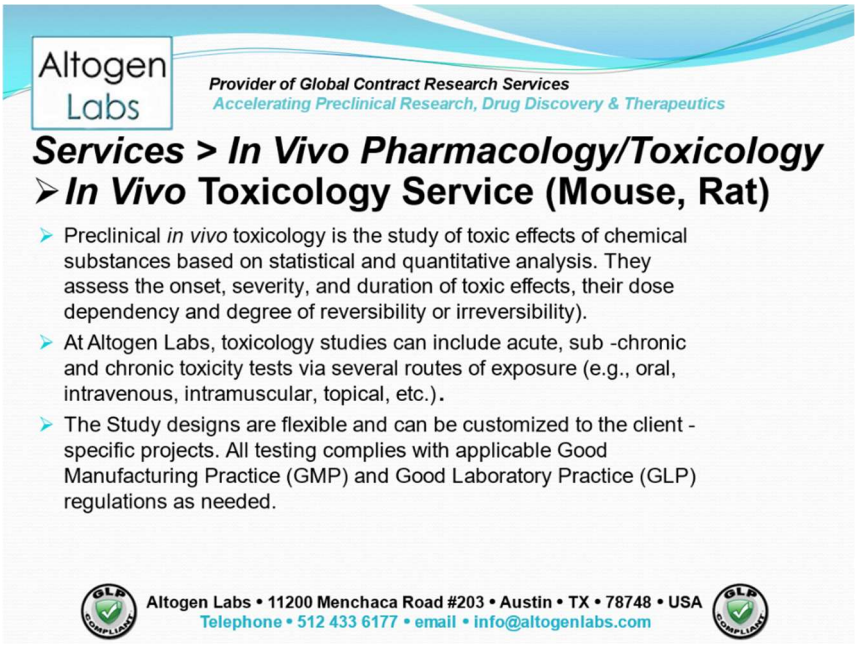

Figure 5. *In vivo* toxicology services available at Altogen Labs (Altogen Labs.)

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Keywords: 4T1, breast cancer, allograft, breast, *in vivo*, cancer, preclinical, research, *in vivo* pharmacology, orthotopic, metastatic, syngeneic

Other Available Altogen Labs Validated Xenograft Models:

BT474 Xenograft Model: https://altogenlabs.com/xenograft-models/breast-cancer-xenograft/bt474-xenograft-model/

Hs578T Xenograft Model: https://altogenlabs.com/xenograft-models/breast-cancer-xenograft/hs578t-xenograft-model/

MCF7 Xenograft Model: https://altogenlabs.com/xenograft-models/breast-cancer-xenograft/mcf7-xenograft-model/