Validated ZR-75-1 Xenograft Model:

Subcutaneous, Orthotopic, And Metastatic Xenograft Tumor Model

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Advancing Breast Cancer Treatments with Xenograft Models

Breast cancer is one of the most common and aggressive malignancies worldwide, with various subtypes exhibiting distinct genetic and phenotypic characteristics. While treatment advancements have improved outcomes for some patients, certain subtypes, such as triple-negative breast cancer (TNBC), continue to present significant challenges due to the lack of targeted therapies. To bridge the gap between preclinical research and clinical application, xenograft models have become a key method for studying breast cancer biology and evaluating therapeutic strategies. These models involve the transplantation of human breast cancer cells or tissues into immunocompromised mice, providing a more accurate representation of tumor growth, metastasis, and drug responses than traditional cell culture systems. Patient-derived xenografts (PDXs) are particularly valuable, as they retain the genetic and histological features of the original patient tumor, offering a more personalized approach to drug testing. Additionally, cell line-derived xenografts (CDXs), provide reproducible models for preclinical drug screening. CDXs are widely used for assessing the efficacy of new therapies and are critical for evaluating drug response in a controlled environment. Xenograft studies, both PDX and CDX, are essential for screening potential anti-cancer therapies, including chemotherapy, targeted treatments, and immunotherapies, and play a critical role in preclinical drug development.

ZR-75-1 Cell Line

The ZR-75-1 cell line was isolated from the mammary gland tissue of a 63-year-old White female patient diagnosed with ductal breast carcinoma. This epithelial cell line is characterized by its ability to grow as adherent monolayers and is widely used in breast cancer research due to its relevance to human disease. ZR-75-1 cells exhibit estrogen receptor (ER) positivity, making them an ideal model for studying hormone-responsive breast cancer. They also express other key markers such as progesterone receptors and HER2, allowing for the investigation of therapies targeting these pathways. The ZR-75-1 cell line provides a reliable *in vitro* platform to explore tumor biology, including cell proliferation, invasion, and response to various treatment modalities. It is frequently utilized for drug screening, including hormonal therapies, targeted therapies, and chemotherapy. Additionally, the ZR-75-1 cell line supports studies of molecular mechanisms involved in breast cancer metastasis and drug resistance. As an established model in cancer research, ZR-75-1 remains crucial for advancing the understanding of breast cancer pathophysiology and treatment.

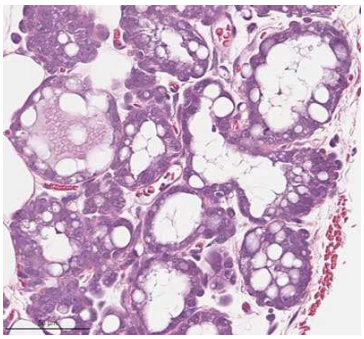

Figure 1. Tumor Histology. H&E stained section of a subcutaneously-implanted ZR-75-1 tumor (Altogen Labs).

Altogen Labs Validated ZR-75-1 Xenograft Model

At Altogen Labs, ZR-75-1 cells are maintained in exponential growth phase under aseptic conditions to ensure optimal cell health. Prior to any procedures, cells are trypsinized, and cell viability is assessed using a trypan blue exclusion assay, with a required viability threshold of 98%. The cell suspension is then adjusted to the appropriate density for injection. For tumor establishment, each mouse is subcutaneously injected into the right flank with $1x10^6$ cells in 100 µL of a Matrigel-ZR-75-1 cell suspension, ensuring a consistent and controlled environment for tumor growth. Injection sites are palpated up to three times a week to monitor tumor development. Tumors are allowed to grow to an average size of 50-150 mm^3, with measurements taken using digital calipers to ensure consistency.

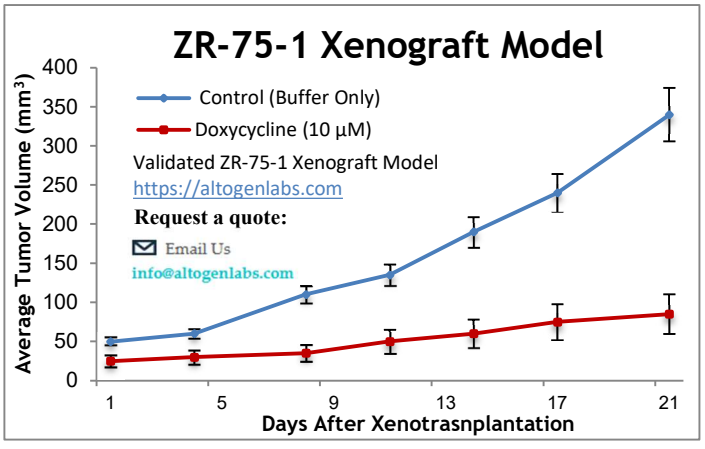

Figure 2. ZR-75-1 breast cancer xenografted in athymic mice, mean values +/- SEM (Altogen Labs).

Once tumors are established, animals are randomized into treatment groups according to the experimental design, with test compounds administered following a pre-established schedule. Mouse body weights are recorded three times a week to monitor general health, while tumor sizes are measured and recorded daily for accurate tracking of tumor growth and response to treatment. The study concludes when tumors reach 2,000 mm^3 or when the predetermined size limit specified in the approved IACUC protocol is met. At the end of the study, a final necropsy is conducted, and tissue collections are performed for downstream analysis, including molecular and histological studies. Tumors are excised, weighed, and documented using digital imaging for precise measurements. Tumor and tissue samples are either stabilized in RNAlater for RNA analysis, snap-frozen in liquid nitrogen (LN2) for protein and genomic analysis, or prepared for histology to examine tumor morphology and response to treatment at a cellular level.

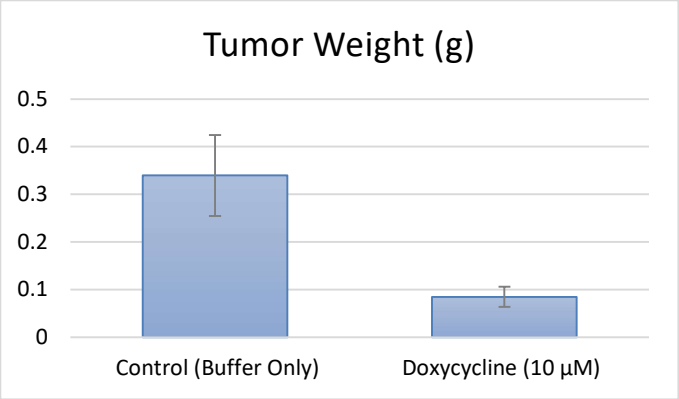

Figure 3. Tumor weight of ZR-75-1 cells in control, buffer only mice and doxycycline treated mice at end of the study (Altogen Labs).

Targeting Glycolysis in Chemotherapy: The Role of DCXR in ZR-75-1 Breast Cancer Cells

The ZR-75-1 breast cancer cell line serves as a crucial model for studying the oncogenic role of dicarbonyl/L-xylulose reductase (DCXR) in tumor progression. DCXR is significantly upregulated in breast cancer tissues and enhances glycolysis, fueling cell proliferation and cell cycle progression. Experimental knockdown of DCXR in ZR-75-1 cells led to suppressed proliferation, reduced glycolytic activity, and cell cycle arrest at the G1 phase, reinforcing its role as a driver of cancer cell metabolism. Conversely, DCXR overexpression in breast cancer cells promoted glycolysis and accelerated the S-phase transition, highlighting its oncogenic potential. Further, *in vivo* xenograft models using ZR-75-1 cells demonstrated that DCXR silencing reduces tumorigenicity, making it a promising therapeutic target. The study also revealed that the glycolysis inhibitor 2-deoxy-D-glucose (2-DG) effectively blocked the pro-tumorigenic effects of DCXR, suggesting a potential metabolic intervention strategy. Understanding the metabolic dependencies of ZR-75-1 cells through DCXR provides new insights into breast cancer treatment strategies focused on disrupting cancer cell energy metabolism.

Exploring Treatment Efficacy with the Subcutaneous ZR-75-1 Model

The subcutaneous ZR-75-1 model is a commonly used preclinical model for studying human breast cancer in a non-invasive setting. In this model, ZR-75-1 cells, derived from a human breast adenocarcinoma, are injected into the flank of immunocompromised mice. This implantation method results in the formation of tumors that can be easily monitored for growth and response to treatments. The subcutaneous location allows for straightforward measurements of tumor size and volume, making it a valuable model for high-throughput drug screening and therapeutic assessments. Researchers often use the subcutaneous ZR-75-1 model to evaluate the effects of chemotherapy, hormone therapies, and targeted treatments. Though less reflective of the tumor microenvironment compared to orthotopic models, this model provides important insights into tumor biology and the potential efficacy of cancer therapies. The subcutaneous ZR-75-1 model is widely recognized for its reproducibility and convenience, making it essential for preclinical breast cancer research.

Studying Metastasis with the ZR-75-1 Metastatic Model

The metastatic ZR-75-1 model is used in nonclinical research for studying the metastatic progression of human breast cancer. In this model, ZR-75-1 cells, derived from a human breast adenocarcinoma, are injected into immunocompromised mice, typically via the tail vein, to simulate hematogenous dissemination of cancer cells. This model allows researchers to track the spread of cancer to distant organs such as the lungs, liver, and bones, closely mimicking the metastatic process observed in human breast cancer patients. The metastatic ZR-75-1 model is widely used to evaluate the effectiveness of anti-metastatic therapies and to understand the molecular mechanisms underlying tumor dissemination. Its ability to assess both primary tumor growth and secondary metastatic lesions makes it invaluable in cancer research. The model also supports studies of tumor-host interactions in distant organs and the development of resistance to therapy. Its reproducibility and ability to simulate a range of metastatic events make the metastatic ZR-75-1 model an essential platform for evaluating therapeutic strategies aimed at reducing metastasis in breast cancer.

Evaluating Novel Therapies Using the ZR-75-1 Orthotopic Breast Cancer Model

The orthotopic ZR-75-1 model is a widely used preclinical model for studying human breast cancer *in vivo*. Derived from a human breast adenocarcinoma, ZR-75-1 cells are implanted into the mammary fat pad of immunocompromised mice to closely replicate the tumor microenvironment seen in patients. This model allows for the evaluation of tumor growth, metastasis, and therapeutic responses in a setting that mirrors the natural progression of breast cancer. Researchers commonly use the orthotopic ZR-75-1 model to assess the efficacy of novel drug candidates, including hormonal therapies, targeted therapies, and combination treatments. The model's ability to mimic human breast cancer characteristics makes it invaluable for preclinical testing and biomarker discovery. Additionally, it serves as a platform for understanding tumor-host interactions, such as angiogenesis and immune response. Its ability to facilitate both localized and metastatic studies provides a comprehensive method for evaluating treatment strategies across different stages of cancer progression.

Case Study: ZR-75-1 Xenografts Reveal PI3K Inhibition as a Key Strategy Against CDK4/6 Therapy Resistance

A study conducted by O'Brien, *et al*., published by *Breast Cancer Research* journal, investigates the mechanisms underlying acquired resistance to CDK4/6 inhibitors in ER+/HER2− breast cancer and explores therapeutic strategies to overcome this challenge. Through the development of preclinical models, the researchers identified that resistance is frequently driven by the loss of Rb or a diminished reliance on Rb signaling, resulting in cross-resistance to CDK4/6 inhibitors. Despite this resistance, PI3K/mTOR signaling remains persistently activated, presenting a viable therapeutic target. Notably, ZR-75-1 xenografts, engineered to develop resistance to alpelisib and fulvestrant, played a pivotal role in demonstrating that PI3K inhibition effectively halts tumor progression, even in CDK4/6-resistant models. Treatment with the p110α-selective PI3K inhibitor, alpelisib, induced robust tumor regression in both PIK3CA-mutant and wild-type ER+/HER2− breast cancers, underscoring the therapeutic potential of PI3K pathway blockade. Furthermore, the addition of CDK4/6 inhibitors to PI3K:ER-targeted therapy successfully reversed resistance in tumors that had progressed on PI3K inhibition alone, highlighting the interdependency of these signaling pathways. These findings provide strong preclinical support for the clinical evaluation of PI3K inhibitors, such as alpelisib, in patients with ER+/HER2− breast cancer who progress on CDK4/6-based therapies. Moreover, the data suggest that upfront triple combination therapy, simultaneously targeting PI3K, CDK4/6, and ER; may serve as a powerful strategy to delay or even prevent the emergence of therapeutic resistance. The study underscores the critical role of ZR-75-1 xenografts in modeling resistance and optimizing next-generation treatment approaches for endocrine-resistant breast cancer.

The Role of ZR-75-1 in Breast Cancer Research Using 3D Models

Nonclinical breast cancer research has greatly benefited from three-dimensional (3D) culture models, including organoids, which closely mimic the tumor microenvironment compared to traditional two-dimensional (2D) models. The ZR-75-1 breast cancer cell line is particularly valuable in these studies, providing insights into luminal-type breast cancer biology. 3D models and organoids allow researchers to study the interactions between tumor cells and the extracellular matrix (ECM), revealing critical transcriptional changes that drive tumor progression, epithelial-to-mesenchymal transition (EMT), and drug resistance. Unlike conventional 2D cultures, organoids derived from ZR-75-1 cells better replicate *in vivo* tumor characteristics, allowing researchers to use them for personalized medicine and drug discovery. These advanced models help identify novel therapeutic targets and improve our understanding of breast cancer heterogeneity, offering new opportunities for developing more effective treatments. By preserving the structural and genetic complexity of patient tumors, organoids provide a more accurate platform for testing targeted therapies and predicting treatment responses. As research continues to refine these models, the combination of ZR-75-1 cells and organoid technology holds great promise for revolutionizing breast cancer treatment and precision medicine.

Autophagy-Driven Cell Death in Breast Cancer

Another study conducted by Dankó T, *et al*., published by *International Journal of Molecular Sciences*, investigates the synergistic effects of rapamycin and doxycycline in breast cancer, with a particular focus on metabolic stress and autophagy-dependent cell death. The combination therapy demonstrated significant anti-tumor activity *in vitro* and *in vivo*, effectively inhibiting proliferation in approximately two-thirds of the tested breast cancer cell lines. The ZR-75-1 luminal B breast cancer model played a pivotal role in elucidating the therapeutic mechanism, exhibiting heightened sensitivity to the dual treatment compared to monotherapies. While short-term exposure to rapamycin and doxycycline produced reversible effects, continuous treatment induced selective autophagy and mitophagy, ultimately driving tumor cell death. Mechanistically, rapamycin inhibited mTOR signaling, while doxycycline disrupted mitochondrial function, collectively impairing cellular energy metabolism. Notably, this process occurred independently of apoptosis or necrosis, distinguishing it from conventional cytotoxic therapies. In ZR-75-1 xenografts, sustained treatment led to pronounced tumor regression,

whereas drug withdrawal resulted in tumor resurgence, underscoring the necessity of prolonged metabolic inhibition. The results further support the therapeutic potential of concurrently targeting mTOR and mitochondrial function to suppress tumor adaptation and resistance. This study provides a compelling rationale for exploring metabolic interventions as a novel approach in breast cancer treatment.

Metabolic Signatures of ZR-75-1 Oncogenes in Tumor Growth

ZR-75-1 is a hormone receptor-positive breast cancer cell line that exhibits a unique metabolic phenotype driven by its oncogenic profile. Unlike highly proliferative triple-negative breast cancer cells, ZR-75-1 cells display a stronger dependence on glycolysis rather than oxidative phosphorylation. This metabolic reliance suggests that oncogenic signaling within these cells prioritizes biosynthetic pathways fueled by glycolytic intermediates over mitochondrial respiration. Notably, ZR-75-1 cells demonstrate resistance to transaminase inhibition but exhibit high sensitivity to glutamate dehydrogenase (GDH) inhibition, aligning with their lower proliferation rates. This metabolic pattern reflects the cell line's role as a model for less aggressive breast cancer subtypes. The metabolic plasticity of ZR-75-1 underscores the adaptability of hormone receptor-positive cancers, influencing therapeutic strategies targeting metabolic vulnerabilities. Understanding its oncogene-driven metabolic landscape may lead to more precise treatment options for similar breast cancer subtypes.

Immuno-oncology Xenograft Models

Altogen Labs is a leading preclinical research organization specializing in the evaluation of innovative pharmacological and biological therapies, including cancer treatments, medical compounds, vaccines, cosmetics, and natural products. With a team of skilled scientists, the company offers state-of-the-art laboratory services, employing cutting-edge technologies to drive oncology research and expedite drug development. Altogen Labs is known for its specialized immuno-oncology services, utilizing humanized and immunodeficient rodent models engrafted with peripheral blood mononuclear cells (PBMC), CD34+ hematopoietic stem cells, and induced pluripotent stem cells (iPSC) to assess immune responses, drug efficacy, and toxicity. One of the company's key strengths is its extensive portfolio of over 100 in-house validated xenograft models, including Cell Line Derived Xenografts (CDX), patient-derived xenografts (PDX), *in vitro* patient-derived cell cultures (PDC), and patient-derived organoids (PDOrg), providing clinically relevant platforms for predicting drug efficacy. Additionally, Altogen Labs conducts thorough toxicity studies, assessing acute, sub-chronic, and chronic toxic effects to ensure the safety and long-term tolerability of compounds.

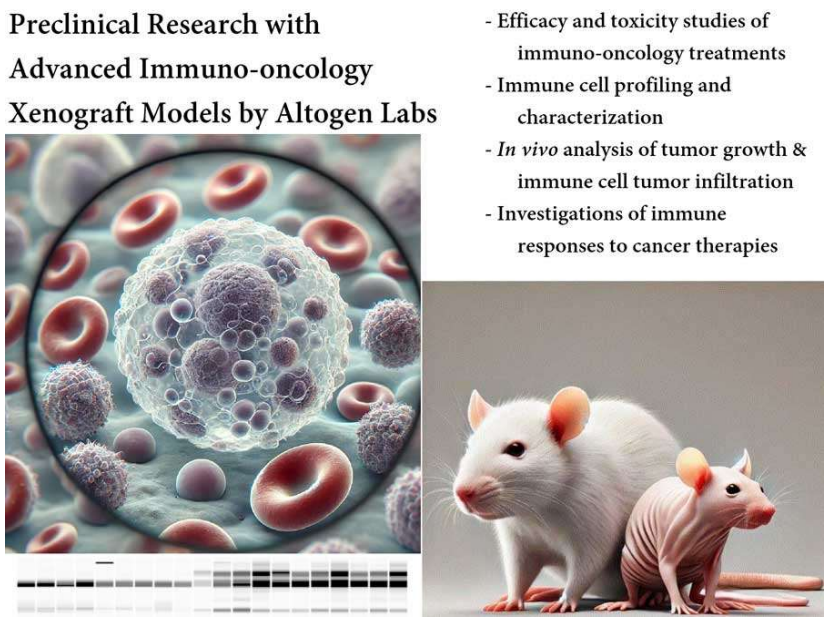

Figure 4. Advanced immune-oncology services available at Altogen Labs (Altogen Labs).

Altogen Labs provides an extensive range of laboratory services, including the ZR-75-1 xenograft model, to support comprehensive cancer research. The ZR-75-1 model, in particular, is highly valuable for studying estrogen receptor-positive breast cancer, offering a relevant and reliable platform for assessing treatment efficacy. This cell line, derived from a human breast adenocarcinoma, closely mirrors the tumor's biological behavior and provides insights into the molecular pathways of cancer. In addition to tumor growth and metastasis studies, Altogen Labs offers the development of genetically engineered cell lines for protein overexpression or RNAi-based cell lines for long-term gene silencing, which are essential for investigating the roles of specific proteins and gene products in cancer progression. Researchers can also perform quantitative gene expression analysis using RT-PCR for mRNA and protein expression analysis with the WES system (ProteinSimple), ensuring precision in molecular target evaluation and treatment outcome studies.

Altogen Labs also offers a variety of specialized services tailored to the ZR-75-1 model, including tumor growth delay (TGD) and tumor growth inhibition (TGI) studies. The laboratory supports a wide range of dosing regimens, with the flexibility to adjust frequency, duration, and administration routes such as intravenous, subcutaneous, intratumoral, intraperitoneal, and oral gavage. These methods, including advanced micro-injection techniques and pump-controlled IV injections, provide a comprehensive approach to evaluating therapeutic responses. To better simulate the metastatic process, alternative cell engraftment techniques, such as orthotopic transplantation and tail vein injection, are also available, allowing researchers to study the effects of therapies on both primary tumors and metastases. Beyond tumor growth studies, Altogen Labs offers a full suite of complementary services, including blood chemistry analysis to monitor animal health, toxicity and survival assessments, and detailed gross necropsies for tissue examination. Histopathology and advanced imaging techniques, such as fluorescence-based whole-body imaging, can also be employed to track tumor development and evaluate therapeutic outcomes. A positive control group, using cisplatin or other chemotherapeutic agents, can be included to validate the effectiveness of the tested treatments. Altogen's multifaceted approach ensures robust and reliable data for researchers investigating new breast cancer therapies.

Figure 5. Available *in vivo* xenograft services at Altogen Labs for ZR-75-1 (Altogen Labs.)

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Keywords: ZR-75-1, breast cancer, xenograft, breast, *in vivo*, cancer, preclinical, nonclinical, research, *in vivo* pharmacology, PDX, CDX, organoids, orthotopic, metastatic