Validated BT474 Xenograft Model:

Subcutaneous, Metastatic, and Orthotopic Xenograft Tumor Model

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Modeling Breast Cancer with Xenografts for Drug Development

Breast cancer is one of the most prevalent malignancies worldwide, driven by genetic, hormonal, and environmental factors. It is classified into subtypes based on receptor expression, including HER2-positive, hormone receptor-positive, and triple-negative breast cancer, each requiring distinct therapeutic approaches. Despite advances in treatment, drug resistance and metastasis remain significant challenges, making it essential to have reliable preclinical models to evaluate novel therapies. Xenograft models, where human breast cancer cells or tumor tissues are implanted into immunodeficient mice, are crucial in translational oncology. These models recapitulate tumor growth, hormone dependence, and metastatic behavior, enabling the study of targeted therapies such as HER2 inhibitors, endocrine therapy, and antibody-drug conjugates. Cell line-derived xenografts (CDXs), which use established human breast cancer cell lines, provide a cost-effective and reproducible platform for evaluating treatment efficacy, although they may not fully capture the complexity of individual patient tumors. By utilizing xenografts, researchers can assess tumor progression, drug efficacy, and mechanisms of resistance, ultimately refining therapeutic strategies to improve breast cancer treatment.

BT474 Cell Line

The BT474 cell line is a well-established HER2-positive, estrogen receptor-positive (ER+), and progesterone receptor-positive (PR+) breast cancer model is derived from an invasive ductal carcinoma from a 60-year-old Caucasian female patient. BT474 cells exhibit strong overexpression of the HER2 (c-ErbB-2) oncogene, making them a key model for studying HER2-targeted therapies such as trastuzumab and antibody-drug conjugates. Due to their hormone receptor positivity, these cells are also valuable for investigating endocrine therapy responses and resistance mechanisms. When implanted into immunodeficient mice, BT474 forms tumor xenografts, allowing researchers to study tumor growth, drug efficacy, and metastasis in a controlled preclinical setting. These xenografts help evaluate novel anti-cancer treatments, particularly those targeting HER2 and hormone receptor pathways. BT474 cells give researchers insight on translational oncology and contribute significantly to the development of precision therapies for HER2-positive breast cancer.

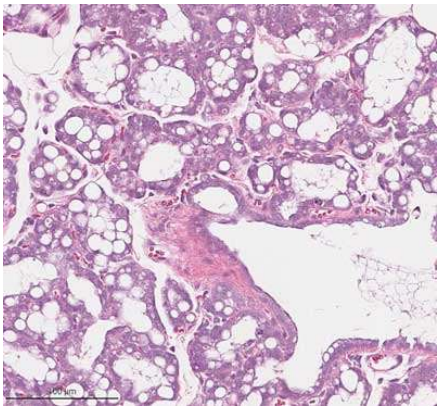

Figure 1. Tumor Histology. H&E stained slide of subcutaneously-implanted BT474 tumor (Altogen Labs).

Altogen Labs Validated BT474 Xenograft Model

At Altogen Labs, in preclinical xenograft studies, BT474 cells are maintained under aseptic conditions in exponential growth prior to injection. The cells are trypsinized, and cell viability is assessed using a trypan blue exclusion assay, ensuring that 98% of cells remain viable. The BT474 cell suspension is then adjusted to the appropriate density. Each mouse (nu/nu, 12 weeks old) receives a single subcutaneous injection into the right flank containing one million cells in a 100-microliter volume of a Matrigel-BT474 cell suspension. The injection sites are palpated up to three times a week until tumors are established, and tumor growth is monitored using digital calipers until the average size reaches 50-150 mm³. Once tumors are established, animals are randomized into appropriate treatment cohorts, and the test compound is administered according to the treatment schedule, ensuring consistency in dosing and timing for each group. Mouse

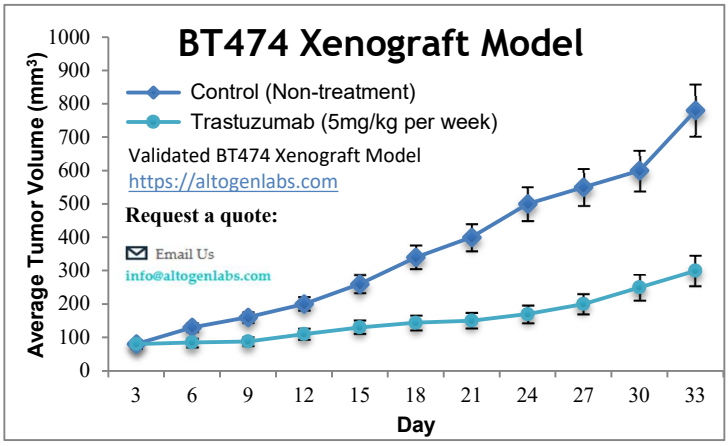

Figure 2. BT474 breast cancer xenografted in immunocompromised mice, mean values +/- SEM (Altogen Labs).

weights are recorded three times a week to monitor overall health and any potential adverse effects of the treatment, while tumor sizes are measured daily using digital calipers to track growth progression. The study concludes when the tumor size reaches 2,000 mm^3, or when the predetermined size limit specified by the approved IACUC protocol is met. If the animals reach the endpoint, a final necropsy is performed, and tumors are excised, weighed, and documented through digital imaging for accurate records. This also includes a gross necropsy to assess the overall health of the animals and collect tissues for downstream analysis, ensuring all data is captured for further study. Tumors and other tissues are preserved appropriately in RNAlater for RNA analysis, snap-frozen in liquid nitrogen for protein analysis, or prepared for histology to investigate tissue morphology and to facilitate further molecular investigations into treatment efficacy and tumor progression.

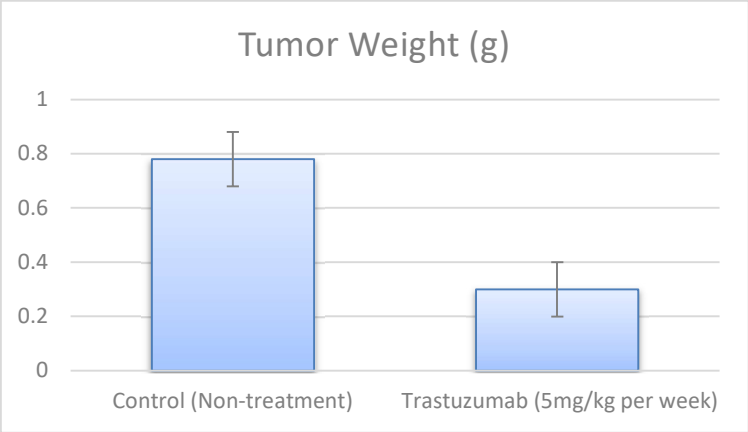

Figure 3. Tumor weight of BT474 cells in control group mice and trastuzumab treated mice at end of the study (Altogen Labs).

The Subcutaneous BT474 Model for HER2-Positive Breast Cancer Research

The subcutaneous BT474 tumor model is a widely used preclinical xenograft model for evaluating therapies targeting breast cancer. In this model, human BT474 breast cancer cells, which express HER2, are injected subcutaneously, under the skin, of immunocompromised mice. Once injected, the cells form tumors that closely resemble human breast cancer in terms of growth pattern and molecular characteristics. Tumor development is monitored by palpation and measurement using calipers, with tumor size tracked over time to assess the efficacy of experimental treatments. The BT474 model allows researchers to evaluate the impact of novel therapies, including monoclonal antibodies and targeted therapies, specifically those aimed at HER2-positive tumors. The model is particularly valuable for studying tumor growth, metastasis, and the molecular mechanisms underlying treatment resistance. After treatment, tumors are excised and analyzed for histological changes, tumor volume, and molecular markers, providing critical insights into therapeutic efficacy. The subcutaneous BT474 model offers a robust and reproducible platform for preclinical cancer research and drug development.

The Metastatic BT474 Model and Investigating HER2-Positive Cancer Spread

The metastatic BT474 model is a preclinical xenograft model used to study the metastasis of HER2-positive breast cancer. In this model, BT474 cells, which overexpress the HER2 receptor, can be introduced through two primary methods: orthotopic injection into the mammary fat pad or intravenous injection into the bloodstream of immunocompromised mice. The intravenous injection allows for the study of hematogenous metastasis, enabling cancer cells to circulate and potentially seed secondary tumors in distant organs such as the lungs, liver, and lymph nodes. This model closely mimics the clinical scenario of metastatic spread and provides insights into the dynamics of tumor dissemination. Researchers can track both primary tumor growth and metastatic progression through imaging techniques like caliper measurements, bioluminescence, or histological analysis. The metastatic BT474 model is ideal for evaluating therapeutic strategies, including treatments aimed at inhibiting metastasis or targeting specific molecular pathways, such as HER2. By studying the metastatic potential of BT474 tumors, this model offers valuable information on tumor progression, resistance mechanisms, and the efficacy of novel therapies in controlling metastatic burden.

Studying Tumor Growth with the Orthotopic BT474 Model

The orthotopic BT474 model is a preclinical xenograft model that closely mimics human breast cancer by implanting BT474 cells directly into the mammary fat pad of immunocompromised mice. This model is particularly valuable for studying the progression of HER2-positive breast cancer, as BT474 cells overexpress the HER2 receptor, which is a key target in targeted therapies. The orthotopic approach better recapitulates the tumor microenvironment and metastasis patterns seen in human breast cancer, providing more clinically relevant data. Tumor growth is monitored through imaging techniques such as caliper measurement or bioluminescence, with tumor progression tracked over time. The model enables researchers to evaluate the efficacy of various treatments, including chemotherapy, targeted therapies, and immunotherapies, specifically tailored to HER2-positive tumors.

Case Study: BT474 Cell Line Demonstrates Efficacy of High Drug-Loading Antibody–Vinca Conjugates

A study conducted by Yurkovetskiy AV, *et al*., published by *Cancer Research* journal, investigates a novel polymer-based antibody-drug conjugate (ADC) platform using poly-1-hydroxymet hylethylene hydroxymethyl-formal (PHF), known as Fleximer, to enhance targeted cancer therapy. Unlike traditional ADCs, which balance drug potency with pharmacokinetic stability, this new approach enables a high drug-to-antibody ratio (DAR) without compromising pharmacological properties. The Fleximer ADC, conjugating trastuzumab with a vinca drug derivative, was tested against HER2-positive cancers, including BT474 breast cancer xenografts. The BT474 model demonstrated significant tumor regression with the ADC, confirming its antigen-specific accumulation and superior efficacy compared to unconjugated drugs or non-targeting controls. Pharmacokinetic analysis showed sustained plasma stability and effective tumor penetration, further validating its therapeutic potential. The study concludes that Fleximer-based ADCs offer a promising advancement in ADC technology, enabling high drug loading while maintaining optimal targeting and pharmacokinetics.

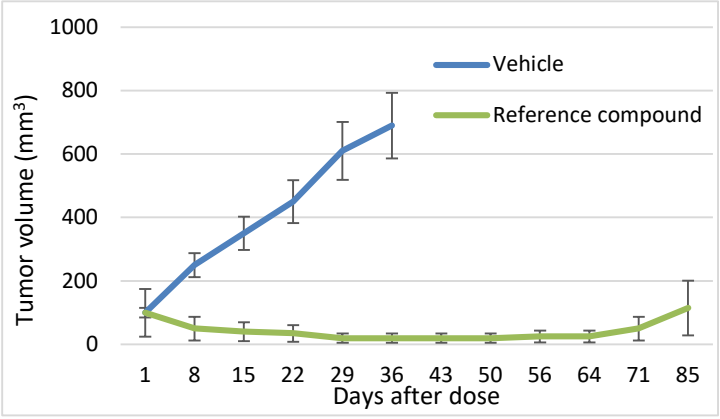

Figure 4. Treatment with the reference compound (15.6 mg/kg) resulted in significant inhibition of tumor growth in BT474 xenograft models.

Additional Case Study: BT474 Breast Cancer Model Validates Dual PIM/PI3K Inhibition as a Novel Therapy

Another study done by Kennedy SP, *et al.*, published by *Oncogene* journal, evaluates the preclinical efficacy of IBL-302, a novel inhibitor targeting PIM and PI3K/mTOR pathways, in breast cancer models, including HER2+ and trastuzumab-resistant cell lines. BT474, a HER2+/PIK3CA-mutated breast cancer cell line, was used extensively in both *in vitro* and *in vivo* assessments. IBL-302 effectively inhibited tumor growth in BT474 xenografts, demonstrating significant reductions in tumor volume compared to controls. Mechanistically, IBL-302 suppressed key survival pathways by reducing phosphorylation of AKT, mTOR, and BAD, thereby inducing apoptosis in BT474 cells. Additionally, combining IBL-302 with trastuzumab enhanced the anti-proliferative effect, even in trastuzumab-resistant BT474 variants, supporting its role in overcoming resistance mechanisms. *In vivo*, BT474 xenograft tumors treated with IBL-302 exhibited notable tumor regression, reinforcing its therapeutic potential. These findings suggest that dual inhibition of PIM and PI3K/mTOR pathways using IBL-302 could be a promising approach for treating HER2+ breast cancer, particularly in patients with resistance to existing therapies.

Understanding BT474: Sensitivity and Resistance in Breast Cancer Treatment

BT474 is a human breast cancer cell line that overexpresses the HER2/neu (ErbB2) receptor, making it a model system for studying HER2-positive breast cancer. It has been extensively used to investigate the effects of targeted therapies, including trastuzumab, pertuzumab, and the tyrosine kinase inhibitor lapatinib. Studies show that trastuzumab effectively induces cell-cycle arrest in BT474 cells, though resistance mechanisms can emerge. The presence of epidermal growth factor (EGF) or heregulin (HRG) can counteract the growth inhibition effects of targeted therapies, emphasizing the complexity of HER2 signaling. Lapatinib is a potent inhibitor of BT474 cell proliferation, but its effectiveness can be diminished by compensatory signaling from growth factors. Combining multiple HER2-targeting agents, such as trastuzumab, pertuzumab, and lapatinib, has been shown to enhance therapeutic efficacy. These findings highlight the need for combination therapies and personalized treatment approaches to overcome drug resistance in HER2-positive breast cancer.

BT474 Breast Cancer Cells: A Model for HER2-Driven Tumorigenesis

BT474 is characterized by strong overexpression of the c-ErbB-2 (HER2/neu) oncogene, a feature observed in approximately 30% of human breast cancers. This overexpression is associated with increased tumorigenicity, resistance to apoptosis, and potential resistance to anti-estrogen therapies. Unlike many other HER2-positive cell lines, BT474 is also estrogen receptor (ER)-positive, making it a valuable model for studying hormone-dependent breast cancer. *In vivo*, BT474 tumors exhibit high proliferative activity but also undergo significant apoptosis, likely influenced by Bcl-2 expression and the absence of wild-type p53. These tumors are capable of metastasizing to regional lymph nodes and forming micro-

metastases in the lungs. The dual expression of HER2 and hormone receptors makes BT474 an essential model for testing targeted therapies, including HER2 inhibitors, endocrine therapies, and novel combination treatments.

For the BT474 xenograft model, Altogen Labs offers a wide array of customizable options to suit various research needs. Researchers can choose from several endpoints such as Tumor Growth Delay (TGD), which measures the time taken for tumors to reach a certain size, or Tumor Growth Inhibition (TGI), which evaluates the reduction in tumor size in response to treatment. The dosing regimen is flexible, with the option for different dosing frequencies, ranging from once to twice daily, for varying durations based on study requirements. Dosing can be performed via a variety of routes, including intravenous, intratracheal, continuous infusion, intraperitoneal, intratumoral, oral gavage, topical, intramuscular, subcutaneous, and intranasal, allowing for precise targeting of the treatment and mimicking clinical administration methods. Advanced techniques such as micro-injection and pump-controlled IV injection are also available for high precision dosing.

Figure 5. Altogen Labs *in vivo* xenograft services for BT474 (Altogen Labs).

Additionally, Altogen Labs offers multiple tumor engraftment options to ensure the most appropriate and reliable model for specific research objectives. These include orthotopic transplantation, where tumors are implanted in the mammary fat pad to closely resemble human tumor behavior, as well as tail vein and left ventricular injection for metastasis studies, which allow for the study of secondary spread to distant organs. Other injection sites like intraperitoneal injection are also available. For tumor analysis, immunohistochemistry is employed to evaluate tumor biomarkers, and various assays are available for lipid distribution, metabolic profiling, and blood chemistry analysis. The lab also offers a comprehensive health observation program to monitor toxicity and survival, with an optional broad health monitoring protocol. Gross necropsies, histopathology, and a positive control group using

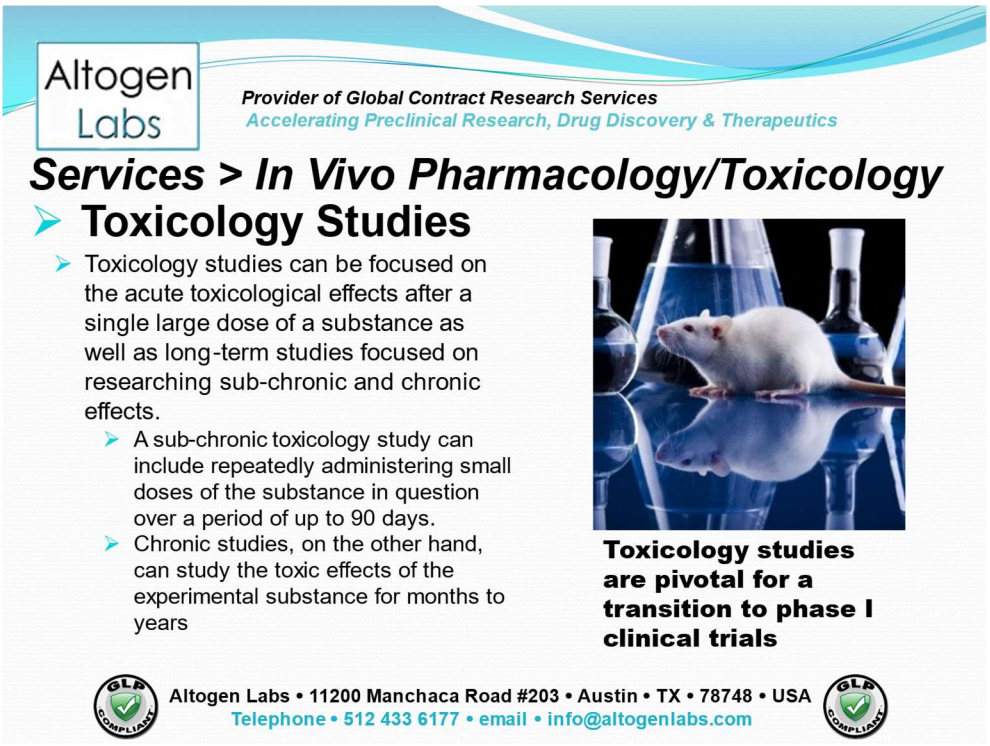

Figure 6. *In vivo* toxicology studies available at Altogen Labs (Altogen Labs).

cyclophosphamide (50 mg/kg via intramuscular injection daily) are provided for comparison with experimental treatments. Imaging studies, including fluorescence-based whole-body imaging and MRI, are available for non-invasive monitoring of tumor growth and metastasis. These diverse options ensure that Altogen Labs can provide a tailored approach to meet the specific needs of cancer research studies.

Advancements in Organoid Technology for Personalized Cancer Therapy

Organoids are three-dimensional cultures created from patient tumor samples that maintain critical characteristics of the original tumor, including genetic and phenotypic diversity. Unlike traditional 2D cell cultures, organoids preserve complex tissue architecture and can be efficiently expanded from primary patient material, offering significant advantages for personalized cancer research and drug testing. While xenograft and allograft models capture tumor-stroma and immune interactions, organoids provide a faster, more scalable platform for assessing therapeutic responses. Recent advances in organoid technology have led to the development of patient-derived tumor organoid (PDTO) biobanks, which serve as valuable living resources for studying cancer progression and drug resistance. These models are particularly beneficial for high-throughput drug screening, allowing researchers to discover potential treatments tailored to the unique profiles of individual tumors.

References:

Diermeier-Daucher S, Breindl S, Buchholz S, Ortmann O, Brockhoff G. Modular anti-EGFR and anti-Her2 targeting of SK-BR-3 and BT474 breast cancer cell lines in the presence of ErbB receptor-specific growth factors. *Cytometry A*. 2011 Sep;79(9):684-93. doi: 10.1002/cyto.a.21107. Epub 2011 Jul 22. PMID: 21786419.

Kennedy SP, O'Neill M, Cunningham D, Morris PG, Toomey S, Blanco-Aparicio C, Martinez S, Pastor J, Eustace AJ, Hennessy BT. Preclinical evaluation of a novel triple-acting PIM/PI3K/mTOR inhibitor, IBL-302, in breast cancer. *Oncogene*. 2020 Apr;39(14):3028-3040. doi: 10.1038/s41388-020-1202-y. Epub 2020 Feb 10. PMID: 32042115; PMCID: PMC7118022.

Van Slooten HJ, Bonsing BA, Hiller AJ, Colbern GT, van Dierendonck JH, Cornelisse CJ, Smith HS. Outgrowth of BT-474 human breast cancer cells in immune-deficient mice: a new in vivo model for hormone-dependent breast cancer. *Br J Cancer.* 1995 Jul;72(1):22-30. doi: 10.1038/bjc.1995.271. PMID: 7599056; PMCID: PMC2034136.

Yurkovetskiy AV, Yin M, Bodyak N, Stevenson CA, Thomas JD, Hammond CE, Qin L, Zhu B, Gumerov DR, Ter-Ovanesyan E, Uttard A, Lowinger TB. A Polymer-Based Antibody-Vinca Drug Conjugate Platform: Characterization and Preclinical Efficacy. *Cancer Res*. 2015 Aug 15;75(16):3365-72. doi: 10.1158/0008-5472.CAN-15-0129. Epub 2015 Jun 25. PMID: 26113086.

Keywords: BT474, breast cancer, xenograft, breast, *in vivo*, cancer, preclinical, research, *in vivo* pharmacology, PDTO, CDX, orthotopic, metastatic, organoid

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/