Validated HT1080 Xenograft Model:

Subcutaneous, Metastatic, And Orthotopic Xenograft Tumor Model

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Harnessing Xenografts to Study Sarcoma Growth and Therapy Response

Sarcomas are a diverse group of malignant tumors that arise from mesenchymal tissues, including bone, muscle, fat, and connective tissues. They are relatively rare but often aggressive, with limited treatment options for advanced or metastatic disease. Xenograft models, in which human sarcoma cells or patient-derived tumor tissues are implanted into immunodeficient mice, play a critical role in preclinical research by providing a biologically relevant platform for studying tumor growth, progression, and therapeutic response. Subcutaneous xenografts offer a convenient and reproducible method for evaluating drug efficacy, while orthotopic and patient-derived xenograft (PDX) models better recapitulate the tumor microenvironment and metastatic behavior. Cell-line derived xenografts (CDXs) serve as a foundational tool for testing standardized treatment regimens, providing a reproducible system for evaluating drug responses across multiple experimental conditions. These models are particularly valuable for testing novel targeted therapies, chemotherapeutic agents, and immunotherapies in a controlled setting before clinical trials. Additionally, xenografts enable researchers to investigate mechanisms of tumor resistance, biomarker discovery, and personalized treatment approaches. Advances in imaging technologies, such as bioluminescence and MRI, have further enhanced the ability to monitor tumor dynamics *in vivo*.

HT1080 Cell Line

The HT1080 cell line is a widely used human fibrosarcoma model derived from the connective tissue of a patient with fibrosarcoma. As an epithelial-like adherent cell line, HT1080 cells exhibit rapid proliferation and the ability to form tumors when implanted into immunodeficient mice, making them valuable for cancer research and drug development. These cells express key oncogenic mutations, which contributes to their aggressive phenotype and makes them a useful model for studying tumor invasion, metastasis, and therapeutic responses. HT1080 cells are commonly used in assays evaluating extracellular matrix degradation, angiogenesis, and cell migration due to their high invasive potential. Additionally, they serve as a platform for testing novel chemotherapeutic agents, targeted therapies, and gene-editing technologies. Their robust and reproducible growth characteristics allow for consistent *in vitro* and *in vivo* studies, supporting advancements in fibrosarcoma research. HT1080 cells have been instrumental in understanding the molecular mechanisms underlying sarcoma progression and resistance to treatment.

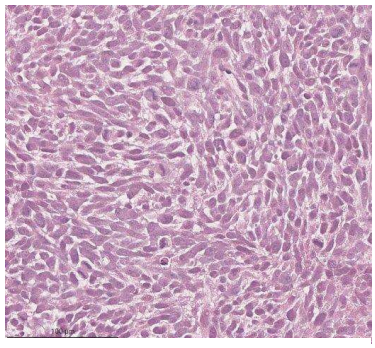

Figure 1. Tumor Histology. H&E stained slide of subcutaneously-implanted HT1080 tumor (Altogen Labs).

Altogen Labs Validated HT1080 Xenograft Model

In preclinical studies at Altogen Labs, HT1080 cells are maintained under aseptic conditions in the exponential growth phase. The cells are then trypsinized, and viability is assessed using a trypan blue exclusion assay, requiring a viability of 97-98% before proceeding. The HT1080 cell suspension is adjusted to the appropriate density, and each mouse receives a single subcutaneous injection of $1x10^6$ cells in 150-200 µL of a Matrigel-HT1080 cell suspension into the right flank. Injection sites are palpated up to three times per week until tumors are established, reaching an average size of 50-150 mm^3, as measured by digital calipers. Once tumors reach the target size, animals are randomized into treatment groups, and the test compound is administered according to the pre-established treatment schedule. Mice weights are recorded two to three times per

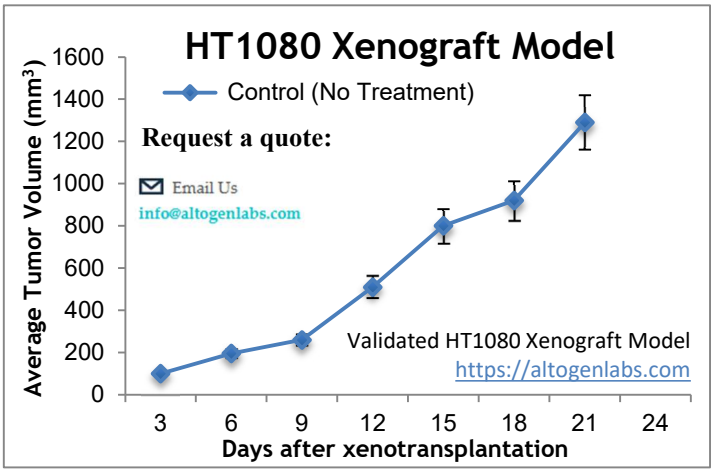

Figure 2. HT1080 fibrosarcoma xenografted in athymic mice, mean values +/- SEM (Altogen Labs).

week, while tumor measurements are documented daily. The study continues until tumors reach 2,000 mm³ or the predetermined size limit specified by the approved IACUC protocol. At the end of the study, a final necropsy is performed, and tissues are collected for further analysis. Tumors are excised, weighed, and digitally imaged, with samples either stabilized in RNAlater, snap-frozen in liquid nitrogen, or processed for histological evaluation.

Case Study: HT1080 Fibrosarcoma Cells Reveal Thiabendazole as a Novel Anti-Cancer Agent

In a study by Cha HJ, *et al*., published by *PLOS Biology* journal, researchers investigated the role of HT1080 fibrosarcoma cells in identifying thiabendazole (TBZ) as a vascular disrupting agent (VDA) with potential anti-cancer applications. Researchers discovered that TBZ, an antifungal drug in clinical use for over 40 years, inhibits angiogenesis and disrupts tumor vasculature, making it a promising candidate for cancer therapy. Using HT1080 xenografts in mice, TBZ treatment significantly reduced tumor growth and vascular density without directly affecting tumor cell proliferation in vitro. The study demonstrates that TBZ acts by targeting endothelial cell behavior rather than inducing apoptosis, differentiating it from other VDAs. Mechanistically, TBZ exerts its effects through microtubule disruption and Rho kinase activation, leading to endothelial cell disassembly and inhibition of angiogenesis. Additionally, its FDA approval and established safety profile make TBZ an attractive candidate for rapid clinical translation as an adjunct therapy for highly vascularized tumors.

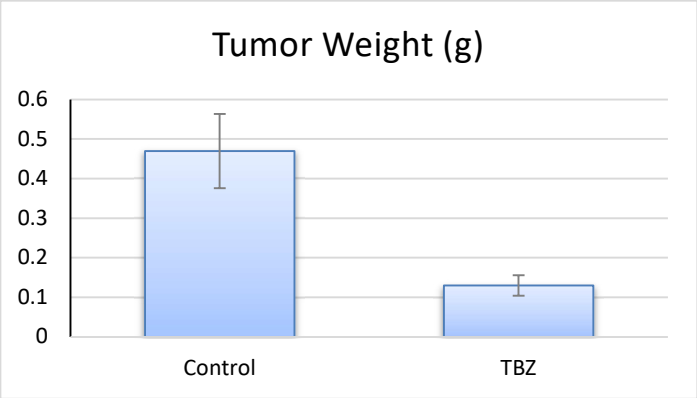

Figure 3. TBZ (50 mg/kg) suppressed the growth of HT1080 fibrosarcoma xenograft tumors.

Additional Case Study: HT1080 and the Discovery of a Mutant IDH1 Inhibitor for Cancer Therapy

Another study conducted by Zheng M, *et al*., published by *Oncotarget* journal, explores the potential of clomifene, a selective estrogen receptor modulator, as a novel inhibitor of mutant isocitrate dehydrogenase 1 (IDH1), a key driver in certain cancers; including HT1080 fibrosarcoma. The study found that clomifene selectively inhibits mutant IDH1 activity, leading to a dose-dependent reduction in D-2-hydroxyglutarate (D-2HG) production, a known oncometabolite that drives tumor progression. In HT1080 xenograft mouse models, oral administration of clomifene significantly suppressed tumor growth, further supporting its therapeutic potential. Knockdown experiments in HT1080 cells revealed that reducing mutant IDH1 expression decreased the sensitivity to clomifene, confirming that its anti-cancer effects are mutant IDH1-dependent. Additionally, clomifene treatment reversed histone hypermethylation, a hallmark of IDH1-mutant cancers, suggesting epigenetic restoration as a mechanism of action. Given its established safety profile as an FDA-approved drug, clomifene represents a promising candidate for repurposing in IDH1-mutant cancers, potentially accelerating its translation into clinical applications.

Evaluating Fibrosarcoma Therapy Using the HT1080 Subcutaneous Model

The subcutaneous HT1080 model is a widely used preclinical platform for studying fibrosarcoma tumor growth and evaluating therapeutic efficacy. In this model, HT1080 cells are injected subcutaneously, typically into the hind flank of immunodeficient mice, allowing for the development of measurable, localized tumors. This approach provides a highly reproducible system for assessing tumor progression, drug response, and biomarker expression under controlled conditions. Due to its accessibility, the subcutaneous model enables frequent tumor monitoring using caliper measurements and advanced imaging techniques. It is commonly employed in preclinical screening chemotherapeutic agents, targeted therapies, and combination treatments to determine their impact on tumor size and growth kinetics.

Advancing Fibrosarcoma Research with Orthotopic HT1080 Xenografts

The orthotopic HT1080 model provides a biologically relevant platform for studying fibrosarcoma within its native tissue environment. In this model, HT1080 cells are implanted into anatomically appropriate sites, such as the retroperitoneal space or intramuscular compartments, to better mimic the tumor microenvironment observed in human sarcomas. This approach allows for the investigation of tumor growth, invasion, and interactions with surrounding stromal and vascular components. Compared to subcutaneous models, orthotopic HT1080 xenografts more accurately replicate disease

progression, including local invasion and metastatic dissemination. These models are particularly valuable for assessing novel therapeutics that target tumor-microenvironment interactions, angiogenesis, and metastatic pathways. Advanced imaging techniques such as bioluminescence and MRI enable real-time monitoring of tumor development and treatment responses. While technically more challenging than subcutaneous implantation, the orthotopic HT1080 model offers a superior preclinical system for evaluating the efficacy of targeted therapies and improving translational cancer research.

Modeling Sarcoma Metastasis with HT1080

The metastatic HT1080 model is utilized for studying the mechanisms of fibrosarcoma dissemination and evaluating anti-metastatic therapies. In this model, HT1080 cells are introduced into immunodeficient mice via intravenous, intracardiac, or orthotopic injection, allowing for the development of spontaneous or experimental metastases in distant organs such as the lungs, liver, and lymph nodes. This system closely mimics the metastatic cascade observed in human sarcomas, including intravasation, circulation, extravasation, and colonization at secondary sites. The model is widely used to investigate key regulators of metastasis, such as cell adhesion molecules, extracellular matrix remodeling, and hypoxia-driven pathways. Additionally, it serves as a preclinical platform for testing novel therapeutics, including inhibitors of invasion, angiogenesis, and immune-based strategies. Real-time imaging modalities, such as bioluminescence and fluorescence labeling, enable longitudinal tracking of metastatic progression and treatment response.

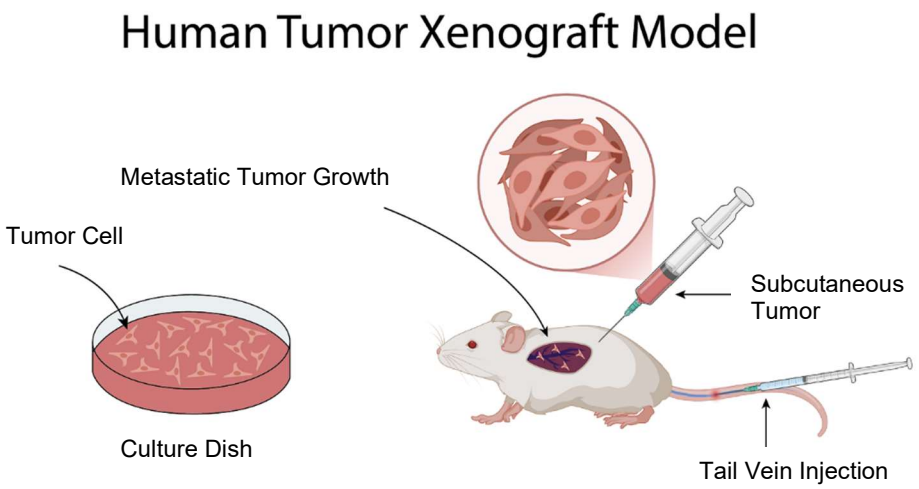

Figure 4. Illustration of metastatic implantation of HT1080 cells (Altogen Labs).

HT1080 Fibrosarcoma Cells and Ferroptosis: A Novel Therapeutic Target

HT1080 fibrosarcoma cells serve as a crucial model for studying ferroptosis, a unique form of regulated cell death driven by iron-dependent lipid peroxidation. Recent research has demonstrated that conjugated fatty acids (CFAs), including α-eleostearic acid (ESA), induce ferroptosis in HT1080 cells through the chaperone-mediated autophagy (CMA) degradation of glutathione peroxidase 4 (GPX4). GPX4 is a key enzyme that prevents lipid peroxidation, and its degradation sensitizes HT1080 cells to oxidative stress. The mechanism involves mitochondrial targeting of CFAs, leading to reactive oxygen species (ROS) generation, lipid peroxidation, and ultimately, ferroptosis cell death. This pathway is dependent on LAMP2A, a key regulator of CMA, as its knockout confers resistance to CFA-induced ferroptosis. *In vivo* studies using HT1080 xenografts further confirm that oral administration of ESA-rich oils suppresses tumor growth through increased lipid peroxidation and GPX4 degradation. These findings highlight the vulnerability of HT1080 fibrosarcoma cells to ferroptosis and suggest CFA-based ferroptosis induction as a potential therapeutic approach for targeting fibrosarcoma and other aggressive cancers.

HT1080 Handling Procedures and Subculturing Protocol

To ensure the highest level of viability, researchers thaw the HT1080 cell vial and initiated culture as soon as the vial is received. In cases where immediate culturing was not feasible, the frozen culture is stored in the liquid nitrogen vapor phase rather than at -70°C, as the latter could result in a loss of cell viability. The vial is thawed by gentle agitation in a 37°C water bath, with precautions taken to keep the O-ring and cap out of the water to minimize contamination risk. The thawing process is completed rapidly, within approximately 2 minutes. Once thawed, the vial is removed from the water bath, decontaminated by dipping or spraying with 70% ethanol, and transferred to a sterile environment to maintain aseptic conditions throughout the procedure. The contents are then transferred to a centrifuge tube containing 9.0 mL of complete culture medium and spun at 125 x g for 5 to 7 minutes. After discarding the supernatant, the cell pellet is resuspended in the recommended complete medium, taking care to avoid excessive alkalinity during recovery. Prior to adding the cell suspension, the culture vessel containing the complete medium is placed in the incubator for at least 15 minutes to allow

the medium to stabilize at the desired pH range of 7.0 to 7.6. The cultures are then incubated at 37°C with a 5% CO_2 in air atmosphere, as recommended for optimal growth conditions.

Researchers use Corning T-75 flasks for subculturing the HT1080 cells, ensuring that the volumes of dissociation medium used were adjusted appropriately for different-sized culture vessels. First, the culture medium is removed and discarded, and the cell layer is briefly rinsed with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to eliminate any serum containing trypsin inhibitors. Researchers then add 2.0 to 3.0 mL of the Trypsin-EDTA solution to the flask and observe the cells under an inverted microscope. The cell layer typically disperses within 5 to 15 minutes, but if the cells are difficult to detach, they are incubated at 37°C to facilitate the process. To avoid clumping, the flask is not agitated by shaking or hitting. Once the cells detach, 6.0 to 8.0 mL of complete growth medium is added, and the cell suspension is gently aspirated by pipetting. The appropriate aliquots of the cell suspension are transferred to new culture vessels, and the cultures are incubated at 37°C. A sub-cultivation ratio of 1:4 to 1:8 is maintained, with medium renewal performed every 2 to 3 days to ensure optimal cell growth and maintenance.

The HT1080 xenograft model provides a robust platform for preclinical studies focused on fibrosarcoma progression and therapeutic evaluation. At Altogen Labs, researchers assess key experimental endpoints such as Tumor Growth Delay (TGD) and Tumor Growth Inhibition (TGI) to evaluate treatment efficacy. The HT1080 cell line supports various dosing methods, including intraperitoneal, intravenous, and subcutaneous administration, allowing for tailored therapeutic approaches. Advanced techniques such as imaging and tumor site-specific injections enable precise monitoring of tumor growth and response to treatment. Comprehensive analyses, including immunohistochemistry, molecular profiling, survival studies, and histopathological evaluations, provide critical insights into fibrosarcoma biology and therapy resistance. This model also facilitates the study of tumor angiogenesis, extracellular matrix remodeling, and metastasis, offering a clinically relevant system for drug development. The HT1080 xenograft model is particularly valuable for evaluating targeted therapies and studying invasive tumor behavior, making it an essential tool in sarcoma research and drug discovery.

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