Validated SF-268 Xenograft Model:
Subcutaneous Xenograft Tumor Model

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Understanding Brain Cancer through Xenograft Models

Brain cancer, a highly complex and aggressive form of malignancy, presents significant challenges in diagnosis, treatment, and patient survival. The most common types of brain cancer include gliomas, meningiomas, and medulloblastomas, with glioblastoma multiforme (GBM) being the most prevalent and deadly among adult brain tumors. These cancers are characterized by their rapid growth, invasive nature, and resistance to conventional therapies such as surgery, radiation, and chemotherapy. The blood-brain barrier (BBB) further complicates treatment, limiting the effectiveness of systemic therapies. To better understand brain cancer's molecular underpinnings and evaluate potential treatment strategies, researchers have turned to xenograft models, including Patient-Derived Xenografts (PDXs) and Cell Line-Derived Xenografts (CDXs). PDXs involve transplanting tumor tissue directly from a patient into an immunodeficient mouse, preserving the tumor's original characteristics and genetic diversity, which makes them particularly valuable for studying the heterogeneity of brain cancers and testing personalized therapies. On the other hand, CDXs, which are established from cultured tumor cell lines, offer reproducible and standardized models for drug testing and mechanistic studies. These models are indispensable for advancing brain cancer research, offering a reliable platform for the preclinical evaluation of novel therapies and the development of targeted treatments.

SF-268 Cell Line

The SF-268 cell line is a widely studied human glioma model that was originally thought to be derived from a 24-year-old female patient with astrocytoma. However, subsequent genetic analysis revealed the presence of a Y chromosome, suggesting that the cell line was actually established from a male patient. SF-268 is characterized by its highly anaplastic nature, meaning it lacks distinct mature morphology and exhibits rapid, uncontrolled cell proliferation. This aggressive growth pattern, along with its poor differentiation, makes it an ideal model for studying glioma biology and testing potential therapies for this type of brain cancer. Due to its high proliferative capacity, SF-268 is often used in drug screening and research focused on tumor progression and invasion. The cell line is also valuable in evaluating therapeutic approaches aimed at targeting malignant gliomas, particularly those that are resistant to traditional treatments. SF-268 has been extensively used in studies exploring the molecular mechanisms driving glioma aggressiveness, including genetic mutations and signaling pathway alterations.

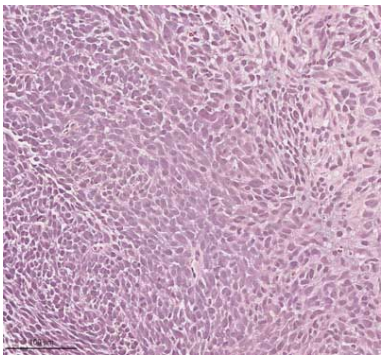

Figure 1. Tumor Histology. H&E stained section of a subcutaneously-implanted SF-268 tumor (Altogen Labs).

Altogen Labs Validated SF-268 Xenograft Model

At Altogen Labs, in preclinical studies, SF268 cells are cultured until they reach the exponential growth phase before being collected for injection. Viable cell counts are determined using trypan blue, and cell concentrations are quantified with a hemocytometer. The cell concentration is adjusted to ensure each mouse (athymic BALB/C or NOD/SCID, 10-12 weeks old) receives a subcutaneous injection in the hind leg, with 1 million SF-268 cells suspended in a 50% Matrigel solution for a 100 µL injection volume. In-study animals are monitored through palpation until tumor establishment is confirmed. Randomized treatment groups are formed based on an average tumor size of 100-150 mm³. The administration of test compounds is carried out in alignment with the treatment schedule, and tumor growth is tracked with daily measurements, alongside regular monitoring of the mouse's body weight (three times weekly).

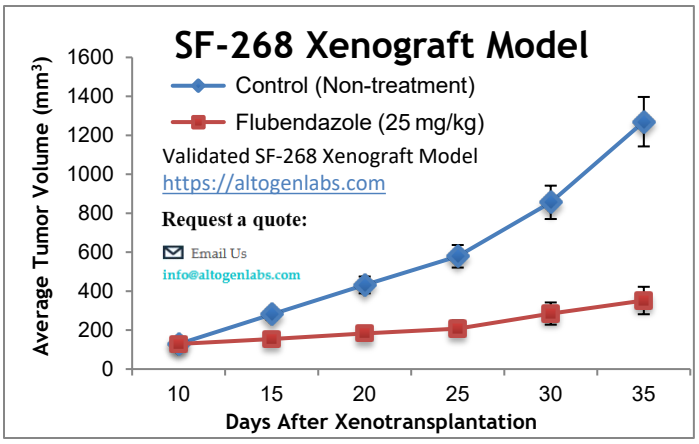

Figure 2. SF-268 brain cancer xenografted in immunocompromised mice, mean values +/- SEM (Altogen Labs).

The study concludes when the tumor size reaches 2,000 mm³ or the approved IACUC protocol's size limit is reached. Xenograft models, including both Cell Line-Derived Xenografts (CDXs) and Patient-Derived Xenografts (PDXs), are crucial tools in cancer research. CDXs involve injecting established cell lines into immunodeficient rodents, while PDX models use tumor cells directly derived from patient samples, offering a more accurate representation of human tumors. These models are essential for evaluating the efficacy of chemotherapy and other therapeutic strategies. Brain tumors, one of the deadliest types of cancer, are commonly studied in these models, particularly as brain cancers are often diagnosed in children. Advances in technology have enabled the identification of distinct subgroups of brain tumors, which can be studied using xenograft models. PDXs, in particular, retain the original features of the patient's tumor, providing significant advantages for personalized medicine and therapeutic testing.

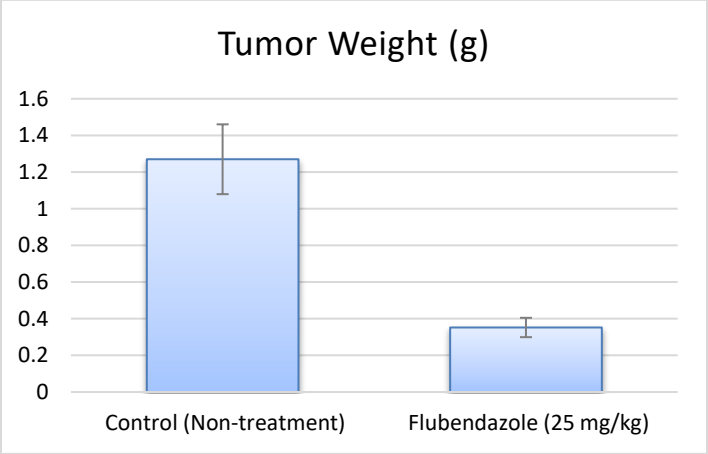

Figure 3. Tumor weight of SF-268 cells in control, buffer only mice and flubendazole treated mice at end of the study (Altogen Labs).

SF268 Glioma Model Reveals PI3K Inhibition as a Promising Therapeutic Strategy

Gliomas, aggressive primary brain tumors, often exhibit deregulation of the phosphatidylinositol 3-kinase (PI3K) signaling pathway, making it an attractive therapeutic target. The SF268 cell line, derived from human glioma, serves as a vital model system for studying the molecular effects of PI3K pathway inhibition. Using the selective PI3K inhibitor PI-103, researchers demonstrated potent suppression of PI3K pathway signaling in SF268 cells, evidenced by reduced phosphorylation of key proteins such as AKT, p70S6 kinase, and ribosomal protein S6. Notably, treatment with PI-103 induced a reversible cell cycle arrest at the G1 phase in SF268 cells, characterized by decreased expression of cyclin D1 and CDC6 and an increase in the cell cycle inhibitor p27Kip1. Interestingly, rather than triggering apoptosis, PI-103 induced significant autophagic responses, marked by extensive cytoplasmic vacuolation and LC-3 protein processing. Furthermore, when used in combination with common chemotherapy agents such as temozolomide, PI-103 displayed synergistic or additive effects, enhancing therapeutic outcomes in preclinical glioma models including SF268. These findings illustrate the potential of selective PI3K inhibitors to alter oncogenic signaling and improve therapeutic strategies in gliomas.

SF268 Cells Illuminate Potential of Neurological Therapeutics Against Glioma

Glioblastoma remains one of the most aggressive brain tumors, resistant to conventional therapies due to mechanisms such as the protective blood-brain barrier and intrinsic cellular resistance. To address this, existing drugs for neurological disorders capable of crossing the blood-brain barrier were evaluated for their potential antitumor effects using various neural tumor cell lines, notably including the SF268 glioma cell line. SF268 cells, characterized by resistance to temozolomide due to MGMT overexpression, served as a crucial model for studying novel drug activities. Among tested drugs, levomepromazine and fingolimod demonstrated significant antitumor effects on SF268 cells, notably reducing proliferation. Further assays showed that drugs such as levetiracetam, haloperidol, and valproic acid exhibited potent synergistic effects when combined with temozolomide, significantly enhancing anti-proliferative activity in SF268 cells. The cell death mechanisms identified included apoptosis induced by haloperidol, valproic acid, and levomepromazine, and autophagy induced by biperiden and dextromethorphan in SF268 cells. Additionally, fingolimod notably inhibited SF268 cell migration, a critical factor in glioma invasiveness. Collectively, these findings highlight SF268 as a valuable model for understanding resistance mechanisms and evaluating new therapeutic strategies in glioma treatment.

Evaluating Glioblastoma Therapies with the SF-268 Subcutaneous Model

The subcutaneous SF-268 xenograft model is a well-established preclinical platform for studying glioblastoma and other central nervous system tumors. In this model, SF-268 cells are injected subcutaneously into immunodeficient mice, typically in the hind flank, allowing for controlled tumor growth monitoring. This model is widely used due to its reproducibility, ease of tumor measurement, and suitability for evaluating the efficacy of novel anticancer therapies. SF-268 tumors exhibit aggressive growth, making them ideal for assessing tumor progression, drug response, and resistance mechanisms. The subcutaneous model enables researchers to perform tumor growth inhibition (TGI) and tumor growth delay (TGD) studies, along with comprehensive pharmacokinetic and toxicity analyses. Additionally, tumor samples can be

collected for histopathological evaluation, gene expression studies, and protein analysis to gain insights into tumor biology. This model provides a valuable tool for screening potential therapies before advancing to more complex orthotopic or metastatic models.

Case Study: Flubendazole Suppresses Glioma Growth by Targeting SF-268 Cell Proliferation

Flubendazole, an FDA-approved antiparasitic medication, exhibits notable anticancer effects against glioma, particularly through significant inhibition of proliferation and increased apoptosis. In a study by Zhou, X., *et al.*, published by *Cell Death Discovery* journal, the glioma cell line SF-268 played a crucial role in demonstrating these effects, where flubendazole significantly inhibited its proliferation and clonogenic ability *in vitro*. *In vivo* experiments showed that flubendazole effectively suppressed tumor growth of SF-268 cells in xenograft models without affecting mice body weight or behavior, suggesting low toxicity. Notably, immunohistochemical analysis revealed reduced Ki-67 expression in SF-268 xenograft tumors, confirming diminished cellular proliferation. However, flubendazole did not significantly affect the migration capability or epithelial-mesenchymal transition markers in SF-268 cells. Mechanistic studies indicated that flubendazole triggered cell cycle arrest at the G2/M phase in SF-268 cells by increasing P53 expression while reducing cyclin B1 and p-cdc2 expression. Additionally, flubendazole induced apoptosis in SF-268 cells by downregulating anti-apoptotic Bcl-2 family proteins and upregulating cleaved caspases and PARP-1, reflecting activation of intrinsic apoptotic pathways. Collectively, these findings highlight SF-268 glioma cells as an important model demonstrating flubendazole's potential as a novel therapeutic agent for glioma via its dual impact on cell cycle and apoptotic mechanisms.

Additional Case Study: SF268 Glioma Cells Illuminate the HOTTIP-miR-10b Axis in TMZ Resistance

This study explores the role of long non-coding RNA HOTTIP in the resistance of glioma cells to temozolomide (TMZ), a commonly used chemotherapy drug. Researchers utilized multiple glioma cell lines, notably highlighting SF268 due to its pronounced resistance to TMZ, making it an ideal model for investigating underlying resistance mechanisms. They identified significantly elevated expression of lncRNA HOTTIP in TMZ-resistant SF268 cells, indicating its critical role in resistance. The upregulation of HOTTIP was associated with increased proliferation, migration, angiogenesis, and metastatic markers in glioma cells. Mechanistically, SF268 cells displayed enhanced epithelial-to-mesenchymal transition (EMT), characterized by elevated ZEB1 and ZEB2 expression and decreased E-cadherin levels, mediated by increased miR-10b expression. Crucially, inhibition of miR-10b reversed EMT changes and resensitized SF268 cells to TMZ, confirming the functional link between HOTTIP, miR-10b, and TMZ resistance. These findings underscore the SF268 glioma cell line as a valuable model for studying the molecular mechanisms underpinning therapy resistance, particularly highlighting the HOTTIP-miR-10b-EMT signaling axis as a promising target for overcoming chemoresistance in glioma.

Chemotherapy: RES529 Enhances Glioma Therapy through TORC Inhibition

Glioblastoma (GBM) is an aggressive primary brain tumor characterized by poor prognosis and resistance to current therapies. Targeting key signaling pathways is crucial in developing new therapeutic strategies. The dual inhibitor RES529, which targets TORC1 and TORC2 signaling complexes, has shown promise in preclinical GBM models. Notably, RES529 significantly inhibited proliferation and tumor growth in GBM cell lines, particularly in the SF268 glioma model, which is often used as a representative glioma cell line. When combined with anti-angiogenic drugs like bevacizumab and sunitinib, RES529 enhanced therapeutic efficacy by delaying tumor recurrence and reducing angiogenesis and vasculogenic mimicry, two processes critical for glioma survival and invasion. Importantly, RES529 penetrates the blood-brain barrier, an essential property for treating brain tumors. Collectively, these findings suggest that RES529, especially when evaluated in the context of SF268 glioma cells, represents a promising approach to improving glioblastoma treatment outcomes.

Oncogenic YAP Activity and its Negative Regulation in SF-268 Cells

SF-268 is a glioma cell line frequently used as a model system to study oncogenic signaling in human cancers, particularly glioblastoma. A key feature of SF-268 cells is their elevated expression of the YAP oncogene due to genomic amplification, making them heavily reliant on YAP activity for proliferation and survival. YAP acts as a transcriptional co-activator downstream of the Hippo signaling pathway, promoting tumorigenesis when dysregulated. In SF-268 cells, high levels of YAP expression drive the activation of pro-growth and survival gene programs, directly contributing to cancer progression. The non-receptor tyrosine phosphatase PTPN14 is identified as a crucial negative regulator of YAP, binding directly and limiting its oncogenic activity by promoting cytoplasmic retention of YAP. Reduced expression of PTPN14 can mimic the effects of YAP activation, enhancing malignant transformation and survival under detached conditions (anoikis resistance), thus indicating its potential tumor-suppressive role. These interactions highlight a critical regulatory network

within SF-268 cells, emphasizing the importance of the Hippo pathway and associated proteins like YAP and PTPN14 in glioma pathology.

Animal handling and maintenance at Altogen Labs are conducted in compliance with Institutional Animal Care and Use Committee (IACUC) regulations and adhere to all Good Laboratory Practice (GLP) requirements to ensure the highest standards of ethical research. All animals are housed in a controlled vivarium environment, where they undergo an acclimation period before study initiation. Mice are sorted based on body mass to maintain consistency across study groups and are monitored daily for tumor development, overall health, and clinical signs of distress. Veterinary staff and research personnel conduct routine health checks to ensure animal welfare and data integrity throughout the study. Detailed documentation, including experimental procedures, health reports, and study observations, is meticulously maintained to provide a comprehensive dataset for analysis. Clients receive an all-inclusive study report that encompasses methods, results, discussions, raw data, and statistical analysis, ensuring transparency and reproducibility of research findings.

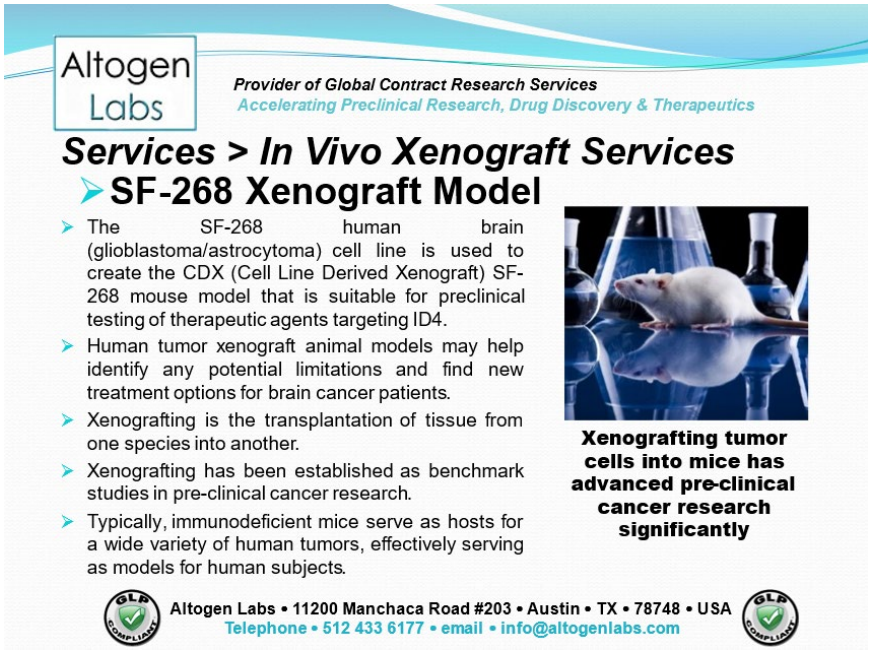

Figure 4. Available *in vivo* xenograft services at Altogen Labs for SF-268 (Altogen Labs).

The SF-268 cell line, derived from a central nervous system tumor, is widely utilized in preclinical brain cancer research due to its highly invasive nature and resistance to chemotherapy and radiation. The SF-268 xenograft model serves as a critical tool for studying tumor progression and therapeutic responses in a biologically relevant *in vivo* system. This model enables the investigation of tumor growth mechanisms, metastasis, and responses to various treatments, including chemotherapy, radiation, and immunotherapy. Altogen Labs offers multiple study options for the SF-268 xenograft model, including tumor growth delay (TGD) and tumor growth inhibition (TGI) studies, diverse dosing regimens and administration routes, immunohistochemistry, blood chemistry analysis, toxicity and survival assessments, and imaging studies such as fluorescence-based whole-body imaging. Additionally, alternative cell engraftment sites, including orthotopic transplantation and metastasis models, can be utilized. Positive control groups with cyclophosphamide treatment are available, along with metabolic and lipid distribution assays to support oncology research.

Figure 5. *In vivo* toxicology services provided by Altogen Labs (Altogen Labs).

Patient-Derived Organoids as Key Models in Precision Oncology

Organoids are three-dimensional (3D) culture systems that closely mimic the genetic and phenotypic complexity of patient-derived tumors. Unlike traditional two-dimensional cell cultures, organoids maintain the intricate tissue architecture and cellular heterogeneity found within original tumors, preserving genetic alterations, cellular differentiation patterns, and phenotypic diversity. Derived directly from primary patient samples, organoids offer a reliable and efficient approach for expanding tumor cells while maintaining their essential biological characteristics. This makes them particularly valuable in personalized oncology research, where treatment efficacy can vary significantly between individual tumors. While traditional xenograft and allograft models in animals offer important insights by incorporating tumor-stroma interactions and immune responses, organoids uniquely bridge the gap by enabling high-throughput and rapid assessment of therapeutic responses *in vitro*. Consequently, the establishment of patient-derived organoid libraries represents a major advancement, facilitating high-throughput drug screening and enabling precision medicine strategies tailored to individual patient tumor profiles. Their genetic stability, combined with ease of scalability and preservation of primary tumor heterogeneity, positions organoids as pivotal tools for the advancement of cancer therapeutics and personalized medicine strategies.

References:

Gravina GL, Mancini A, Colapietro A, Delle Monache S, Sferra R, Pompili S, Vitale F, Martellucci S, Marampon F, Mattei V, Biordi L, Sherris D, Festuccia C. The Brain Penetrating and Dual TORC1/TORC2 Inhibitor, RES529, Elicits Anti-Glioma Activity and Enhances the Therapeutic Effects of Anti-Angiogenetic Compounds in Preclinical Murine Models. *Cancers (Basel)*. 2019 Oct 21;11(10):1604. doi: 10.3390/cancers11101604. PMID: 31640252; PMCID: PMC6826425.

Guillard, S., Clarke, P. A., te Poele, R., Mohri, Z., Bjerke, L., Valenti, M., … Workman, P. (2009). Molecular pharmacology of phosphatidylinositol 3-kinase inhibition in human glioma. *Cell Cycle*, *8*(3), 443–453. https://doi.org/10.4161/cc.8.3.7643

Li Z, Li M, Xia P, Lu Z. HOTTIP Mediated Therapy Resistance in Glioma Cells Involves Regulation of EMT-Related miR-10b. *Front Oncol*. 2022 Mar 24;12:873561. doi: 10.3389/fonc.2022.873561. PMID: 35402278; PMCID: PMC8987496.

Michaloglou C, Lehmann W, Martin T, Delaunay C, Hueber A, Barys L, Niu H, Billy E, Wartmann M, Ito M, Wilson CJ, Digan ME, Bauer A, Voshol H, Christofori G, Sellers WR, Hofmann F, Schmelzle T. The tyrosine phosphatase PTPN14 is a negative regulator of YAP activity. *PLoS One*. 2013 Apr 16;8(4):e61916. doi: 10.1371/journal.pone.0061916. PMID: 23613971; PMCID: PMC3628344.

Zhou, X., Liu, J., Zhang, J. *et al.* Flubendazole inhibits glioma proliferation by G2/M cell cycle arrest and pro-apoptosis. *Cell Death Discovery* 4, 18 (2018). https://doi.org/10.1038/s41420-017-0017-2

Keywords: SF-268, brain cancer, xenograft, brain, *in vivo*, cancer, preclinical, research, *in vivo* pharmacology, organoids, glioblastoma, CDX, PDX

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/

HCC1954 Xenograft Model: https://altogenlabs.com/xenograft-models/breast-cancer-xenograft/hcc1954-xenograft-model/

T-47D Xenograft Model: https://altogenlabs.com/xenograft-models/breast-cancer-xenograft/t-47d-xenograft-model/

ZR-75-1 Xenograft Model: https://altogenlabs.com/xenograft-models/breast-cancer-xenograft/zr-75-1-xenograft-model/