Validated SF295 Xenograft Model:
Subcutaneous Xenograft Tumor Model

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Leveraging Xenograft Models for Brain Cancer Drug Testing

Brain cancer encompasses a wide range of malignancies that arise from different cell types within the brain, with gliomas, including glioblastoma multiforme (GBM), being among the most prevalent and aggressive. Xenograft models, particularly patient-derived xenografts (PDXs) and cell line-derived xenografts (CDXs), play a critical role in preclinical research to study brain cancer biology and evaluate novel therapeutic approaches. CDXs are established by implanting cultured cancer cell lines into immunocompromised mice, providing a consistent and reproducible model for drug testing. PDXs, on the other hand, involve the implantation of primary tumor tissue directly from a patient into immunodeficient mice, retaining much of the genetic and phenotypic heterogeneity of the original tumor. These models are invaluable for mimicking the complex interactions between brain tumor cells and the microenvironment, allowing for more accurate predictions of treatment efficacy. PDX models are particularly useful for testing personalized medicine strategies and identifying potential biomarkers for targeted therapies in brain cancer.

SF295 Cell Line

The SF295 cell line was established in the late 1980s from a 67-year-old female patient diagnosed with glioblastoma, a highly aggressive form of malignant astrocytoma. As one of the most widely used models in brain cancer research, SF295 is characterized by a late-stage phenotype, making it important for studying advanced glioblastoma biology. The cell line is homozygous for both PTEN and TP53 mutations, which are common alterations in glioblastoma and contribute to its oncogenic properties, including tumor progression and resistance to therapy. Glioblastomas, as highlighted by the American Brain Tumor Association, are the leading cause of cancer-related fatalities in children, underscoring the importance of such models for therapeutic research. SF295 serves as a valuable model for evaluating new treatment strategies, particularly in understanding the molecular mechanisms driving glioblastoma's aggressiveness. Its utility in drug screening and mechanistic studies makes it essential for preclinical glioma research, especially in the context of targeting genetic mutations like PTEN and TP53.

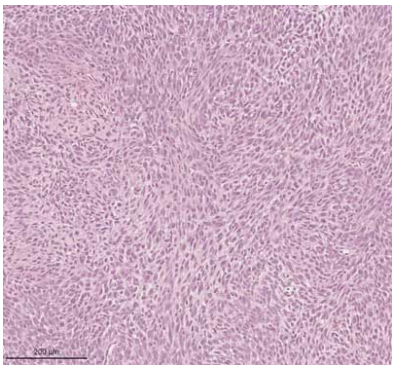

Figure 1. Tumor Histology. H&E stained section of a subcutaneously-implanted SF295 tumor (Altogen Labs).

Altogen Labs Validated SF295 Xenograft Model

At Altogen Labs, in preclinical studies, SF295 cells are cultured under conditions that promote exponential growth, with preparation achieved through trypsinization. Viable cell counts are determined using trypan blue exclusion, and the cell suspension is diluted to the required working concentration. For xenograft establishment, one million SF295 cells (1 x 10^6 cells) in 100 µL of 50% Matrigel solution are subcutaneously injected into the hind leg flank of 10 to 12-week-old athymic BALB/C or NOD/SCID mice. The mice receive a single injection, and tumor establishment is monitored closely. Following tumor formation, animals are grouped into cohorts for treatment according to the scheduled protocol. Tumor growth is assessed daily, and animal weights are recorded up to three times a week. Once tumor size reaches predetermined limits, the study concludes, and tumors are excised, weighed, and imaged for documentation.

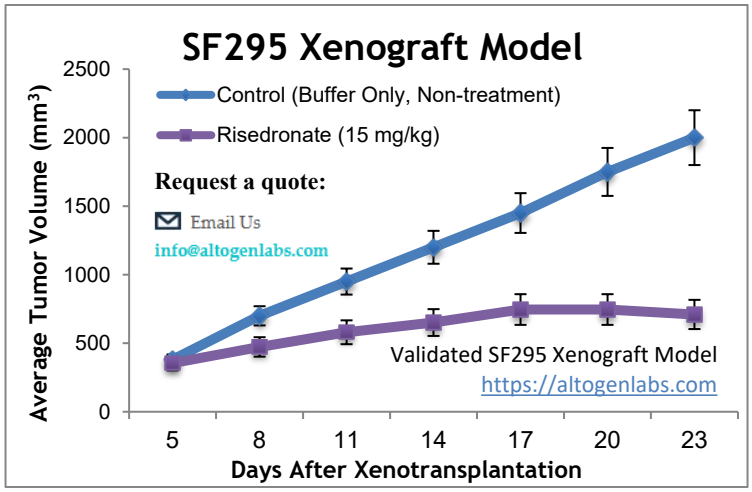

Figure 2. Tumor volume measurements of SF295 glioblastoma xenografts in immunocompromised mice following xenotransplantation. Data are presented as mean tumor volume (± SEM) over time (Altogen Labs).

Downstream analysis of excised tumors involves tissue collection, which is then snap frozen, immersed in RNAlater, or processed for protein, RNA, and DNA isolation. Xenograft models, including both subcutaneous and orthotopic models in immunocompromised mice, are pivotal in assessing the effectiveness of new cancer therapies. These models, employed for evaluating clinically approved anti-cancer agents, require meticulous planning, from choosing the appropriate animal model to tumor growth monitoring, treatment administration, and final analysis (histology, gene and protein expression). Altogen Labs offers extensive laboratory services, utilizing over 90 Cell Line Derived Xenograft (CDX) models and more than 30 Patient-Derived Xenograft (PDX) models, complemented by quantitative gene expression analysis (RT-PCR) and protein expression profiling using the WES system (ProteinSimple). Experimental dosing begins when the mean tumor volume reaches a specific size, typically between 75-125 mm^3, marking the initiation of treatment for staged studies.

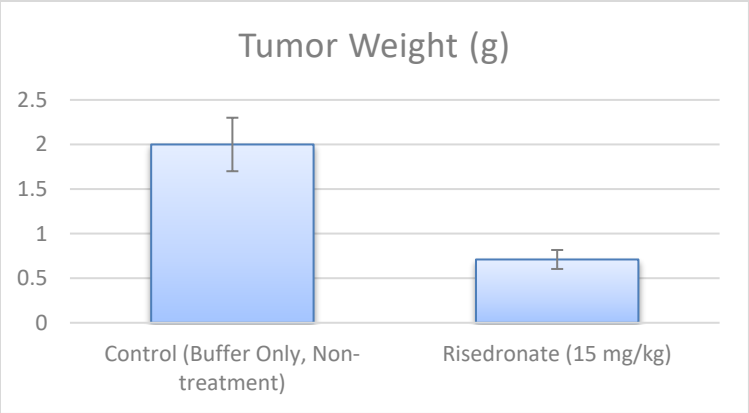

Figure 3. Final tumor weights in SF295 xenograft-bearing mice treated with risedronate (15 mg/kg) compared to non-treated controls (buffer only). Results indicate a significant reduction in tumor mass in the treated group, supporting the potential anti-tumor effect of risedronate in this glioblastoma model (Altogen Labs).

SF295 Subcutaneous Xenograft Model for Glioblastoma Research

The subcutaneous SF295 xenograft model is widely used in preclinical research to study glioblastoma, a highly aggressive form of brain cancer. Derived from a 67-year-old female patient with glioblastoma, SF295 cells are implanted subcutaneously into immunocompromised mice, typically BALB/C or NOD/SCID strains, to create a tumor microenvironment that mimics the human disease. The model allows for the evaluation of tumor growth dynamics, therapeutic efficacy, and molecular responses to treatment. Tumor volume is monitored regularly, and once tumors reach a predetermined size, they are excised and analyzed for histological and molecular markers. This model is particularly valuable for testing novel drug candidates, assessing drug toxicity, and understanding the genetic alterations characteristic of glioblastoma, such as mutations in PTEN and TP53. By using the SF295 model, researchers can identify promising therapies and better understand glioblastoma progression and resistance mechanisms. It provides a reliable and reproducible platform for developing targeted treatments for this challenging cancer.

Oncogenic Alterations and Drug Resistance in SF295 Glioblastoma Cells

The SF295 cell line, derived from a human glioblastoma, serves as a key model in understanding drug resistance mechanisms in cancer research. Notably, SF295 cells exhibit mutations in key oncogenes, including PTEN and TP53, which contribute to tumor progression and resistance to therapy. The resistance phenotype in SF295 cells is prominently linked to decreased expression of DNA topoisomerase I (Top1), a critical enzyme targeted by many chemotherapy drugs such as camptothecin and its analogs. In studies involving the SF295 sublines SF295/hCPT50 and SF295/BN50, which were selected for resistance to topoisomerase inhibitors, the reduced Top1 expression is primarily regulated at the transcriptional level. This reduction in Top1 impairs the formation of DNA-protein crosslinks, a key process in the action of Top1 inhibitors, thus conferring resistance to these drugs.

In addition to the loss of Top1 expression, the SF295 cell line demonstrates cross-resistance to multiple topoisomerase I inhibitors, but remains sensitive to other classes of drugs, including topoisomerase II inhibitors like mitoxantrone and etoposide. This highlights the significance of Top1 in mediating resistance and suggests the potential use of Top2 inhibitors in treating SF295 models with acquired resistance to Top1-targeting agents. Despite the development of resistance, the SF295 model remains essential for investigating the molecular mechanisms of glioblastoma and testing new therapeutic strategies, particularly in overcoming Top1-related resistance.

Case Study: Mechanisms of Resistance to Homocamptothecins in SF295 Glioblastoma Cells

In a study conduced by Liao Z, *et al.*, published by *Molecular Pharmacology* journal, researchers focused on the development of two resistant human glioblastoma cell lines, SF295/hCPT50 and SF295/BN50, derived from the SF295 parental line. These resistant lines were created by exposing the SF295 cells to increasing concentrations of homocamptothecin (hCPT) and diflomotecan (BN80915), respectively. The SF295/hCPT50 and SF295/BN50 cell lines demonstrated 15- to 22-fold resistance to hCPT and BN80915, as well as cross-resistance to other topoisomerase I inhibitors, such as topotecan and camptothecin, while maintaining sensitivity to topoisomerase II inhibitors like mitoxantrone and etoposide. This resistance was attributed to a reduction in the expression of DNA topoisomerase I (Top1) protein, primarily at the transcriptional level, rather than due to changes in drug transporter expression or mutations in the target enzyme. The study further investigated potential mechanisms of resistance, such as the role of drug transporters like P-glycoprotein (Pgp), multidrug resistance-associated protein 1 (MRP1), and ABCG2. However, these transporters were not overexpressed in the resistant cell lines, suggesting that they did not contribute to the observed resistance. Additionally, treatment with DNA methyltransferase or histone deacetylase inhibitors did not restore Top1 expression, ruling out epigenetic modifications as a cause for the reduced Top1 levels. Furthermore, the resistant cells exhibited slower S-phase progression and a slower growth rate, which may contribute to the reduced efficacy of hCPTs.

Additional Case Study: Targeting DNA Repair Pathways to Overcome TMZ Resistance in Glioblastoma

A different study conducted by Li J., *et al.*, published by *Cancers* journal, evaluated strategies to overcome temozolomide (TMZ) resistance in glioblastoma (GBM) by enhancing NAD+ bioavailability and inhibiting poly(ADP-ribose) glycohydrolase (PARG). TMZ, a standard treatment for GBM, faces resistance due to high expression of MGMT (O6-methylguanine-DNA methyltransferase) or defects in the mismatch repair (MMR) pathway. The authors hypothesize that increasing NAD+ levels with the precursor dihydronicotinamide riboside (NRH) and inhibiting PARG can potentiate TMZ's effects. The combination of TMZ, NRH, and PARG inhibitor (PARGi) significantly enhanced cytotoxicity in TMZ-resistant GBM cell lines, including those with MGMT overexpression or MSH6 mutations. This approach led to a hyperaccumulation of poly(ADP-ribose) (PAR), an important signal in the base excision repair (BER) pathway, inhibiting DNA repair. This resulted in elevated DNA damage and apoptosis, highlighting the potential of NRH + PARGi combination therapy in overcoming TMZ resistance. The regimen was effective across multiple GBM cell lines, suggesting its broad applicability for treating resistant GBM.

Synergistic Antitumor Effects of BACPTDP in Combination with Gemcitabine

BACPTDP is a water-soluble camptothecin pro-drug designed to exploit the acidic tumor microenvironment for enhanced drug uptake and activity. It demonstrates potent antitumor effects across a range of cancer types, including glioma, where it exhibits comparable efficacy to irinotecan in SF295 glioma xenografts. The drug’s mechanism relies on its ability to remain active under the acidic condition’s characteristic of many solid tumors, where it is selectively taken up and retained. BACPTDP’s therapeutic activity is further enhanced when combined with other chemotherapeutic agents like gemcitabine, especially in models such as pancreatic cancer, where it shows synergistic effects. This synergy is notably observed when gemcitabine is administered prior to BACPTDP, significantly improving therapeutic outcomes. Additionally, BACPTDP's potential for reduced toxicity and improved tumor targeting makes it a promising candidate for treating cancers with hypoxic, acidic microenvironments, such as pediatric neuroblastoma and pancreatic carcinoma.

Metabolic Reprogramming in SF295 Glioma Cells Following Schweinfurthin Treatment

Schweinfurthins, a class of natural compounds, exhibit potent anticancer activity in certain cell lines, with SF295 glioma cells showing remarkable sensitivity at nanomolar concentrations. These compounds induce significant metabolic disruptions in SF295 cells, particularly affecting pathways involved in energy production, such as the tricarboxylic acid (TCA) cycle and oxidative phosphorylation. In addition to metabolic changes, schweinfurthins activate stress-response and signaling pathways, including PDGF and Toll receptor signaling, that are crucial for cellular adaptation to treatment. However, not all cell lines exhibit the same sensitivity; for instance, A549 lung cancer cells are more resistant to schweinfurthins. This resistance is linked to the differential regulation of pathways such as Hedgehog signaling, which is upregulated in A549 cells after treatment. In contrast, SF295 cells do not show such activation, suggesting that the Hedgehog pathway plays a key role in mediating resistance. Interestingly, inhibition of the Hedgehog pathway using specific inhibitors can enhance the sensitivity of resistant cells to schweinfurthin treatment, offering a potential strategy to overcome resistance and improve therapeutic outcomes.

The SF295 cell line, derived from human glioblastoma, is a pivotal model used in cancer research, especially for studying brain tumors like glioblastoma multiforme (GBM). Altogen Labs utilizes the SF295 xenograft model to investigate the efficacy of various treatments, including chemotherapy, radiation, and targeted therapies for brain tumors. The animal handling and maintenance at Altogen Labs are IACUC-regulated and GLP-compliant, ensuring high standards of ethical and scientific rigor. Following acclimation to the vivarium environment, mice are sorted based on body mass and closely monitored for tumor growth and clinical signs. Tumor measurements are taken daily, and whole-body mouse weights are recorded up to three times a week. Upon reaching predetermined tumor size limits, tumors are excised, weighed, and documented, with detailed experimental procedures, health reports, and data provided to clients. This comprehensive service from Altogen Labs includes all-inclusive reports containing methods, results, discussion, raw data, and statistical analysis, enabling robust assessments of therapeutic efficacy.

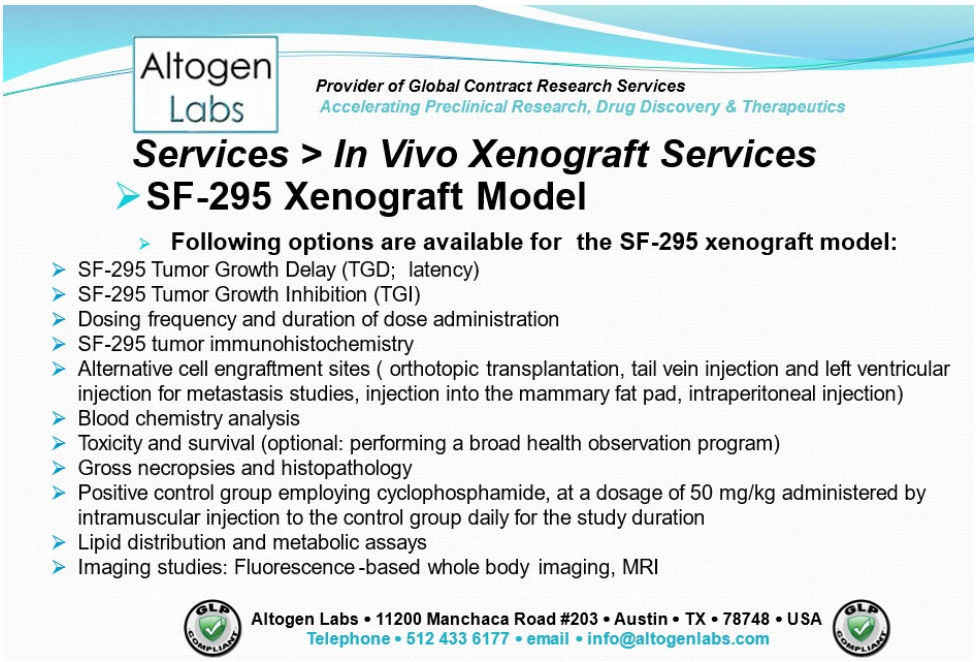

Figure 4. Available *in vivo* xenograft services at Altogen Labs for SF295.

Altogen Labs offers a wide array of preclinical research services using over 90 standard Cell Line Derived Xenograft (CDX) models and more than 30 Patient-Derived Xenograft (PDX) models, including the SF295 glioblastoma model. Their offerings include tumor growth delay (TGD), tumor growth inhibition (TGI), and extensive analysis of tumor biology, such as histology, gene expression, and protein analysis. Altogen Labs provides flexibility in dosing methods, including intravenous, intratumoral, oral, and advanced micro-injection techniques. Their services also extend to advanced imaging studies, immunohistochemistry (IHC), blood chemistry analysis, and histopathology, ensuring that researchers have all the necessary parts for comprehensive cancer research. By supporting in-depth exploration of therapeutic responses, Altogen Labs plays a crucial role in the development of new treatments for glioblastoma and other cancers.

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

Subculturing SF295 Cells

To subculture SF295 cells, begin by ensuring that the recommended medium is on hand, as cells should not be thawed until the appropriate medium is available. The SF295 Expansion Medium consists of MEM supplemented with 2 mM L-Glutamine and 10% FBS. First, remove the vial of frozen SF295 cells from liquid nitrogen and place it in a 37°C water bath. Monitor the vial closely until the cells are fully thawed. Rapid and complete thawing is essential for maximum cell viability. Once thawed, disinfect the outside of the vial with 70% ethanol and immediately transfer the cells to a sterile 15 mL conical tube using a 1 or 2 mL pipette, ensuring no bubbles are introduced. Slowly add 9 mL of SF295 Expansion Medium dropwise to the tube, taking care to avoid osmotic shock. Gently mix the cell suspension by pipetting up and down twice, again ensuring no bubbles form. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells. Afterward, decant the supernatant and remove residual cryopreservative (DMSO), then resuspend the cells in 15 mL of SF295 Expansion Medium. Transfer the cell mixture to a T75 tissue culture flask and incubate at 37°C in a humidified incubator with 5% CO_2.

For subculturing, it is important not to allow the cells to grow to confluency; they should be passaged at approximately 80-85% confluence. Start by carefully removing the medium from the flask, then rinse the cells with 10 mL of 1X PBS and aspirate the rinse. Apply 5-7 mL of Trypsin and incubate for 5 minutes at 37°C. Gently tap the side of the flask to ensure complete cell detachment. After detachment, add 5-7 mL of SF295 Expansion Medium to neutralize the trypsin, then gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube and centrifuge at 300 x g for 3-5 minutes. Discard the supernatant, loosen the cell pellet, and resuspend the cells thoroughly in 2-5 mL of SF295 Expansion Medium. Count the cells using a hemocytometer, then plate them to the desired density, with a typical split ratio of 1:5 to 1:6.

Organoids as a Scalable Model for Cancer Therapy Evaluation

Organoids are three-dimensional *in vitro* models derived from patient tumor samples that maintain the key genetic, phenotypic, and architectural features of the original tumor. Unlike traditional two-dimensional cell cultures, organoids preserve the complex tissue architecture of the tumor and can be efficiently expanded from primary patient material, offering a more physiologically relevant system for cancer research. This ability to retain tumor-specific characteristics makes organoids a valuable for personalized cancer research and drug testing. While xenograft and allograft models are effective in providing insights into tumor-stroma and immune interactions, organoids offer a faster, scalable platform to assess therapeutic responses. Recent advancements in organoid technology have led to the establishment of patient-derived tumor organoid (PDTO) biobanks, which serve as living resources for studying cancer progression and resistance. These models are particularly valuable for high-throughput drug screening, facilitating the identification of therapies that are tailored to the specific molecular profiles of individual tumors.

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