Validated SK-N-AS Xenograft Model:

Subcutaneous, Orthotopic, And Metastatic Xenograft Tumor Model

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Xenograft Models in Neuroblastoma Research

Neuroblastoma is the most common extracranial solid tumor in children and a leading cause of pediatric cancer-related mortality, particularly in its high-risk, treatment-resistant forms. Characterized by genetic heterogeneity and variable clinical behavior, neuroblastoma presents significant challenges in both therapeutic development and outcome prediction. Traditional *in vitro* systems are limited in their ability to replicate the complex tumor microenvironment, prompting the widespread adoption of xenograft models to improve translational relevance in preclinical research. Cell line-derived xenografts (CDXs) and patient-derived xenografts (PDXs) allow for *in vivo* investigation of tumor growth, metastasis, drug sensitivity, and resistance mechanisms. Models such as SK-N-AS, which represent non-MYCN-amplified neuroblastoma, provide valuable insight into aggressive tumor phenotypes and support the evaluation of targeted therapies. The integration of xenografts into neuroblastoma research enhances the biological fidelity of preclinical studies, facilitates biomarker discovery, and contributes to the advancement of personalized treatment strategies for this complex pediatric malignancy.

SK-N-AS Cell Line

The SK-N-AS cell line is a human neuroblastoma model derived from metastatic bone marrow and is commonly used to study high-risk, non-MYCN-amplified neuroblastoma. Although it lacks MYCN amplification, SK-N-AS exhibits genetic alterations such as 1p deletion and 11q loss, both of which are linked to poor clinical outcomes. The cell line is p53 wild-type and displays notable resistance to conventional chemotherapies including vincristine and doxorubicin, making it a valuable tool for investigating drug resistance. Prior studies have explored the involvement of anti-apoptotic proteins, hypoxia-inducible factors, and drug efflux transporters in these resistant phenotypes. SK-N-AS has also been used in immune-oncology research, revealing low responsiveness to immune checkpoint blockade and evidence of immune evasion through cytokine secretion and impaired antigen presentation. However, despite extensive characterization of its genetic and signaling landscape, significant gaps remain regarding the epigenetic and metabolic adaptations that support resistance and immune escape.

Altogen Labs Validated SK-N-AS Xenograft Model

SK-N-AS cells are harvested during the exponential growth phase using trypsinization and assessed for viability using a Guava PCA flow cytometry assay, ensuring a minimum viability of 97 to 98 percent. The cell concentration is adjusted to the appropriate density required for *in vivo* injection. A suspension containing one million SK-N-AS cells mixed in a 1:1 ratio with Matrigel is prepared for subcutaneous injection. Athymic nude mice (Foxn1nu/Foxn1+), aged 10 to 12 weeks, each receive a single 100-microliter injection in the hind limb. Following inoculation, tumor establishment is monitored by palpation up to three times weekly. Once tumors reach a volume of 50 to 150 mm³, caliper measurements begin, and the in-life portion of the study is initiated. Mice are randomized into treatment cohorts as specified by the client, and the test compounds are administered accordingly. Tumor volumes and body weights are monitored daily to assess treatment efficacy and systemic effects.

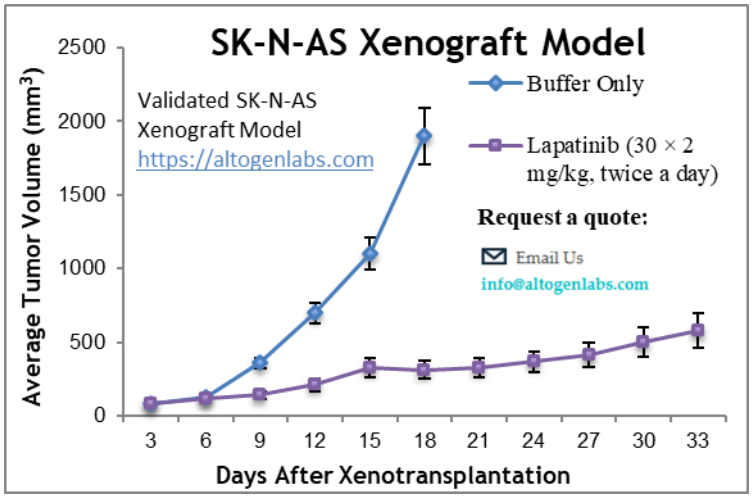

Figure 1. Average tumor volume in SK-N-AS neuroblastoma cancer xenografts in immunocompromised mice and treated with either buffer control or lapatinib. Lapatinib significantly inhibited tumor growth compared to control, mean values +/- SEM (Altogen Labs).

The study concludes when tumors reach the predetermined endpoint volume. At termination, tumors are resected, digitally imaged, and weighed. Client-directed tissue processing includes formalin fixation for histology, snap freezing, or isolation of nucleic acids for downstream analyses. Altogen Labs offers fully customizable SK-N-AS xenotransplantation services, including metabolic profiling and histopathological evaluation. The SK-N-AS xenograft model, classified as a cell line-derived xenograft (CDX), is frequently employed as a high-risk neuroblastoma model characterized by elevated expression of Polo-like kinase 1 (PLK-1). This model is particularly valuable in preclinical investigations of PLK-1 inhibitors such as BI 2536, as well as conventional tumor growth inhibitors including lapatinib, trastuzumab, and rosiglitazone. Through expertise in xenotransplantation, Altogen Labs provides reliable and translationally relevant preclinical models to support oncology drug discovery and mechanistic studies.

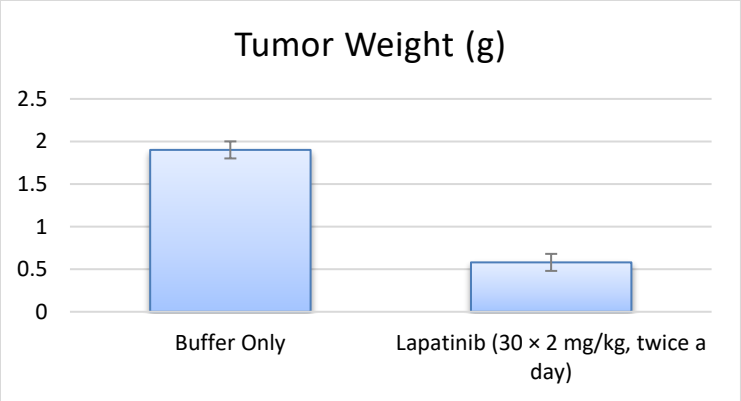

Figure 2. Final tumor weights of SK-N-AS xenografts collected at study endpoint from mice treated with buffer only or lapatinib. Lapatinib treatment resulted in a significant reduction in tumor mass compared to control (Altogen Labs).

Subcutaneous SK-N-AS Xenografts in Neuroblastoma Research

Subcutaneous xenograft transplantation using the SK-N-AS neuroblastoma cell line provides a robust and reproducible model for studying high-risk, non-MYCN-amplified neuroblastoma *in vivo*. SK-N-AS cells, which exhibit chemoresistance, wild-type TP53 expression, and chromosomal alterations including 1p and 11q loss, are injected subcutaneously into immunocompromised mice to generate rapidly growing tumors suitable for pharmacological evaluation. This model has been widely used to investigate drug responses, particularly to Polo-like kinase 1 inhibitors such as BI 2536 and volasertib, which have shown significant anti-tumor activity in preclinical studies. Although the subcutaneous environment does not fully replicate the anatomical context of neuroblastoma, the model remains valuable for its ease of use, high engraftment rates, and compatibility with longitudinal tumor measurements. Our studies have demonstrated that SK-N-AS xenografts display distinct metabolic reprogramming in response to targeted therapies, including alterations in oxidative phosphorylation and glycolysis that may underlie resistance. By integrating histological, transcriptomic, and metabolic analyses, this model serves as an essential platform for preclinical screening, mechanistic discovery, and drug prioritization in early-stage neuroblastoma research.

Modeling Site-Specific Tumor Dynamics with SK-N-AS Orthotopic Xenografts

Orthotopic xenograft transplantation using the SK-N-AS neuroblastoma cell line provides a biologically relevant *in vivo* model for studying high-risk, non-MYCN-amplified neuroblastoma within its native microenvironment. By injecting SK-N-AS cells into the adrenal gland or paraspinal region of immunodeficient mice, researchers replicate the anatomical context of tumor growth, enabling more accurate evaluation of tumor-stromal interactions, local invasion, angiogenesis, and therapeutic response. Although SK-N-AS has a relatively low metastatic potential compared to MYCN-amplified lines, orthotopic implantation has produced localized tumors and, in some cases, limited metastatic spread. These models have been used to investigate context-sensitive therapies, including anti-angiogenic agents and spatially targeted drug delivery methods, with imaging techniques such as MRI and bioluminescence facilitating non-invasive tumor tracking. While technically demanding, orthotopic SK-N-AS models offer enhanced physiological relevance and have been critical in identifying biomarkers of therapeutic resistance and local progression. When combined with multi-omics analyses, this model significantly contributes to the refinement of neuroblastoma treatment strategies and advances translational cancer research.

SK-N-AS Metastasis Models for Drug Testing

Metastatic xenograft transplantation using the SK-N-AS neuroblastoma cell line provides a vital platform for modeling the systemic progression of high-risk, non-MYCN-amplified neuroblastoma. Although SK-N-AS cells exhibit modest intrinsic metastatic capacity, their injection via intravenous or intracardiac routes into immunodeficient mice has enabled the development of experimental models that recapitulate multi-organ dissemination, including colonization of the liver, lungs, and bone marrow. These models have been instrumental in studying metastatic organotropism, drug distribution, and resistance mechanisms. Transcriptomic analyses of SK-N-AS-derived metastatic lesions have revealed dysregulation of

adhesion molecules, chemokine signaling, and epithelial-to-mesenchymal transition pathways associated with invasive behavior. Moreover, they provide a clinically relevant context for evaluating novel therapies targeting residual disease and tissue-specific metastases, particularly in pharmacologically challenging niches such as bone marrow. Our own investigations using metastatic SK-N-AS models have demonstrated site-specific differences in therapeutic response to epigenetic and immunomodulatory agents. While technical variability remains a limitation, these models offer critical insight into the biology of neuroblastoma metastasis and are essential for advancing the preclinical development of anti-metastatic strategies.

COX-2 as a Driver of Neuroblastoma Bone Lesions

The SK-N-AS neuroblastoma cell line serves as a valuable model for investigating bone metastasis in pediatric cancer, offering insight into how tumor cells colonize and degrade bone tissue. When introduced into the circulation of immunocompromised mice, SK-N-AS cells consistently form osteolytic lesions driven by enhanced osteoclast activity, elevated cyclooxygenase-2 (COX-2) expression, and increased secretion of prostaglandin E2 (PGE2). These factors promote both bone resorption and angiogenesis, creating a microenvironment favorable to tumor expansion. Inhibiting COX-2 significantly reduces bone destruction, osteoclast recruitment, and vascular endothelial growth factor (VEGF) expression, highlighting its central role in metastatic progression. Notably, this pathway appears to operate independently of parathyroid hormone-related protein (PTHrP), suggesting mechanistic diversity among neuroblastoma subtypes. While the experimental design offers strong physiological relevance through intracardiac injection and co-culture systems, broader validation across additional cell lines is needed. Overall, SK-N-AS provides a clear framework for understanding tumor-bone interactions and supports further research into COX-2 inhibition as a therapeutic strategy to limit neuroblastoma metastasis and its skeletal complications.

Case Study: SK-N-AS Xenografts Respond to Targeted Therapy

The SK-N-AS neuroblastoma cell line, characterized by a non-MYCN-amplified high-risk phenotype, has been shown by Sprüssel A, *et al*., in a study published by *Journal of Clinical Medicine*, to respond effectively to targeted inhibition of KDM1A, a histone demethylase implicated in tumor progression and poor clinical outcomes. Treatment with the small molecule inhibitor NCL-1 reduced cell viability, suppressed proliferation, and induced apoptosis in SK-N-AS both *in vitro* and *in vivo*. Notably, NCL-1 also increased expression of neuronal differentiation markers such as NTS and MAP2 and promoted neurite outgrowth, suggesting a phenotypic shift from undifferentiated to differentiated cellular states. In subcutaneous xenograft models, NCL-1 significantly suppressed SK-N-AS tumor growth without inducing observable toxicity, highlighting its potential as a well-tolerated therapeutic. In addition to tumor-intrinsic effects, the study demonstrated an anti-angiogenic response, including a decrease in PECAM1-positive endothelial cells and disrupted vascular architecture within the tumors, indicating a broader role for KDM1A in regulating tumor vasculature.

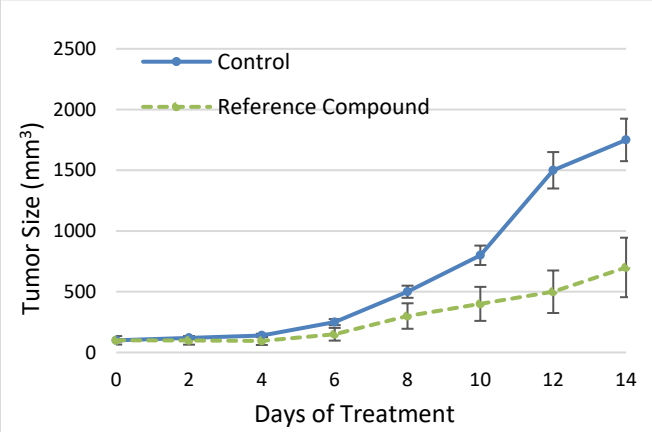

Figure 3. Treating SK-N-AS xenografted tumors with a reference compound (5 mg/kg, i.p.) resulted in significant inhibition of tumor growth compared to the control group over a 14-day period.

Patterns within the data underscore a dual mechanism of action for KDM1A inhibition in SK-N-AS: the induction of neuronal differentiation and impairment of angiogenic support. The use of both *in vitro* cell-based assays and *in vivo* xenograft models adds robustness to the findings, although the reliance on a single compound and limited exploration of resistance pathways may constrain generalizability. The observed downregulation of HIF1A in hypoxic tumor regions further supports the link between KDM1A activity and hypoxia-driven tumor adaptation. By targeting KDM1A, the study provides evidence that SK-N-AS cells depend on this epigenetic regulator to maintain their proliferative, undifferentiated state. These findings open new avenues for therapeutic intervention in non-MYCN-amplified neuroblastoma. Future studies should focus on combination therapies that pair KDM1A inhibitors with agents targeting complementary pathways such as angiogenesis or metabolic reprogramming to further enhance treatment efficacy.

Epigenetic Reprogramming of SK-N-AS Cells

SK-N-AS neuroblastoma cells can be epigenetically reprogrammed to acquire stable stem cell-like properties, offering a valuable model for studying tumor-initiating behavior in non-MYCN-amplified neuroblastoma. Upon transient treatment with DNA methylation inhibitors, SK-N-AS cells upregulate core stemness factors such as SOX2, POU5F1, and KLF4, and express surface markers like CD133 and CXCR4. These induced cells maintain sphere-forming capacity and stem-like characteristics for extended periods in culture without further treatment. In xenograft models, even small numbers of reprogrammed SK-N-AS cells are capable of initiating tumors with high efficiency, producing undifferentiated large-cell neuroblastomas that histologically resemble the most aggressive human cases. This phenotype is marked by large vesicular nuclei, high mitotic index, and uniform cell morphology, features associated with poor clinical prognosis.

Several key patterns emerge from this reprogramming model. Increased expression of stemness genes correlates with elevated tumorigenic potential, enhanced MYC protein expression, and aggressive histopathological features. CXCR4, a chemokine receptor often linked to metastatic signaling, is strongly expressed in reprogrammed SK-N-AS tumors but absent in tumors derived from non-reprogrammed cells, suggesting a role in maintaining the tumor-initiating phenotype. Moreover, these reprogrammed cells display dramatically increased sensitivity to heat shock protein 90 (Hsp90) inhibitors, with rapid downregulation of MYC following treatment. This suggests a therapeutic vulnerability that may be exploited in targeting stem-like neuroblastoma populations. While the approach highlights the plasticity of neuroblastoma cells under epigenetic influence, further investigation is needed to clarify the molecular mechanisms that stabilize this phenotype and determine whether similar vulnerabilities exist across diverse genetic backgrounds. This model underscores the importance of epigenetic state in regulating tumor initiation and provides a foundation for developing therapies directed at aggressive, undifferentiated neuroblastoma.

Additional Case Study: SK-N-AS and Resistance to Glutamine Inhibition

In their study published by *PLOS One* journal, Olsen RR, *et al*. investigate glutamine metabolism as a therapeutic vulnerability in neuroblastoma, using the SK-N-AS cell line as a representative model of low Myc expression. Among six neuroblastoma cell lines analyzed, SK-N-AS exhibited resistance to apoptosis when treated with 6-diazo-5-oxo-L-norleucine (DON), a glutamine antagonist, showing primarily cytostatic rather than cytotoxic effects. In contrast, high-Myc-expressing lines such as IMR32 underwent apoptosis under the same treatment. When N-Myc was overexpressed in SK-N-AS, DON sensitivity increased substantially, suggesting that Myc status modulates the apoptotic response to glutamine inhibition. *In vivo*, SK-N-AS xenografts in mice demonstrated reduced tumor proliferation following DON administration, though marked apoptosis was observed only with elevated Myc levels or higher drug concentrations. These findings position SK-N-AS as a key model for understanding Myc-independent resistance to metabolic therapy and emphasize the selective nature of glutamine-targeted interventions.

The correlation between Myc expression and DON efficacy was further validated through combination therapy with the Bcl-2 family inhibitor ABT-263, which showed additive effects in SK-N-AS and strong synergy in Myc-high lines. This reinforces the idea that Myc status serves as a predictive marker for therapeutic response. The study's methods, employing diverse *in vitro* and *in vivo* systems, including SK-N-AS xenografts, were robust and well-controlled, though limited by small cohort sizes and the use of a legacy compound with known toxicities. Nonetheless, the research offers valuable insights into resistance mechanisms in low-Myc tumors and supports combination approaches for improving treatment outcomes. Further investigation into compensatory pathways in SK-N-AS and the development of next-generation glutamine metabolism inhibitors are warranted to enhance therapeutic potential.

FZD2 Signaling Drives SK-N-AS Tumor Growth

Frizzled2 (FZD2) signaling has emerged as a critical regulator of neuroblastoma cell behavior, particularly in high-risk forms of the disease. In SK-N-AS cells, which lack MYCN amplification, FZD2 influences both β-catenin-dependent and β-catenin-independent Wnt signaling pathways. These dual-signaling axes orchestrate various oncogenic behaviors, including proliferation, migration, and survival. Baseline signaling analysis reveals that SK-N-AS cells exhibit elevated total and active β-catenin, MYC expression, and phosphorylated ERK, in contrast to MYCN-amplified lines which display higher cyclin D1 and LRP6 phosphorylation. Functionally, FZD2 promotes SK-N-AS cell proliferation through canonical Wnt signaling components such as LRP6, β-catenin, and MYC, while also engaging non-canonical effectors like PKC and ERK. siRNA-mediated silencing of FZD2 in SK-N-AS significantly reduces tumor cell proliferation and migration *in vitro* and suppresses tumor growth *in vivo* xenografts, accompanied by lower β-catenin activity, decreased Ki67 proliferation indices, and reduced angiogenesis. These findings highlight a complex regulatory network in which FZD2 integrates inputs from multiple Wnt ligands to drive oncogenesis in SK-N-AS cells through distinct molecular cascades. The downregulation of MYC and cyclin D1 upon FZD2 knockdown, along with increased PKC and ERK phosphorylation, suggests a signaling

shift that impairs tumor growth. Rac1 activity, a marker of non-canonical pathway engagement, is also attenuated in response to FZD2 blockade, correlating with reduced cell motility. While the study underscores the role of FZD2 as a potential therapeutic target, it also raises questions about pathway compensation and receptor redundancy in the tumor microenvironment. Further research should investigate how targeting FZD2 influences signaling crosstalk, particularly the balance between Wnt-driven proliferation and differentiation programs in neuroblastoma subtypes like SK-N-AS.

Chromosomal Adaptation and Drug Resistance in Neuroblastoma

The SK-N-AS neuroblastoma cell line, which lacks MYCN amplification, provides an instructive model for understanding how drug resistance and oncogene regulation evolve under chemotherapeutic pressure. Upon long-term exposure to cisplatin, SK-N-AS cells (designated SK-N-ASCDDP) exhibit increased MYCN mRNA expression alongside chromosomal alterations, specifically gain of the 2p arm where the MYCN gene resides. This increase in MYCN expression occurs despite the absence of classical gene amplification and appears to result from clonal expansion of subpopulations with extra 2p copies, as visualized by interphase and metaphase FISH. Notably, this gain of 2p was not detected by MLPA, likely due to its averaging of signal across heterogeneous cell populations, underscoring the importance of using multiple methods for genomic analysis. The correlation between MYCN gene copy gain and elevated transcript levels in SK-N-ASCDDP supports the hypothesis that 2p gain confers a selective advantage during cisplatin treatment, potentially facilitating resistance mechanisms.

Patterns in the data further highlight how increased MYCN expression in SK-N-AS cells is not necessarily associated with amplification in the classical sense but may still result in functional consequences for tumor progression and drug resistance. While MYCN amplification is a known marker of poor prognosis, modest increases in copy number such as those seen in 2p gain can still enhance transcriptional activity and influence treatment outcomes. In SK-N-ASCDDP, MYCN overexpression does not appear to result from acute cisplatin exposure but is rather linked to long-term selection pressures, suggesting a cumulative adaptive mechanism. This phenomenon emphasizes the plasticity of neuroblastoma cells in modulating oncogene dosage to survive cytotoxic stress. The study also reinforces that high MYCN expression, even without amplification, can contribute to aggressive tumor behavior when combined with defects in apoptotic regulation. These insights broaden the understanding of MYCN-associated resistance beyond amplification status and underscore the need for therapeutic strategies that target both MYCN transcriptional regulation and genomic stability in neuroblastoma subtypes like SK-N-AS.

The SK-N-AS cell line is a human neuroblastoma model widely used in preclinical research to investigate tumor biology and evaluate therapeutic agents. Originally derived from a bone marrow metastasis in a neuroblastoma patient, SK-N-AS cells are commonly employed in xenograft studies to assess tumor growth dynamics and treatment response *in vivo*. Successful xenograft modeling requires careful consideration of variables including animal strain selection, route of administration, dosing schedule, and downstream analyses such as histology, mRNA and protein expression profiling. Altogen Labs supports such studies through its comprehensive suite of preclinical services, offering over 120 validated CDX and PDX models. For researchers studying gene function in tumor progression, Altogen Labs also develops custom-engineered SK-N-AS cell lines with stable protein overexpression or RNAi-mediated gene silencing to model oncogene and tumor suppressor function.

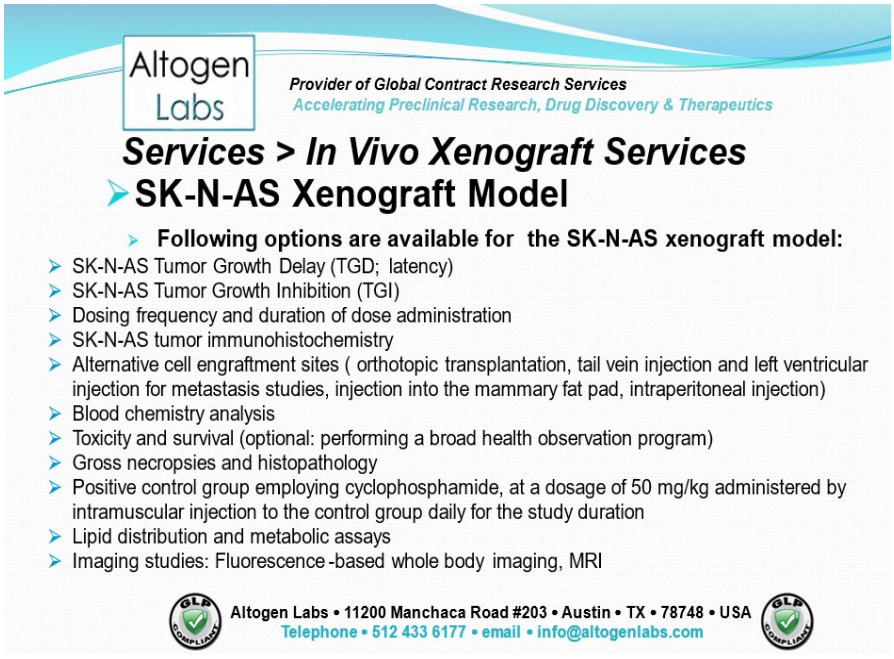

Figure 4. *In vivo* SK-N-AS xenograft services at Altogen Labs, including tumor growth studies, diverse dosing routes, immunohistochemistry, toxicology, and imaging.

All *in vivo* studies at Altogen Labs are performed in GLP-compliant, IACUC-regulated facilities with full transparency and client access to experimental protocols, raw data, statistical analysis, and final reports.Altogen Labs offers a variety of experimental designs tailored to the SK-N-AS xenograft model. These include tumor growth delay (TGD) and tumor growth inhibition (TGI) studies, with flexible options for dosing frequency, duration, and administration route, including intravenous, intraperitoneal, intratumoral, oral gavage, intranasal, subcutaneous, and orthotopic delivery. Additional capabilities include immunohistochemical analysis of tumor tissues, blood chemistry profiling, and toxicity assessment through broad health monitoring. Detailed necropsies and histopathological evaluations are available to assess systemic effects and tissue-specific responses. These customizable services enable researchers to generate high-resolution data on SK-N-AS tumor biology, drug efficacy, and safety, advancing translational research in neuroblastoma.

Figure 5. Comprehensive *in vivo* pharmacology and toxicology services offered by Altogen Labs, including acute and chronic toxicity studies, pharmacokinetics, immunotoxicology, reproductive toxicity, absorption studies, and related preclinical testing capabilities.

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