Validated SW620 Xenograft Model:

Subcutaneous, Orthotopic, And Metastatic Xenograft Tumor Model

By Altogen Labs, 11200 Menchaca Road, Suite 203, Austin, TX 78748
Phone: (512) 433-6177 | Email: info@altogenlabs.com

Xenograft Insights into Colorectal Cancer Progression

Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide, driven by its high incidence, metastatic potential, and resistance to therapy. Despite progress in targeted treatments and early detection, outcomes for patients with advanced or recurrent disease remain poor due to the molecular heterogeneity of CRC and the complex interplay between tumor cells and their microenvironment. Xenograft models, particularly those utilizing immunocompromised mice implanted with human colon cancer cells, have become essential in preclinical research for evaluating tumor biology, therapeutic efficacy, and drug resistance. While cell line-derived xenografts offer experimental consistency, patient-derived models better preserve the genetic and histological features of primary tumors. However, limitations persist in fully replicating immune interactions and metastatic processes. This study addresses these challenges by using the metastatic SW620 cell line to investigate the role of long non-coding RNAs in promoting epithelial-to-mesenchymal transition and immune evasion.

SW620 Cell Line

The SW620 cell line, derived from a lymph node metastasis of a Duke's stage C colorectal adenocarcinoma, serves as a widely utilized model for studying metastatic progression in colorectal cancer. Characterized by mutations in KRAS (G12V) and TP53, as well as aberrant Wnt/β-catenin signaling due to APC loss, SW620 exhibits enhanced invasive properties compared to its primary tumor counterpart, SW480. It displays a mesenchymal phenotype marked by reduced E-cadherin and elevated vimentin expression, along with upregulation of metastasis-associated genes such as MMP7, CXCR4, and CD44. Additionally, this cell line demonstrates increased expression of ABC transporters, contributing to chemoresistance, and shows enrichment of hypoxia-related and pro-inflammatory pathways including IL-8, VEGFA, and HIF1A. Despite its extensive use, significant gaps remain in understanding the metabolic adaptations, tumor–stroma interactions, and the regulatory role of non-coding RNAs, particularly long non-coding and circular RNAs, in SW620-mediated metastasis.

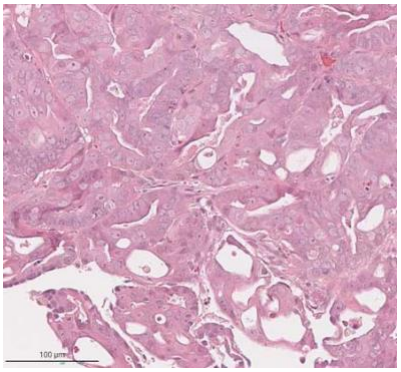

Figure 1. Tumor Histology. H&E stained section of a subcutaneously-implanted SW620 tumor (Altogen Labs).

Altogen Labs Validated SW620 Xenograft Model

Xenograft animal models are a cornerstone of preclinical oncology research, providing critical insights into the *in vivo* efficacy of investigational anti-cancer agents. These models involve the transplantation of human tumor cells into immunocompromised mice or rats, either subcutaneously or orthotopically, to mimic tumor growth in a physiologically relevant environment. Such systems allow for the controlled assessment of tumor progression, drug response, and resistance mechanisms. All clinically approved chemotherapeutic agents have undergone evaluation using xenograft platforms, which require careful planning, including the selection of appropriate cell lines, host animals, routes of administration, dosing regimens, and tumor measurement protocols to ensure translational relevance.

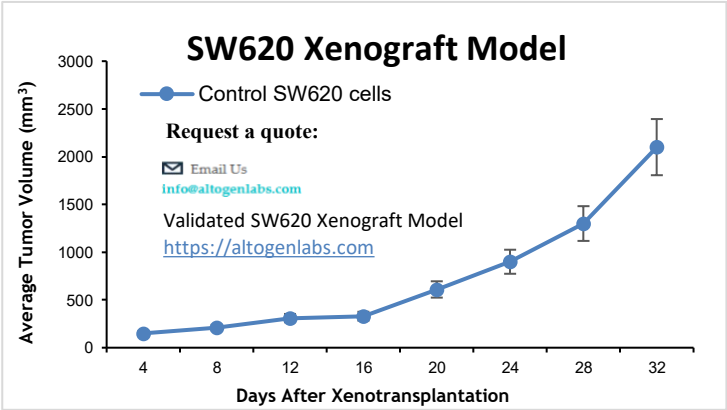

Figure 2. Tumor growth curve of the SW620 xenograft model of colorectal cancer in immunocompromised mice. Tumor volumes were measured at regular intervals, and data are presented as mean ± standard error of the mean (SEM). Study performed using Altogen Labs in-house validated SW620 xenograft model.

In this study, SW620 cells, derived from a metastatic colorectal adenocarcinoma, are maintained under conditions that promote exponential growth before inoculation. After confirming cell viability using trypan blue exclusion, cells are suspended in Matrigel and injected into the hind leg flank of 10-week-old athymic BALB/c (Nu/Nu) mice, with each injection delivering 1 x 10^6 cells in 0.1 to 0.2 mL. Tumor formation is monitored by palpation and digital caliper measurements until average volumes reach 100 to 150 mm^3, at which point animals are randomized into treatment cohorts. The test compound is administered according to a predetermined dosing schedule. Tumors are measured daily and body weights recorded three times per week. Mice are humanely euthanized when tumors reach 2,000 mm^3 or at 35 days post-injection. T umors are then excised, weighed, photographed, and processed for downstream analyses, including snap freezing, RNAlater preservation, formalin fixation for histology, or nucleic acid extraction.

Subcutaneous SW620 Xenografts in Metastatic CRC Research

Subcutaneous xenograft transplantation remains a foundational technique in preclinical cancer research, providing a reliable and reproducible platform for assessing tumor growth dynamics, therapeutic efficacy, and molecular alterations *in vivo*. The SW620 cell line, derived from a lymph node metastasis of a Duke's stage C colorectal adenocarcinoma, has been widely utilized in this context due to its aggressive phenotype and capacity to model advanced-stage colorectal cancer. When injected into immunodeficient mice, such as athymic nude or NOD/SCID strains, SW620 cells consistently form tumors within 10 to 14 days, particularly when delivered in a Matrigel matrix. These xenografts demonstrate hallmark features of metastatic tumors, including poorly differentiated histology, elevated mesenchymal markers, and hypoxia-induced signaling. Notably, they express high levels of pro-angiogenic cytokines such as VEGFA and IL-8, further underscoring their utility in modeling a hypoxic and invasive tumor microenvironment.

While subcutaneous models lack the tissue-specific architecture and metastatic behavior seen in orthotopic systems, they offer distinct advantages for high-throughput drug screening and mechanistic investigations. In the case of SW620, subcutaneous xenografts enable direct analysis of pathways involved in epithelial-to-mesenchymal transition and immune evasion. Our research has leveraged this model to explore the regulatory roles of long non-coding RNAs, revealing molecular networks that contribute to tumor progression and treatment resistance. The accessibility of subcutaneous tumors permits precise caliper measurements and facilitates tissue collection for histopathological and molecular analyses across time points. As such, this model continues to play a pivotal role in elucidating the molecular drivers of metastatic colorectal cancer and remains a critical component of the translational oncology.

SW620 Orthotopic Model for Metastatic CRC Research

Orthotopic xenograft transplantation is a powerful approach for modeling colorectal cancer with enhanced biological relevance, as it permits tumor establishment within the native anatomical site. In contrast to subcutaneous models, orthotopic implantation into the colon or rectum replicates critical aspects of the tumor microenvironment, including tissue architecture, stromal composition, and patterns of invasion. The SW620 cell line, derived from a metastatic colorectal adenocarcinoma, is particularly well-suited for orthotopic transplantation due to its aggressive phenotype and intrinsic capacity for local invasion and distant dissemination. This model supports the evaluation of tumor growth, epithelial-to-mesenchymal transition, angiogenesis, and metastatic potential in a setting that closely mirrors the clinical behavior of advanced colorectal cancer.

When introduced into the colonic or rectal wall of immunocompromised mice, SW620 cells consistently form tumors that demonstrate features of poorly differentiated adenocarcinoma, including disorganized glandular structures, stromal remodeling, and increased neovascularization. The orthotopic setting promotes interactions with surrounding tissue that influence tumor progression and response to therapy, offering a refined platform for mechanistic studies. Our investigations using this model have revealed that non-coding RNAs play a pivotal role in modulating epithelial plasticity, immune escape, and metastatic efficiency.

Modeling Colorectal Metastasis with SW620 Xenografts

Metastatic xenograft transplantation provides a powerful *in vivo* approach for investigating the biological mechanisms that underlie cancer dissemination and colonization at distant organ sites. These models are particularly valuable for replicating the complex, multi-step processes of metastasis, including intravasation, survival in circulation, extravasation, and secondary tumor formation. The SW620 cell line, derived from a lymph node metastasis of colorectal adenocarcinoma, serves as an ideal system for modeling these phenomena due to its inherently aggressive phenotype, high migratory capacity, and resistance to apoptosis during cellular detachment. Its mesenchymal gene expression profile and ability to survive under hypoxic and anchorage-independent conditions further enhance its utility in studying metastatic behavior.

Metastatic transplantation using SW620 can be performed through several injection routes, such as tail vein, intrasplenic, or orthotopic inoculation, depending on the desired site of metastatic colonization. These methods lead to the development of secondary tumors in organs such as the liver, lungs, and lymph nodes, reflecting patterns observed in advanced colorectal cancer. Resulting metastases exhibit hallmark features of invasive disease, including disorganized tissue architecture, stromal remodeling, and increased vascularization. Experimental use of this model supports the investigation of molecular drivers of metastatic progression, including signaling pathways involved in epithelial-to-mesenchymal transition, survival under stress, and immune evasion. Integration of transcriptomic and functional analyses in this context enables identification of candidate regulators that contribute to systemic dissemination and therapeutic resistance. The SW620 metastatic xenograft model thus offers a robust, clinically relevant platform for elucidating the biological underpinnings of metastasis and advancing preclinical evaluation of anti-metastatic therapies.

Case Study: Targeting FAK Y397 in SW620 Xenografts with Y11

In a study published by *Carcinogenesis* journal, Golubovskaya et al. from Roswell Park Cancer Institute and the University of Florida present a detailed study on Y11, a novel small molecule inhibitor targeting the Y397 autophosphorylation site of focal adhesion kinase (FAK). Utilizing structure-based computational modeling and functional validation, the authors identified Y11 as a selective and potent inhibitor that directly binds the N-terminal domain of FAK. The compound significantly reduced Y397 phosphorylation, impaired clonogenicity, and decreased cell viability in the SW620 colon cancer cell line in a dose-dependent manner. Importantly, Y11 induced detachment and apoptosis without affecting the viability of normal human fibroblasts. *In vivo* experiments using a subcutaneous SW620 xenograft model showed that Y11 treatment markedly suppressed tumor volume and weight, with accompanying reductions in Y397-FAK and activation of apoptosis markers PARP and caspase-3. These findings validate Y11's role as a targeted inhibitor of FAK-mediated signaling and tumor growth.

The data strongly support the authors' hypothesis that Y397 is a critical regulatory site for FAK activation and a viable therapeutic target in metastatic colorectal cancer. Compared to conventional ATP-competitive inhibitors, which often suffer from off-target effects due to conserved kinase domain homology, Y11 exhibits specificity and limited toxicity. The study's methodology, including high-throughput compound screening, *in vitro* kinase assays, Octet binding analysis, and xenograft modeling, demonstrates technical rigor and translational potential. However, limitations include the lack of pharmacokinetic characterization and the use of early treatment initiation post-cell implantation, which may not fully model advanced disease states. Nonetheless, the use of the highly metastatic SW620 line underscores the clinical relevance of this research. The work by Golubovskaya et al., published by Oxford University Press, contributes meaningfully to the field of targeted cancer therapeutics, and lays the foundation for further development of FAK inhibitors that act through allosteric modulation.

Additional Case Study: AKT3 Regulates Stemness and Metabolism in SW620 Cells

In a study published in *Frontiers in Pharmacology* journal by Bai C, et al., the authors investigate the role of AKT isoforms in regulating mitochondrial fitness and cancer stem cell (CSC) phenotypes in colorectal cancer, with a specific focus on the SW620 cell line. Key findings reveal that AKT3, unlike AKT1 and AKT2, plays a central role in maintaining the CSC-like population within SW620 cells. Using siRNA-mediated knockdown of individual AKT isoforms, the authors demonstrate that suppression of AKT3 significantly reduces the $CD44^{+}/CD24^{-}$ cell fraction, impairs tumorsphere formation, and diminishes mitochondrial respiration, without adversely affecting cell viability. Western blot and Seahorse XF analyses show that AKT3 depletion leads to a marked reduction in oxidative phosphorylation, implicating this isoform in mitochondrial integrity and energy metabolism. Moreover, *in vivo* data from SW620 xenografts indicate that silencing AKT3 results in smaller tumor volumes and lower expression of CSC markers, supporting the conclusion that AKT3 sustains tumor-initiating properties in metastatic colorectal cancer cells.

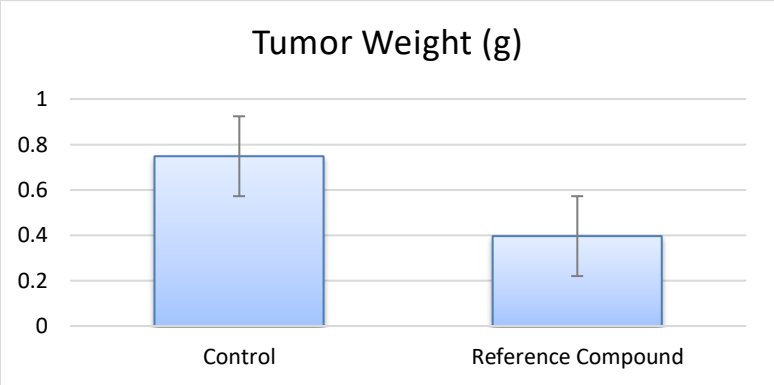

Figure 3. Tumor weights of SW620 xenografts harvested from mice treated with a reference compound (200 mg/kg) or vehicle control (buffer only). Tumor weights were recorded on Day 20 of the study and are presented as mean ± SEM.

A consistent pattern throughout the study is the specificity of AKT3 in modulating mitochondrial function and CSC maintenance, which is not observed upon knockdown of AKT1 or AKT2. These data support the authors' central thesis that AKT isoforms perform non-redundant functions in tumor biology, with AKT3 uniquely contributing to metabolic reprogramming and stemness. The methodology is rigorous, combining *in vitro* metabolic profiling, flow cytometry, and *in vivo* xenograft validation using SW620 cells. However, the study could be strengthened by including additional metastatic CRC cell lines for broader applicability, as well as by addressing potential compensatory mechanisms that may emerge upon long-term AKT3 suppression. The implications of these findings are significant, suggesting that selective targeting of AKT3 may impair the metabolic plasticity of CSCs and reduce metastatic potential in colorectal cancer. Future research should explore the downstream effectors of AKT3 and investigate whether dual inhibition of AKT3 and mitochondrial metabolism can synergistically abrogate CSC function and tumor progression.

ROS-Mediated Chemotherapy Targeting SW620 Cells

Baicalin, a plant-derived flavonoid compound, has demonstrated notable cytotoxic effects against colorectal cancer cells, particularly those with metastatic potential such as the SW620 cell line. These cells, originating from a lymph node metastasis and characterized by a mutated p53 gene, respond to baicalin with marked reductions in viability in both dose- and time-dependent manners. Morphological hallmarks of apoptosis, including nuclear condensation and membrane blebbing, accompany increased sub-G1 DNA content and Annexin V positivity, confirming the activation of programmed cell death. Mechanistically, baicalin triggers both intrinsic and extrinsic apoptotic pathways, as evidenced by the activation of caspase-9 and caspase-8, respectively, along with downstream caspase-3. A key driver of this cytotoxicity is the accumulation of reactive oxygen species (ROS), which initiate oxidative stress and contribute to mitochondrial dysfunction. The pro-apoptotic effect of baicalin is significantly attenuated by ROS scavengers, indicating that oxidative signaling is essential to its mechanism of action.

In animal models bearing SW620 xenografts, systemic baicalin administration substantially reduces tumor volume without apparent toxicity to the host. This supports the relevance of baicalin as a potential chemotherapeutic agent targeting p53-deficient metastatic colorectal cancer. Importantly, the compound's selectivity for cancerous cells over normal cells, combined with its ability to induce ROS-dependent apoptosis, aligns with broader efforts to identify non-toxic, plant-based compounds for oncological applications. In contrast, non-metastatic colorectal cancer cells such as SW480 exhibit more variable responses to chemotherapy, often requiring higher drug concentrations or combination regimens to achieve similar levels of cytotoxicity. This differential sensitivity underscores the importance of tailoring chemotherapeutic strategies to the molecular and genetic context of each tumor type. Further research should focus on defining baicalin's downstream molecular targets, its interactions with conventional chemotherapeutics, and its potential to overcome drug resistance in both primary and metastatic colorectal cancer models.

Genistein-Induced Apoptosis in SW620 Colorectal Cancer Cells

Genistein, a naturally occurring soy isoflavone, has demonstrated potent anticancer activity in metastatic colorectal cancer cells, particularly in the SW620 cell line. Treatment with genistein results in a marked, dose- and time-dependent reduction in cell viability, accompanied by classical indicators of apoptosis, including nuclear condensation, DNA fragmentation, and increased Annexin V positivity. In SW620 cells, genistein induces cell cycle arrest at the G2/M phase through the upregulation of the cyclin-dependent kinase inhibitor p21 and suppression of key mitotic regulators such as cyclin B1 and Cdc2. This disruption of cell cycle progression leads to apoptotic signaling, further evidenced by the activation of caspase-3 and the cleavage of PARP, indicating a caspase-dependent pathway of cell death.

One notable characteristic of genistein is its preferential cytotoxicity toward cancerous cells, with minimal impact on normal colon epithelial cells. This selectivity underscores its potential as a non-toxic chemopreventive agent for colorectal cancer, especially in tumors that exhibit resistance to conventional therapies. SW620, a metastatic and p53-mutant cell line, responds robustly to genistein, making it an ideal model for studying alternative therapeutic strategies. These findings suggest that genistein disrupts critical pathways associated with cell cycle regulation and survival in aggressive colorectal cancers. Further research should focus on elucidating its downstream molecular targets, assessing its pharmacologic properties *in vivo*, and determining its compatibility with existing chemotherapeutic regimens.

Oncogenic Drivers of Metastasis in SW620 Cells

SW620 is a metastatic colorectal cancer cell line characterized by aggressive behavior and dysregulated oncogenic signaling. This line exhibits overexpression of key oncogenes such as c-Met, KRAS, and c-Myc, which contribute to uncontrolled cell proliferation, survival, and invasion. Elevated c-Met expression enhances cellular motility and resistance to apoptosis, while mutations in KRAS sustain activation of downstream pathways including PI3K/AKT and MAPK/ERK, independent of upstream receptor input. The transcription factor c-Myc is also markedly upregulated, promoting increased biosynthetic activity and metabolic reprogramming in favor of rapid cell division. Together, these oncogenic drivers establish a signaling environment that supports malignant progression in SW620 cells.

Additional molecular features of SW620 reinforce its metastatic potential. There is consistent upregulation of cell cycle regulators such as cyclin D1 and CDK4, which facilitate unchecked cell cycle progression. Markers of epithelial-to-mesenchymal transition are also dysregulated, with high vimentin expression and diminished E-cadherin levels, indicating enhanced invasiveness. These coordinated molecular alterations suggest a transitionally reprogrammed phenotype optimized for tumor dissemination. The study's use of gene expression profiling and protein-level assays allows for a comprehensive understanding of transcriptional and post-translational changes, although more granular methods like single-cell sequencing could further elucidate cellular heterogeneity. The oncogene profile of SW620 provides valuable insight into the mechanisms driving metastatic colorectal cancer and highlights potential targets for combinatorial therapy. Future research should investigate the functional interplay among these oncogenes and identify context-specific vulnerabilities for therapeutic intervention.

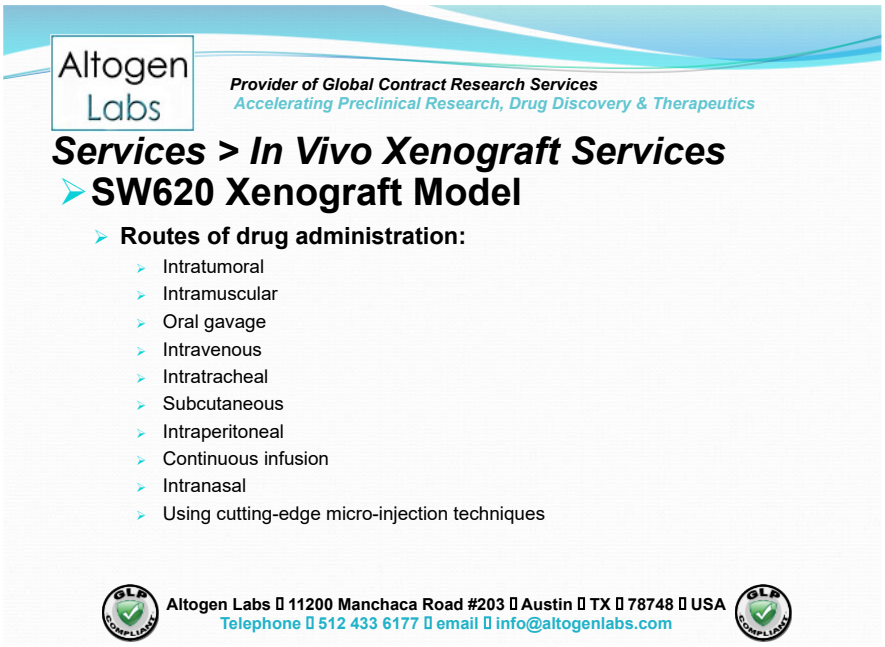

Figure 4. Summary of *in vivo* xenograft services available for the SW620 colorectal cancer model at Altogen Labs, including multiple drug administration routes such as subcutaneous, intratumoral, intravenous, and intraperitoneal injection.

Xenograft animal models are foundational to preclinical cancer research, providing a reliable platform to assess the therapeutic efficacy of experimental drugs against specific tumor types. These models involve the transplantation of human cancer cells into immunocompromised rodents, typically mice or rats, through subcutaneous or orthotopic injection. This approach enables the formation of tumors that replicate key biological and pathological features of human cancers in a living system. All anticancer agents that reach clinical application undergo rigorous testing in such preclinical *in vivo* models, underscoring their importance in translational oncology. The design of xenograft studies encompasses severa l variables, including the selection of a suitable animal model, tumorigenic cell line, administration route, dosing frequency, and analytical methods to monitor tumor progression and biological responses. Common evaluation techniques include caliper-based tumor volume measurement, histopathological analysis, and gene or protein expression profiling. At Altogen Labs, all procedures are carried out in accordance with United States Good La boratory Practice (GLP) standards and are regulated by an Institutional Animal Care and Use Committee (IACUC). Prior to treatment initiation, animals are acclimated to the vivarium, sorted by body weight to ensure uniform distribution among cohorts, and examined daily for tumor development and clinical condition. A complete study report is provided, including detailed methodologies, raw and processed data, statistical interpretation, and health monitoring records. Additional laboratory services include tissue collection, histological staining, protein and RNA isolation, and gene expression analysis to support mechanistic insights.

The SW620 xenograft model represents a robust and adaptable platform for studying metastatic colorectal cancer, particularly for compounds targeting tumor growth and dissemination. Derived from a lymph node metastasis of a colorectal carcinoma, the SW620 cell line is well suited for investigations focused on tumor growth delay, tumor growth inhibition, and therapeutic efficacy under various treatment regimens. Altogen Labs offers multiple administration routes to accommodate different drug properties and research goals. These include intravenous, intratracheal, continuous infusion, intraperitoneal, intratumoral, oral gavage, topical, intramuscular, subcutaneous, and intranasal delivery. Advanced techniques such as pump-controlled intravenous infusion and precision-guided microinjection are also available. To extend the model's relevance, alternative engraftment sites are supported, including orthotopic implantation into the colon, tail vein injection for evaluating hematogenous spread, and mam mary fat pad or left ventricular injections for studying metastasis. This flexibility allows researchers to simulate both primary and secondary tumor development with high fidelity.

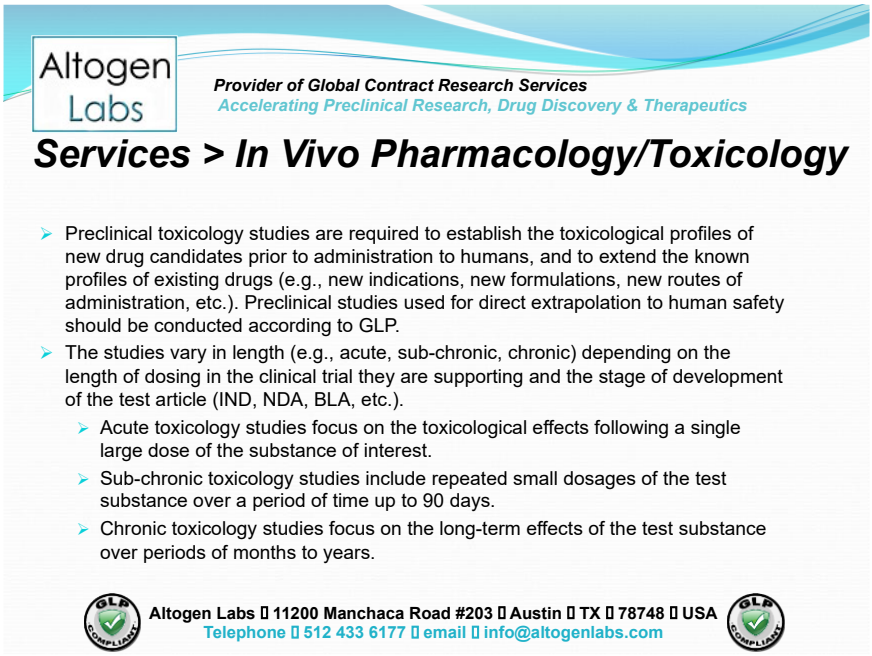

Figure 5. Overview of *in vivo* pharmacology and toxicology services offered by Altogen Labs, including acute, sub-chronic, and chronic toxicity studies conducted under GLP to support IND, NDA, and BLA regulatory submissions.

References:

Bai C, Sun Y, Pan X, Yang J, Li X, Wu A, Qin D, Cao S, Zou W, Wu J. Antitumor Effects of Trimethylellagic Acid Isolated From Sanguisorba officinalis L. on Colorectal Cancer via Angiogenesis Inhibition and Apoptosis Induction. *Front Pharmacol*. 2020 Jan 28;10:1646. doi: 10.3389/fphar.2019.01646. PMID: 32047442; PMCID: PMC6997556.

Chen WC, Kuo TH, Tzeng YS, Tsai YC. Baicalin induces apoptosis in SW620 human colorectal carcinoma cells *in vitro* and suppresses tumor growth *in vivo*. *Molecules*. 2012 Mar 29;17(4):3844-57. doi: 10.3390/molecules17043844. PMID: 22456615; PMCID: PMC6268256.

Golubovskaya VM, Figel S, Ho BT, Johnson CP, Yemma M, Huang G, Zheng M, Nyberg C, Magis A, Ostrov DA, Gelman IH, Cance WG. A small molecule focal adhesion kinase (FAK) inhibitor, targeting Y397 site: 1-(2-hydroxyethyl)-3, 5, 7-triaza-1-azoniatricyclo [3.3.1.1(3,7)]decane; bromide effectively inhibits FAK autophosphorylation activity and decreases cancer cell viability, clonogenicity and tumor growth *in vivo*. *Carcinogenesis*. 2012 May;33(5):1004-13. doi: 10.1093/carcin/bgs120. Epub 2012 Mar 7. PMID: 22402131; PMCID: PMC3334519.

Tobelaim WS, Beaurivage C, Champagne A, Pomerleau V, Simoneau A, Chababi W, Yeganeh M, Thibault P, Klinck R, Carrier JC, Ferbeyre G, Ilangumaran S, Saucier C. Tumour-promoting role of SOCS1 in colorectal cancer cells. *Sci Rep*. 2015 Sep 22;5:14301. doi: 10.1038/srep14301. PMID: 26391193; PMCID: PMC4585755.

Wang C, Dai Z, Fan R, Deng Y, Lv G, Lu G. HSF1 overexpression enhances oncolytic effect of replicative adenovirus. *J Transl Med*. 2010 May 6;8:44. doi: 10.1186/1479-5876-8-44. PMID: 20459615; PMCID: PMC2877000.

Keywords: SW620, colon, colorectal, xenograft, *in vivo*, cancer, preclinical, research, *in vivo* pharmacology, colon cancer, PDX, CDX, metastatic, orthotopic