Validated 786-O Xenograft Model:
Subcutaneous Xenograft Tumor Model

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Advancing Xenograft Models in Kidney Cancer Research

Renal cell carcinoma (RCC), particularly its clear cell subtype, presents a major clinical challenge due to its genetic heterogeneity, high metastatic potential, and resistance to conventional treatments. Although targeted therapies and immunotherapies have improved outcomes, many patients develop resistance or relapse. To address this, xenograft models derived from RCC cell lines or patient tumors are widely used to study tumor growth, angiogenesis, and drug response *in vivo*. These models are essential for bridging the gap between *in vitro* research and clinical application, though limitations such as the absence of immune components and tumor heterogeneity remain. Advancing xenograft methodologies offers the potential to improve preclinical drug evaluation and support the development of more effective, personalized therapies for kidney cancer.

786-O Cell Line

The 786-O cell line, derived from a primary clear cell renal cell carcinoma (ccRCC), is widely used in kidney cancer research due to its loss of VHL function and constitutive HIF-2α activation, which drives angiogenesis and metabolic reprogramming. This makes it a key model for studying hypoxia signaling and evaluating HIF-2α inhibitors like belzutifan. The line exhibits high VEGF expression, glycolytic metabolism, and resistance to chemotherapy, reflecting aggressive ccRCC phenotypes. Genomic alterations include 3p loss and gains in 5q and 7, affecting tumor suppressors such as PBRM1 and BAP1. However, its lack of immune components and tumor heterogeneity limits its use in immunotherapy studies and modeling intra-tumoral diversity. Despite its value in xenograft and drug screening studies, its translation to *in vivo* tumor complexity remains limited, underscoring the need for complementary models that better reflect the tumor microenvironment.

Altogen Labs Validated 786-O Xenograft Model

Altogen Labs offers a validated subcutaneous xenograft model using the 786-O human renal carcinoma cell line to support preclinical evaluation of anticancer therapeutics targeting clear cell renal cell carcinoma. For model development, 786-O cells are cultured under sterile conditions until logarithmic growth is achieved, then harvested and resuspended in serum-free medium or a 1:1 mixture with extracellular matrix gel to enhance tumor establishment. A total of 5 × 10^6 viable cells in 100 microliters are injected subcutaneously into the right flank of female athymic nude mice aged six to eight weeks. Tumor formation typically occurs within one week, and animals are randomized into study cohorts once tumor volumes reach 100 to 150 mm^3. Therapeutic agents are administered according to a predefined dosing schedule and delivery route based on study objectives and compound characteristics. Tumor dimensions are measured twice weekly using digital calipers, and volumes are calculated using the standard formula (length × $width^2$) divided by two. Animal body weight and clinical signs are monitored throughout the study to assess general health and potential compound toxicity.

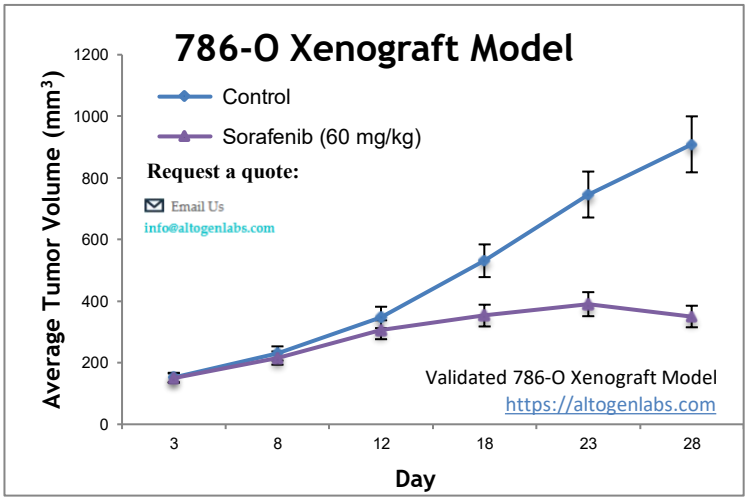

Figure 1. Tumor growth kinetics and chemotherapeutic evaluation of the Altogen Labs in-house validated 786-O xenograft model of renal cancer. Immunodeficient mice bearing subcutaneous 786-O tumors were randomized to receive treatment with sorafenib (60 mg/kg) or vehicle control (buffer only). Data are presented as mean tumor volumes ± standard error of the mean (SEM).

Upon study completion, tumors are excised, weighed, and processed for downstream analyses, including histopathology, immunohistochemistry, and molecular profiling. This xenograft model provides a highly reproducible and biologically relevant system for evaluating tumor growth inhibition, pharmacodynamics, and mechanism of action for a wide range of therapeutic agents. Altogen Labs offers full-service xenograft studies using the 786-O model, including experimental design, treatment administration, data collection, and comprehensive analytical support tailored to client-specific research and regulatory needs. In addition to tumor analysis, blood samples and non-tumor organs can be collected for pharmacokinetic and toxicological assessments. This xenograft model enables detailed characterization of drug efficacy, mechanism of action, and biomarker correlation under controlled *in vivo* conditions. Altogen Labs provides complete study execution, data analysis, and reporting services, ensuring scientifically rigorous, GLP-compliant preclinical studies tailored to the requirements of oncology research and therapeutic development.

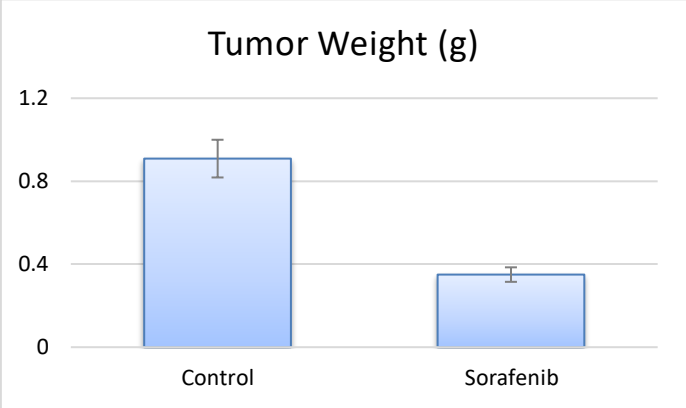

Figure 2. Tumor weights of 786-O xenografts harvested from mice treated with the sorafenib (60 mg/kg) or vehicle control (buffer only). Tumor weights were recorded on Day 28 of the study and are presented as mean ± SEM. The study performed using Altogen Labs in-house validated 786-O xenograft model.

Subcutaneous 786-O Xenografts in Renal Cancer Research

Subcutaneous xenograft transplantation using the 786-O renal cell carcinoma cell line remains a widely utilized preclinical model for studying the tumorigenic behavior and therapeutic responsiveness of clear cell renal cell carcinoma (ccRCC). The 786-O cell line, characterized by inactivation of the von Hippel–Lindau (VHL) tumor suppressor and consequent constitutive expression of hypoxia-inducible factor 2-alpha (HIF-2α), recapitulates key molecular features of advanced ccRCC, including angiogenic signaling and metabolic reprogramming. In this model, 786-O cells are typically injected subcutaneously into immunodeficient mice, such as athymic nude or NOD/SCID strains, leading to reproducible tumor formation that facilitates longitudinal measurement of tumor volume and assessment of therapeutic efficacy. Subcutaneous implantation offers technical simplicity, robust tumor engraftment rates, and accessibility for tumor monitoring, making it an effective platform for evaluating targeted therapies, including HIF-2α inhibitors and anti-angiogenic agents. The model has been instrumental in elucidating mechanisms of drug action and resistance and has supported the preclinical validation of agents now in clinical use. However, a notable limitation is the absence of an intact immune microenvironment, which precludes evaluation of immune-based therapies and limits the model's applicability to immuno-oncology. Additionally, the subcutaneous site does not fully recapitulate the orthotopic tumor microenvironment of the kidney. Despite these constraints, the 786-O subcutaneous xenograft model remains a critical tool in translational kidney cancer research, providing a biologically relevant and experimentally tractable system for preclinical drug development and mechanistic studies.

ER Stress and AKT Inhibition Drive Apoptosis in 786-O Cells

Norcantharidin (NCTD) exhibits potent cytotoxicity in the 786-O renal carcinoma cell line through mechanisms involving mitochondrial dysfunction, endoplasmic reticulum (ER) stress, and inhibition of the PI3K/AKT signaling pathway. NCTD selectively reduced 786-O cell viability in a dose- and time-dependent manner while sparing normal renal epithelial cells, suggesting therapeutic selectivity. Apoptotic induction was confirmed by sub-G1 cell cycle arrest, Annexin V positivity, and cleavage of caspases and PARP, indicating engagement of both intrinsic and extrinsic apoptotic pathways. Mitochondrial depolarization, upregulation of Bax, and downregulation of Bcl-2 and Mcl-1 further implicated mitochondrial signaling in cell death. Concurrently, NCTD triggered robust ER stress, marked by elevated levels of Grp78, CHOP, ATF4, and phosphorylated eIF2α, while pharmacologic inhibition of ER stress via salubrinal partially reversed apoptotic outcomes. Additionally, NCTD suppressed phosphorylated AKT, and constitutive AKT overexpression mitigated apoptosis and restored anti-apoptotic protein expression, suggesting that AKT inactivation acts downstream of ER stress and amplifies cell death signaling.

The study's *in vivo* component confirmed the antitumor effects of NCTD using a 786-O xenograft model, demonstrating significant tumor volume and weight reductions without overt systemic toxicity. Reduced Ki-67 expression in treated tumors supported its antiproliferative activity. Methodologically, the study employed well-validated *in vitro* and *in vivo* models, supported by molecular assays and functional interventions that strengthen causal interpretation. However, the limited number of renal cancer cell lines and absence of long-term or metastasis-focused data narrow the scope of

applicability. Despite this, the study contributes valuable mechanistic insights that may inform strategies for targeting apoptosis resistance in hypoxia-adapted or PI3K/AKT-driven tumors. Although the findings center on renal carcinoma, parallels in ER stress and AKT dysregulation are frequently observed in gastric cancer and suggest potential therapeutic crossover. The SNU16 gastric cancer cell line, while not directly examined, may serve as a relevant platform for future studies exploring similar stress-mediated vulnerabilities and combinatorial treatment approaches.

Synergistic Antitumor Effects of IMO and Everolimus in VHL-Mutant RCC

The combination of a Toll-like receptor 9 (TLR9) agonist and an mTOR inhibitor exerts potent synergistic antitumor effects in renal carcinoma models, particularly those with mutant Von-Hippel Lindau (VHL) status such as the 786-O cell line. While the mTOR inhibitor alone effectively reduces tumor cell viability, the addition of the TLR9 agonist significantly enhances efficacy, even in models where direct *in vitro* signaling inhibition is minimal. *In vivo*, this dual therapy leads to sustained tumor growth inhibition and prolonged survival, associated with suppression of VEGF and HIF-1 expression. These findings indicate that the TLR9 agonist contributes primarily through disruption of angiogenesis and tumor–stroma interactions, rather than direct cytotoxicity to tumor cells. The strong inhibition of downstream effectors such as p70S6K, along with reductions in VEGF secretion, supports a cooperative mechanism that impacts both tumor signaling and vascular support systems. The 786-O model demonstrates the importance of considering the tumor microenvironment when evaluating therapeutic efficacy, especially in VHL-deficient contexts where angiogenesis plays a dominant role. Despite limited *in vitro* response to TLR9 agonism, the *in vivo* antitumor activity suggests a critical contribution from immune modulation and endothelial disruption. Experimental approaches incorporating endothelial function assays and survival analyses reveal the capacity of this combination to impair migration, adhesion, and capillary formation in supporting vasculature. These outcomes reinforce the therapeutic relevance of combining immunomodulatory and antiangiogenic strategies in RCC and suggest broader applicability to other malignancies characterized by hypoxia-driven VEGF dependence.

Dual Mechanisms of SC66 in 786-O Renal Carcinoma

The 786-O renal carcinoma cell line has emerged as a highly informative platform for understanding mechanisms of chemotherapeutic resistance and for testing novel compounds in renal cell carcinoma (RCC). Notably characterized by constitutive activation of the PI3K-AKT-mTOR pathway, 786-O cells model the signaling dynamics frequently observed in aggressive RCC phenotypes. Pharmacological studies reveal that the AKT inhibitor SC66 suppresses 786-O viability, migration, and colony formation, while promoting apoptosis through both AKT-dependent and AKT-independent pathways. Inhibition of AKT signaling correlates with reduced phosphorylation of downstream targets such as S6K1, alongside increased caspase activation and mitochondrial depolarization. In addition, SC66 induces oxidative stress, downregulates SphK1, elevates ceramide levels, and activates JNK signaling, suggesting that multiple pro-apoptotic axes are simultaneously engaged. Importantly, SC66 retains cytotoxic activity in 786-O cells with AKT knockdown or genetic ablation, further supporting the presence of auxiliary mechanisms that bypass canonical AKT blockade. *In vivo*, SC66 effectively reduces the growth of 786-O xenograft tumors, with molecular signatures from the *in vitro* studies mirrored in tumor lysates, including suppression of AKT-mTOR and activation of ceramide-JNK pathways. Methodologically, the 786-O model demonstrates high reliability and translational value due to its consistent *in vitro* behavior and reproducible tumor formation in xenograft systems. However, interpretive caution is warranted, as the model lacks immune components and does not fully recapitulate the tumor microenvironment of human RCC. Experimental approaches such as caspase assays, ROS measurements, and kinase activity profiling provide mechanistic clarity, yet the specificity of SC66's action on off-target systems remains a limitation. While the study employs rigorous controls and statistically sound methods, broader generalization to human clinical outcomes requires validation in immunocompetent and genetically heterogeneous systems. The findings reinforce the critical role of metabolic and stress-response pathways in RCC progression and drug sensitivity. Future research should focus on delineating the upstream regulators of SphK1 and JNK in 786-O cells and evaluating the combinatorial potential of SC66 with immune-modulating agents or mTORC1/2 dual inhibitors. This integrative approach may yield more robust strategies for overcoming therapeutic resistance in renal carcinoma and, by extension, inform applications in other malignancies sharing similar signaling profiles.

Targeting Oncogenic Pathways in 786-O Renal Carcinoma

The 786-O renal carcinoma cell line, derived from a VHL-deficient clear cell carcinoma, exhibits hallmark features of hyperactivated PI3K-AKT-mTOR and BRD4 signaling, both of which contribute to unchecked proliferation, apoptosis resistance, and metastatic potential. Pharmacologic inhibition using a dual-target compound effectively suppressed cell viability, induced apoptosis via caspase activation, and impaired both DNA synthesis and clonogenic survival. Notably, this inhibition provoked downregulation of BRD4-regulated oncogenes such as Myc and Bcl-2, disrupted G1-S phase progression, and significantly curtailed migratory capacity. These effects were specific to malignant cells, as non-

cancerous renal epithelial controls displayed minimal response. *In vivo*, 786-O xenografts demonstrated marked tumor regression and reduced growth rates under dual-inhibition treatment, highlighting the compound's therapeutic potential through simultaneous suppression of oncogenic transcriptional and survival pathways.

Methodologically, the 786-O model presents a reliable system for studying VHL-mutant RCC and allows for reproducible investigation of intracellular signaling dependencies, apoptotic thresholds, and tumorigenic behavior. Standard assays such as BrdU incorporation, PI-FACS cell cycle profiling, TUNEL apoptosis quantification, and Transwell migration analyses enable multidimensional assessment of drug effects. However, limitations persist, including the subcutaneous xenograft model's inability to recapitulate metastatic progression or immune microenvironment interactions. Interpretation must also consider the differential basal activity of targeted pathways between malignant and non-malignant cells, which may affect drug specificity and toxicity profiles. Despite these challenges, insights gained from 786-O have broader implications for tumors reliant on PI3K-AKT-mTOR and Myc-driven programs. In particular, the mechanistic convergence of hypoxia, oncogenic signaling, and epigenetic control underscores the promise of dual-pathway inhibition for solid tumors such as gastric cancer, where similar molecular axes are active. Future research should expand to orthotopic models, integrate immune components, and evaluate cross-applicability of pathway co-targeting in other hypoxia-adaptive malignancies.

Immuno-oncology Xenograft Models

Altogen Labs is a preclinical contract research organization specializing in the investigation and translational development of novel pharmacological and biologic therapeutics, with a focus on oncology, immunotherapy, vaccine platforms, dermatological agents, and bioactive natural products. The facility is staffed by experienced scientific personnel and equipped with advanced instrumentation to support high-throughput *in vivo* and *in vitro* studies. A key area of expertise includes immuno-oncology, with established capabilities utilizing both humanized and immunodeficient murine models engrafted with peripheral blood mononuclear cells (PBMCs), CD34+ hematopoietic stem cells, and induced pluripotent stem cells (iPSCs) to evaluate immune reconstitution, therapeutic efficacy, and safety parameters. Central to Altogen Labs' platform is a curated library of more than 100 internally validated xenograft models, including cell line-derived xenografts (CDX), patient-derived xenografts (PDX), patient-derived cell cultures (PDC), and patient-derived organoids (PDOrg), offering clinically relevant systems for predictive drug response and biomarker discovery. These models support comprehensive efficacy testing for small molecules, biologics, and combination therapies. The laboratory also provides GLP-compliant toxicology services encompassing acute, sub-chronic, and chronic exposure studies to characterize dose-dependent toxicity, identify target organ effects, and determine the therapeutic index of investigational compounds. This integrated approach enables efficient progression from early-stage discovery to IND-enabling studies across a wide range of therapeutic modalities.

Apoptotic Regulation and Therapeutic Vulnerabilities in 786-O Renal Carcinoma

Characterized by loss of functional VHL protein, the 786-O renal carcinoma cell line displays constitutive activation of hypoxia-inducible factors and downstream pro-survival signaling cascades. This molecular phenotype contributes to its resistance to apoptosis and enhanced tumorigenicity. One of the defining therapeutic response patterns in 786-O cells is their sensitivity to norcantharidin-induced apoptosis, which occurs through coordinated disruption of mitochondrial integrity, activation of endoplasmic reticulum stress pathways, and suppression of AKT-mediated survival signaling. Apoptotic markers such as caspase cleavage, mitochondrial membrane depolarization, and modulation of Bcl-2 family proteins are consistently observed, along with increased expression of ER stress-related proteins including CHOP and phosphorylated eIF2α. Furthermore, inhibition of ER stress or forced activation of AKT attenuates the cytotoxic response, indicating a tightly regulated, multi-axis control of apoptosis in this cell line. These mechanistic insights reveal a vulnerability in VHL-deficient renal cancer cells that can be therapeutically exploited through agents that simultaneously target metabolic stress responses and oncogenic survival pathways. Serving as a reproducible and genetically defined model, the 786-O cell line has become a cornerstone in the study of apoptosis regulation in renal carcinoma, particularly within the context of hypoxia-adapted tumors. Its use in both *in vitro* and *in vivo* systems has yielded reliable results across multiple apoptotic assays, including cell viability, flow cytometry, protein immunoblotting, and xenograft tumor regression. However, its lack of metastatic potential and relatively homogeneous genetic background limit its utility in modeling tumor heterogeneity or resistance evolution. These limitations underscore the importance of integrating orthotopic and patient-derived models to validate therapeutic mechanisms. Despite its origin in renal cancer, the apoptotic vulnerabilities uncovered in 786-O cells, particularly the interplay between ER stress and AKT signaling, have broader relevance for other tumor types. Gastric cancers that exhibit aberrant PI3K/AKT activation or dependence on stress-response pathways may similarly respond to dual-targeting strategies that disrupt both metabolic and survival signaling. Future investigations should examine the applicability of these findings in gastric tumor models, assess synergy with immunomodulatory agents, and further define molecular predictors of treatment response.

The 786-O renal cancer xenograft model provided by Altogen Labs offers a biologically accurate and reproducible platform for preclinical evaluation of therapeutic candidates targeting clear cell renal cell carcinoma (ccRCC). Derived from a primary human renal tumor, the 786-O cell line is VHL-deficient and characterized by constitutive stabilization of hypoxia-inducible factor 2α (HIF-2α), a key transcription factor driving angiogenesis, metabolic reprogramming, and tumor progression in renal cancers. Unlike some renal cancer models, 786-O cells lack expression of HIF-1α due to epigenetic silencing, making this model particularly relevant for studying HIF-2α–dependent oncogenesis. When implanted subcutaneously in immunodeficient mice, 786-O cells reliably form tumors with moderate to rapid growth kinetics, typically reaching measurable size within two weeks post-injection. These tumors exhibit prominent vascularity and histological features consistent with clear cell morphology. The 786-O xenograft model is well suited for assessing therapies that target pathways downstream of VHL loss, including VEGF signaling, mTOR activation, and metabolic regulation.

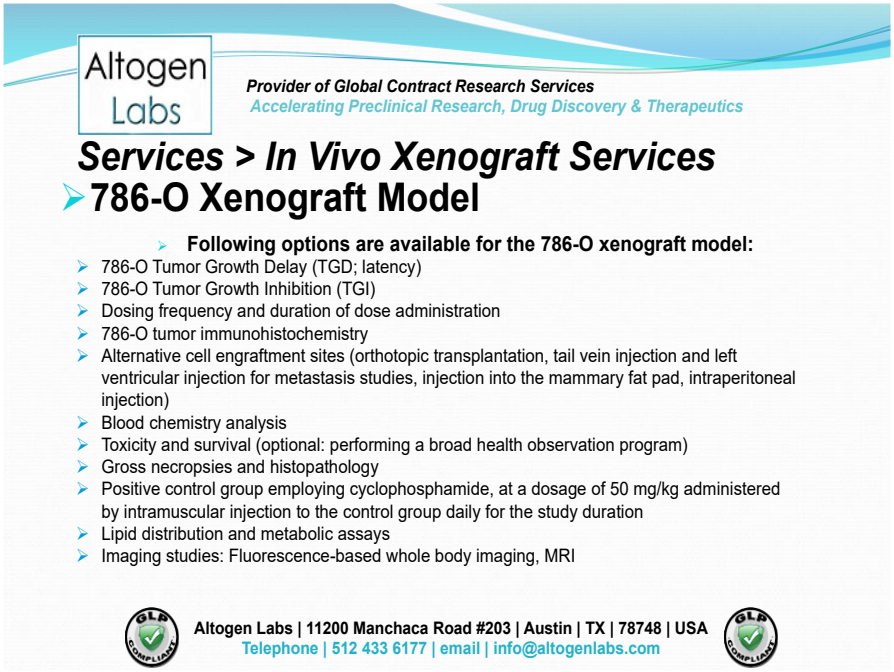

Figure 3. Overview of *in vivo* xenograft study capabilities using the 786-O renal carcinoma model at Altogen Labs. Listed services include tumor growth inhibition and delay assessments, dosing regimen optimization, immunohistochemistry, alternative engraftment routes for metastasis modeling, blood chemistry, toxicology, necropsy, histopathology, and imaging studies. The platform supports comprehensive preclinical evaluation of anticancer agents using the 786-O xenograft system.

Altogen Labs supports a broad range of *in vivo* oncology services using the 786-O xenograft model, including xenograft establishment, test article administration, tumor growth monitoring, survival analysis, and post-treatment tissue collection for pharmacodynamic or histological evaluation. The model enables detailed assessment of drug efficacy and tumor response dynamics in a genetically defined system that recapitulates key aspects of VHL-deficient RCC. Studies conducted with this model typically involve subcutaneous implantation into athymic nude or NOD/SCID mice, with standard endpoints including tumor volume reduction, growth delay, and biomarker modulation. Altogen Labs provides additional capabilities such as immunohistochemistry for angiogenic and apoptotic markers, qPCR or western blotting for pathway-specific targets, and analysis of drug distribution and target engagement. The 786-O model is especially valuable for preclinical testing of HIF-2α inhibitors and VEGF-targeted agents, such as those currently in clinical development or FDA-approved for advanced RCC.

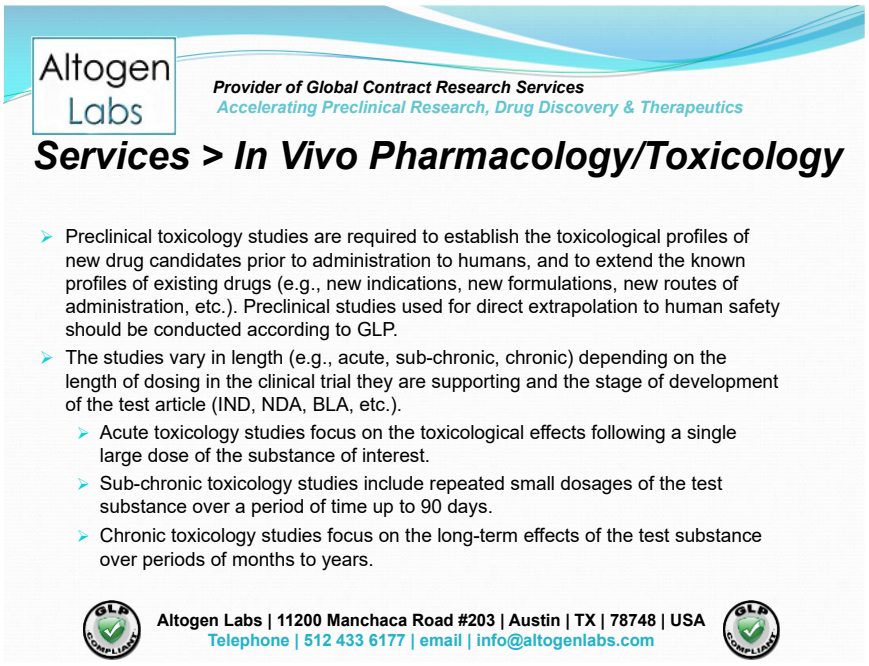

Figure 4. Summary of *in vivo* toxicology study capabilities at Altogen Labs, including acute, sub-chronic, and chronic exposure models designed to assess the safety profiles of investigational compounds in compliance with GLP standards.

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Keywords: 786-O, kidney, renal, xenograft, *in vivo*, cancer, preclinical, research, kidney cancer, *in vivo* pharmacology, renal cancer

Other Available Altogen Labs Validated Renal Cancer Xenograft Models:

A498 Xenograft Model: http://altogenlabs.com/xenograft-models/kidney-cancer-xenograft/a498-xenograft-model/

Caki-1 Xenograft Model: https://altogenlabs.com/xenograft-models/kidney-cancer-xenograft/caki-1-xenograft-model/

G401 Xenograft Model: http://altogenlabs.com/xenograft-models/kidney-cancer-xenograft/g401-xenograft-model/

Renca Xenograft Model: https://altogenlabs.com/xenograft-models/kidney-cancer-xenograft/renca-xenograft-model/

RXF393 Xenograft Model: https://altogenlabs.com/xenograft-models/kidney-cancer-xenograft/rxf393-xenograft-model/