Medical Discovery and Innovation

Preclinical Contract Research Services by Altogen Labs

Altogen Labs (altogenlabs.com), an Austin, TX based contract research organization (CRO) founded in 2009, offers preclinical laboratory services to pharmaceutical, biotechnology, and academic researchers worldwide to support and accelerate medical discovery and research studies. Our Good Laboratory Practice (GLP)-compliant laboratory offers services for drug toxicology, drug efficacy, drug discovery and new medicine development, specifically supporting preclinical investigation with *in vitro* and *in vivo* services including anti-cancer compound screening, IC_{50} assays, stable-cell line engineering, over 80 validated xenograft models, gene function and expression analysis, in-vivo RNAi and tissue-targeted siRNA delivery, transient and stable transfections of cell lines, engineering of RNAi knockdown cell-lines, microfluidics-based liposome encapsulation, and cell-based assay development.

Altogen Labs also offers bioremediation biotechnology products and services. Bioremediation is the process of introducing naturally occurring biomatter, in the form of microbial organisms, fungi, plants, and their endogenous biomolecules, into a contaminated site as a treatment to restore the environment to a less toxic or non-toxic state[1]. Our team of scientists has developed scalable, patent-pending bioremediation products, comprised of bacterial strains optimized for hydrocarbon degradation, to be utilized for restoration of sites contaminated with a wide range of pollutants including crude oil, PAHs, pesticides, acetone, benzene, and other environmental toxins emanating from industrial or chemical wastes and spills.

Altogen Labs offers instant quotes, comprehensive service packages, and highly competitive pricing. We assure our clients that our high-quality products and services will be upheld by our technical support and quality control teams. **Altogen Labs** supports our clients from experimental design through official regulatory or patent submissions with 100% of the intellectual property rights held by our clients.

Meeting Our Clients Needs

Successful research projects require an extremely focused strategy, meticulous planning, material and human resources, and constant vigilance over both the large and small details. Outsourcing of projects is an effective and efficient means of ensuring that limited time resources are invested proportionately into the highest priority facets of the project. It is our goal to partner with our clients to deliver the most efficient and reliable delivery of products and services to ensure that any research project is progressing at the fastest rate possible, and that all preclinical experiments and data have been rigorously studied and analyzed prior to transitioning into clinical research to ensure maximum probability of project success. **Altogen Labs** is committed to satisfying our clients' timely needs while concurrently delivering results of the highest quality and integrity. Our scientists bring years of experience and elite levels of expertise in biochemistry, molecular biology, and drug discovery. **Altogen Labs** has a track record of success and client satisfaction as underscored and endorsed by the following client assessments of our services.

"Altogen is a broad base CRO in regards to being able to do analytical work to animal studies. They are noted for people with good lab hands and come up with innovative solutions to otherwise hard problems. They are not a 'cookie-cutter' CRO but one that actually engages the company and determines what the problem is."

Curt Bilby, PhD, CEO - Terapio Corporation, an Austin, TX-based biopharmaceutical company engineering therapeutics based on the transport protein RLIP76 as effective therapies to combat radiation and chemical-induced oxidative stress.

*"It is helpful to have a partner that can help guide you through and help refine your studies, and **Altogen Labs** and their staff are very experienced in that area and they really are a good partner to work with. Not just from getting the work you want done, but also in helping to find that work and refine your studies to help maximize the chance of getting the results you are looking for."*

Chris Marich, MBA, COO - Savara Pharmaceuticals, a Texas, U.S.-based pharmaceutical company in the clinical stages of development for both small molecule and biologic-based inhalation therapies to treat patients with rare pulmonary conditions.

Preclinical Research

Bringing new pharmaceutical drugs to market is a long, arduous, and costly process with conservative estimates placing the timeline from discovery to approval at 10 years and the financial impact at more than $1B spent[3]. The cost of new drug development has more than doubled over the past decade and is expected to only soar higher in the future[4]. The Drug Development process advances through 3 stages prior to submission to the FDA for review: Discovery and Development, Preclinical Research, and Clinical Research[5]. Before a drug can be

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tested in clinical trials, preclinical studies must unequivocally establish that the Investigational New Drug (IND) is safe to be administered to human subjects in clinical research studies[5]. Specifically, the objective of the preclinical stage of development is to thoroughly profile the toxicity of the IND through experimentation observing good laboratory practices (GLP).

Altogen Labs offers in vitro toxicology studies of INDs to characterize the drug candidate's effects on biological functions and metabolism. Cancer cell lines are used as *in vitro* models for dose-response studies. Our laboratory houses a large catalog of cancer cell lines to be screened for IC_{50} values of candidate drugs. Our research laboratory provides IC_{50} studies by screening candidate drugs against our extensive library of cell lines or against client provided custom cell lines.

For in vivo research, **Altogen Labs** offers xenografting – the method of transplanting human tumor tissue into immunocompromised mice to create a more biologically accurate model to investigate an IND's effect on tumor activity[6]. **Altogen Labs** offers consultation to our clients on xenograft model selection. Xenograft studies are GLP compliant and animal handling at our facility is IACUC-regulated. Our team of scientists is equipped to perform research studies utilizing more than 80 xenograft models including models for brain carcinoma, pancreatic and breast cancer, epidermoid and nasopharyngeal carcinoma, and xenograft studies for melanoma, colon, breast, lung, and prostate cancers. Additionally, over 20 patient PDX xenograft models are also available. Clients employing xenograft studies are provided with detailed experimental procedures, health reports, and experimental data. These studies can be further expanded to include tissue collection, histology, RNA isolation, and gene expression analysis.

Altogen Labs also offers liposome encapsulation services. Liposomes, spherical particles consisting of an aqueous core and at least one lipid bilayer, can be used to encapsulate various types of cargo including DNA, RNA, drugs, and proteins, and serve as efficient delivery vehicles of these agents for in vivo and *in vitro* studies[7]. Liposome delivery has been established as an effective technique for administration of pharmaceuticals and can be performed topically, orally, or via pulmonary or parenteral routes. Liposomes are specifically advantageous for overcoming solubility issues for difficult-to-formulate agents and are utile for difficult-to-deliver agents like small-interfering RNA molecules. Moreover, liposome encapsulation can augment intracellular transport of drugs and may also facilitate specific delivery of drugs to target cells or tissues. **Altogen Labs** offers encapsulation of any species of charged molecule (mRNA, siRNA, shRNA, microRNA, plasmid DNA, and protein) into a custom formulation or one of our standard liposome formulations, such as DMPC : DMPG : Cholesterol and PC : DOTAP : PEG : Cholesterol.

Microfluidization, extrusion, and sonication methods are available to create a homogenous liposome sample with encapsulated cargo molecules of a specified size (we offer a controlled particle size liposomes of 50 – 400 nm size).

Altogen Labs is also experienced in developing and performing *in vitro* and *in situ* immuno and cell-based assays. *In vitro* Enzyme-linked Immunosorbent Assays (ELISAs) are used to characterize antigen or antibody binding activity and are a resourceful tool used in research, diagnostics, and quality control. *In situ*, cell-based ELISA allows for probing an agent's effect on cellular signaling pathways and biochemistry. Furthermore, our expertise in cell-based assays grants the ability to expand these studies into cytotoxic and metabolic analysis. Cellular processes that can be elucidated by these means include receptor activation, receptor binding, cell signaling, and ligand internalization. Our lab services include custom ELISA development, optimization, and analysis of customer provided samples via a robust cell-based ELISA, consisting of the compound of interest tested against the activation of 15 Serine/Threonine kinases and 17 Tyrosine kinases. Our team of scientists have years of experience designing unique and novel strategies for immune and cell-based assays. Our analysis techniques and methods include enzyme immunoassay (EIA), Bio-Plex kinase profiling assays, biomarker analysis, receptor binding, cell signaling, cytotoxicity, cell cycle assays, cell viability, and cell proliferation. Furthermore, **Altogen Labs** can establish a cell-based assay to profile a proprietary compound's quantitative effects on specific biomarkers, cytokines, or phosphoproteins in cell culture supernatant or whole-cell lysates.

In Vivo Toxicology Studies

For clients wanting to progress passed *in vitro* toxicology studies, **Altogen Labs** also offers in vivo preclinical toxicology studies in rat and mouse models. For preclinical toxicology studies, we offer the options of acute, subchronic and chronic toxicity studies.

Acute toxicology studies investigate toxicological effects after a single, large dose of a substance of interest is administered. Longer term subchronic and chronic studies simulate the effects of drugs over a longer course of use and what adverse effects can arise in that timeframe. For subchronic studies, small dosages of the test substance are repeatedly administered over a maximum time period of 90 days. Comparatively, chronic toxicology studies investigate the effects of substances over a longer time course of several months to years. These studies are key in drug development, as they lay the groundwork for further investigation in Phase I clinical trials contingent on the substance yielding favorable results and minimal toxicological, carcinogenic, and mutagenic effects.

Additionally, another focus of *in vivo* toxicology studies is the exploration of how an investigative new drug (IND) is best administered. Delivery of a drug to its target cells in a living system is an intricate process that can be foiled by a myriad of cellular actions. Accordingly, preclinical studies should investigate different drug administration routes to determine how toxicity and efficacy can differ between them. The outcome of these studies should be an optimized procedure for IND administration that is safe, effective, and reduces adverse effects.

Complementary to optimizing drug delivery, identification of possible adverse side effects from IND administration is also crucial in attempting to advance the study into clinical trials. Potential side effects can have a deleterious effect on the future health of the subject and/or its progeny. Potential side effects can be detrimental to the immune system, damaging to the DNA, and harmful to vital organs. Accordingly, additional tests can be performed to study sensitivity, irritation, mutagenic and carcinogenic properties, reproductive toxicity, and immunotoxicity. An INDs application for clinical study can also be augmented by studies of pharmacokinetics/pharmacodynamics (PK/PD), and absorption, distribution, metabolism, and excretion (ADME). **Altogen Labs** provides toxicity reports including blood chemistry, markers bioanalysis, pharmacokinetics, pharmacodynamics, and histopathology results.

Stable Cell Line Engineering

Altogen Labs also offers stable cell line development with a time of completion of 28 days. Access to stable cell

lines engineered to overexpress a biomolecule of interest is extremely valuable for research studies exploring gene function and drug screening. Furthermore, preclinical studies involving mammalian cell lines allows a smoother transition into clinical studies. However, although extremely useful for applications in research, production of stable cell lines and primary cells can be resource draining with respect to cost, labor, and time. Our lab offers several stable cell line engineering services with the following properties: protein-overexpression, RNAi knockdowns, and reporter gene expressing (luciferase, GFP, RFP, YFP). Our Scientists have ample experience in stable cell line generation and we offer more than 150 different cancer cell lines. We develop all stable cell lines concordant with client specifications and accomplish this in a 28 day timeframe. Additionally, we can produce multiple clonal cell lines having varying levels of expression for a biomolecule of interest (low, medium, or high). Our standard cell line generation services include transfection of plasmid DNA (10 – 20 μg), drug selection of clonal cells, colony picking, generation of a stable cell line, expression and functional screening (a minimum of 10 passages), and final validation of construct expression by qRT-PCR and/or Western Blot analysis. This two-method validation of stable cell line development ensures that the gene of interest is both integrated into the genome and concurrently being expressed.

Cell Banking Services

Altogen Labs also offers GLP-compliant cell-banking services. To ensure indefinite longevity to cells, cryogenic storage in liquid nitrogen at -196°C is recognized as the best possible practice. Preserving the integrity of a single cell line in culture for the purposes of research and manufacturing can be troublesome due to factors like sample contamination by microbial species, target phenotype change, genetic drift, and uncertainty with respect to the finite life span of a cell line. Access to a cell bank allows the client to preserve consistency in research and manufacturing as this grants access to a cell reservoir of low passage number, free of microbial contamination, and phenotypically intact for study. **Altogen Labs** complies with regulatory measures to ensure that all procedures, practices, and facilities are up to current GLP standards, and ensures that all cell lines are free of bacterial and mycoplasma contamination. Our protocols use combinations of cryoprotectants and cooling steps to ensure successful cryopreservation of biological materials, cells, and tissues. **Altogen Labs** banks stable cell lines, cells for transfusion, umbilical cord blood cells, stem cells, and tumor/histological cells.

Altogen Labs offers our clients long term cell storage in a secure, controlled, and monitored environment. All cell banking work is performed under certified laminar flow hoods, and cells are grown in calibrated incubators with ultraviolet (UV) irradiated air and water. Replicate cell samples are taken and stored in separate freezers to mitigate the risk of temperature failure. The freezer temperature and liquid nitrogen levels are monitored round the clock. Cryovials can be used to expand a cell population, or shipped on dry ice to any worldwide destination.

RNAi Studies

RNA interference (RNAi) is a specific, *in vitro* gene silencing tool employed for gene function studies in cultured cells. Targeted silencing of gene expression is achieved by the introduction of small-interfering RNA (siRNA) or microRNA (miRNA). An siRNA molecule is a double stranded oligonucleotide, 20-24 base pairs in length, having a hydroxylated, 2' nucleotide overhang at each 3' end and phosphorylated 5' ends. Alternatively, a miRNA is a 22 nucleotide, single stranded RNA molecule that targets and binds to a complementary RNA sequence to yield a double stranded RNA molecule. Typical introduction of siRNAs or miRNAs into the cell via chemical transfection methods can transiently silence expression of target genes for three to seven days.

Although useful, the *in vitro* RNAi experiment is limited in not allowing for gene function study in the organism altogether, as an *in vivo* study would. However, the *in vivo* study presents its own set of challenges in the form of RNA instability, inefficient delivery, incorrect biodistribution, and the intrinsic difficulty of targeting specific tissues. For these reasons, *in vivo* RNAi experiments can be costly and time consuming.

In a typical gene function study, siRNAs and miRNAs are introduced by direct administration or via plasmid DNA and viral vectors, the latter needing cellular processing to become biochemically functional. Generally, the viral vector method is the most efficient in vivo, however, the vectors are relatively difficult to construct and run the risk of immunogenicity issues.

Once administered, the biggest obstacle to an in vivo RNAi study is the specific delivery of the ribonucleotide to the target tissue. Achievement of this would require that the siRNA or miRNA has to endure degradation by endogenous nucleases, evade immune system detection, minimize off target effects, and ultimately, be endocytosed by the target tissue.

Altogen Labs has developed RNAi methods for *in vivo* studies that prevent siRNA degradation in serum via chemical modification and liposome encapsulation. These modifications enhance delivery of the ribonucleotide to the target organ, and trigger significant, tissue-targeted functional effects. Chemical modifications of siRNA and miRNA *in vivo* include 2'MOE, FANA, 2'-Fluoro, and LNAs.

Altogen Labs provides complete *in vivo* siRNA development services including *in vitro* siRNA testing in cell-based assays, encapsulation and tissue-targeted siRNA delivery into animal models (including mouse, rat, and xenograft cancer models). We also offer multiple siRNA administration routes for local and systemic delivery including intravenous, intraperitoneal, intranasal, intratumoral, intratracheal, intradermal, intramuscular, intrathecal, intracerebellar, and intravascular perfusion.

Bioremediation

Bioremediation is the process of using microbes, fungi, green plants, and their endogenous enzymes to restore a contaminated environment to a non-toxic or less toxic state[1]. Bioremediation is one of the biotechnology focus areas of **Altogen Labs**. Our team of scientists has applied techniques in biotechnology to develop patent-pending bioremediation products and services to offer to our clients. Our technology is based on harnessing and enhancing the ability of microorganisms found in soil and water to digest hydrocarbons, thus accelerating the conversion of pollutants to less-toxic or innocuous substances. **Altogen Labs** has developed methods for isolation, selection, and cultivation of highly efficient hydrocarbon-degrading microbes specific to the site of a particular spill. Our technology is scalable, and has been optimized to account for variables like temperature, soil conditions, and oil composition. Through our unique methods, we have developed a library of over 150 strains of natural oil-degrading bacteria. These microbes have been demonstrated in the laboratory and in field tests to be highly efficient for remediation of crude-oil and petroleum saturated earth. Our bioremediation products are fast, safe, and cost-effective for restoring large amounts of soil and liquids that have become hydrocarbon contaminated (oil, petroleum, pesticides, carbon tetrachloride, acetone, benzene,

toluene, dichloroethylene, and other industrial and chemical manufacturing wastes).

At **Altogen Labs**, we recognize that providing products and services in a timely fashion is critical to project success. **Altogen Labs** is committed to meeting our client's expectations while maintaining high quality and integrity.

Altogen Labs offers instant quotes, comprehensive service packages, and highly competitive pricing. It would be our pleasure to discuss the details and goals of your current research project and how we can assist you in meeting them. For price quotes and timeline estimates we can be reached by email, info@altogenlabs.com, and by phone at (512) 433-6177.

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