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CNS DRUG DEVELOPMENT – INTEGRATED SOLUTIONS LESSEN COMPLEXITY

The different parts of the nervous system, including the brain and spinal cord (i.e., central nervous system – CNS) and the peripheral nervous system, are important drug targets for many serious diseases affecting human health. As the body's processing center, the CNS is responsible for all functions of our bodies, including thoughts, emotions, memories, and behaviors. Unsurprisingly, drugs working on the CNS are subject to stringent regulatory requirements, and specialized safety assessments are often mandated. To confirm CNS activity without safety concerns, dedicated nonclinical and early phase clinical studies, with relevant endpoints and biomarkers, and supporting bioanalysis, are needed; and drug manufacturing must be carefully managed via rigorous handling and production processes.

Engaging at the outset with a fully integrated and experienced drug development partner can ensure safety, with timely sharing of data at every step of the drug development plan, and facilitate agile, flexible decision-making and planning.

IN THIS ISSUE

INTRODUCTION

According to Fortune Business Insights[1], the global CNS market is projected to grow from $89.02 billion in 2021 to $166.53 billion in 2028, at a compound annual growth rate (CAGR) of 9.4%. There are several reasons for this, some of which are:

- The worldwide population over the age of 65 is increasing faster than the total population, augmenting the demand for effective treatments of age-related neurodegenerative conditions.
- Psychedelic treatments have demonstrated potential, and are increasingly being studied for difficult-to-treat psychiatric conditions, including depression, psychoses, anxiety, addiction, PTSD, and ADHD.
- Large areas of unmet needs are being addressed by CNS-active drugs in:
  - acute and chronic pain relief, where non-opioid alternatives are of great interest
  - brain diseases, such as stroke, epilepsy, encephalitis, and tumors
  - disorders that involve dysfunction of the immune system, such as autoimmune disorders, allergies, and immune deficiencies
  - preventing rejection of transplanted organs or to treat certain types of cancer

The successful delivery of drugs targeting the treatment of CNS conditions is challenged by the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BSCFB), which protect the CNS from intrusion by harmful substances. Large molecules are less able to penetrate the blood/brain barrier to deliver therapeutic results and thus most CNS-active drugs are small molecules.

Agents working on the CNS include antiemetics, anesthetics, antiparkinsonian drugs, stimulants, sedatives, narcotic and non-narcotic analgesics, muscle relaxants, anticonvulsants, antidepressants, antipsychotics, immunomodulators, and interferons.

The many advancements in developing CNS-active drugs have been hampered by higher failure rates compared to drug discovery in most other therapeutic domains. Identifying CNS experts with a well-established record of conducting rigorous, high-quality research can assure the effective management of challenges and reduce the risks of delaying or failing to advance promising drug candidates.

PRECLINICAL SAFETY AND TOXICITY TESTING OF CNS-ACTIVE MOLECULES

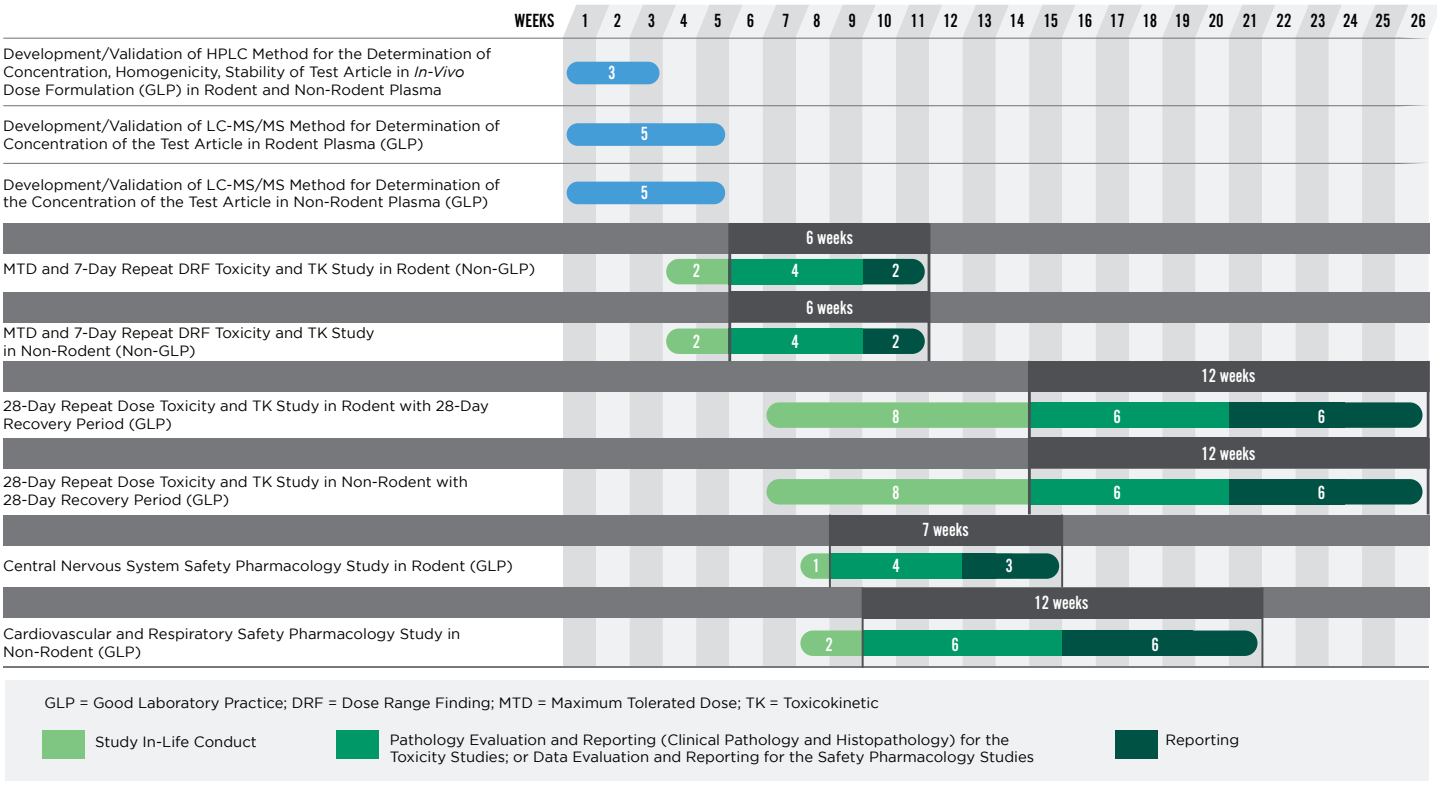
Pharmacology and Toxicology

IND-enabling, nonclinical CNS drug development typically includes pivotal toxicology studies and safety pharmacology studies to evaluate the potential therapeutic effects, define the appropriate route of administration, and establish dosing frequency for later phase studies.

- Carefully designed and well-characterized animal models are crucial for testing disease hypotheses at the organism level and validating human data.
- Robust *in vitro* and *in vivo* studies of drug absorption, distribution, metabolism, and excretion (ADME) build an understanding of toxicities and any possible safety issues of new molecular entities (NME), reducing the risk of failure at the clinical drug development phase.
- Pivotal toxicology studies help to assess novel therapies and advance the best drug candidates to human trials. Collaboration with preclinical partners who have a broad understanding of regulatory expectations regarding CNS drug development and who are experienced in the meticulous execution of well-designed preclinical studies can maximize the chances of a drug's success in the clinic.

The Gantt chart below demonstrates how the in vivo portion of a small molecule drug development program can be completed within six months for submission to regulatory authorities.

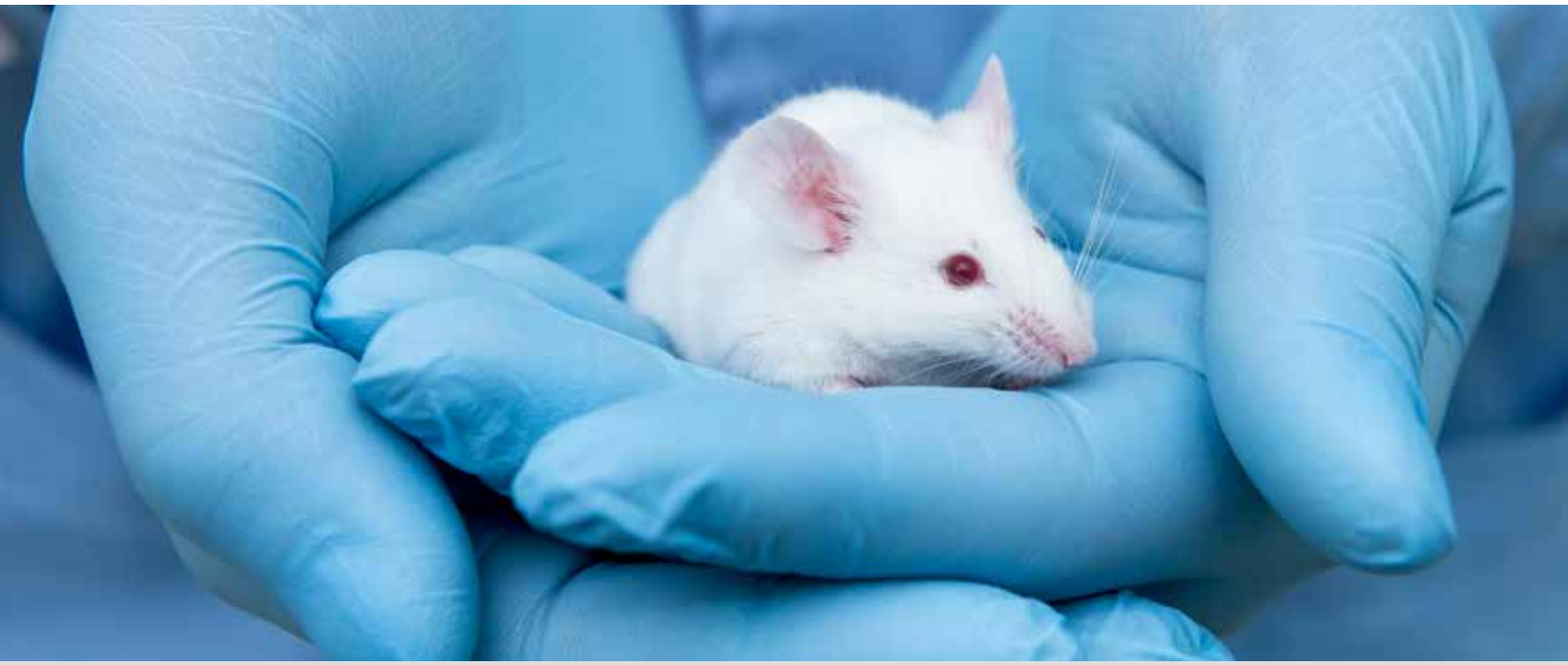
Figure 1.



Animal Models of Disease

Some of the available testing models include *in vivo* studies in rodents (mice and rats) and nonhuman primates (NHPs) for epilepsy, Parkinson's disease, psychiatric disorders, and other brain diseases. A selected list is below:

- **Epilepsy**
  - MTLE mouse and HPD as a biomarker of focal epilepsy
  - GAERS rat and SWD as biomarker of absence epilepsy
  - Endpoint measurements (NHP)
- **Essential Tremor**
  - Harmaline mouse and the ET band as biomarker of essential tremor
- **Sleep Disorders**
  - Endpoint measurements in NHP
- **Parkinson's Disease**
  - 6-OHDA rat (unilateral) and BetaPark biomarker of late-onset Parkinson's disease phase
  - 6-OHDA rat (unilateral) and GammaPark biomarker of L-DOPA induced dyskinesia and AIM scoring
  - Alpha-synuclein rat model and BetaPark as evolutive biomarker of prodromal phase of Parkinson's disease
- **Cognitive Function (Alzheimer's, Schizophrenia)**
  - Pharmaco-induced models of cognitive dysfunction, tested with ASSR and ERP protocols, both used in the clinic
  - Assessment of various cognitive modalities using "trained" NHPs with the Cambridge Neuropsychological Test Automated Battery (CANTAB)
  - Transgenic or surgical models from third-party provider
- **EEG model phenotyping:** from signature identification to biomarker validation and beyond.
- **Neurophysiological biomarkers** with EEG-implanted telemetry devices in NHPs
- **Pharmaco-EEG** studies to assess the effects of psychotropic medications on the bioelectrical activity of the brain in NHPs



Toxicology Studies

Toxicology testing for CNS-active drugs includes the standard requirements in the M3(R2) FDA guidance:

- **Acute toxicity** testing to determine the maximum tolerated dose (MTD) in the test species.
- **Repeat-dose toxicity** testing to characterize adverse effects after repeated daily dosing with the test article. Assessments include changes in morphology, physiology, growth or life span, clinical chemistry, and behavior. The studies provide essential data on the general toxicity, the toxicity to specific target organs, the dose-response relationship, responses to toxic metabolites formed in the organism, delayed responses, cumulative effects, and information on reversibility/irreversibility of the effect.
- **Chronic toxicity** studies to provide data on adverse effects following repeated administration of a test substance over a significant portion of the life span of the test species. Establishing the duration of a chronic study is based on the anticipated human exposure of the drug treatment.

Nerve Conduction Studies (NCS)

Specialized assessments in nerve conduction are sometimes performed in CNS drug development, depending on the specifics of the NME. These key assays, performed in NHPs, can be included in preclinical safety and toxicology programs to assess the peripheral neuropathy (PN) liability of a new drug. NCS or neurography evaluations are non-invasive electrodiagnostic techniques that evaluate nerve injury or degeneration by testing the ability of the nerve to conduct an electrical impulse. The nerve is stimulated at one or more sites along its course, and the electrical response of the nerve is recorded.

There are currently no guidelines standardizing NCS, so sponsors will want to engage with an experienced preclinical partner who can provide invaluable knowledge for selecting the type and number of nerves, and minimum combinations appropriate for their stage of drug development or indication.

Safety Pharmacology Studies

Safety pharmacology studies are focused on identifying adverse effects on physiological functions following a single-dose administration in laboratory animals. The single dose ranges from one that produces minimal to no effect, up to a dose that produces noticeable effects.

The CNS safety protocols under International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use S7A necessitate the inclusion of a thorough and detailed behavioral analysis of home cage activity, the response to handling, open field observation with ancillary measures of basal muscle tone, muscle strength, and tremor in a functional observation battery (FOB), as well as quantitative measurements of three-dimensional activity in an automated photobeam arena.

Assessment of motor function and locomotor activity (LMA) is a predictive bioassay to study the effects of drug substances from pharmacology, neurobiology, and toxicology perspectives. Test article-induced alterations in motor activity will present as abnormal movements, impaired coordination, slowing of responses, or the interference of normal movements by the increased expression of fixed, repetitive, purposeless behavior (drug-induced stereotypies).

Detailed Cage-Side Observations

Cage-side observations allow investigators to assess the presence of test article-related abnormalities on normal animal behaviors, like eating, drinking, sleeping, and ambulation. Regulators are interested in similar clinical neurological endpoints that would affect any veterinary or human patient populations, as seen in Table 1 below.

Table 1. Summary of Measures in the FOB, Type of Data Produced by Each.

Observation	Type of data	Home cage
Posture	Descriptive	x
Convulsions	Descriptive or rank (present/absent, Racine score)	x
Palpebral closure	Ordinal	
Lacrimation	Ordinal	x
Piloerection	Nominal (Y/N)	x
Salivation	Ordinal	x
Vocalization	Nominal (Y/N)	
Rearing	Counts	x
Urination	Count (estimate)	
Defecation	Count	
Gait	Descriptive or rank	x
Arousal	Ordinal	x
Mobility	Ordinal	x
Stereotypy	Descriptive or rank (present/absent, type)	x
Bizarre behavior	Descriptive or rank (present/absent, type)	x

Abbreviations: N=No; Y=Yes.

Open-Field Observation

Open-field observation permits the evaluation of drug-related effects on additional aspects of animal behavior. Animals placed into unfamiliar territory usually respond with a subjective state of anxiety, arousal, or fear. When an animal is placed into the center of the open field, it feels exposed, and will typically move to the wall or corners to minimize detection by predators. Any delay in this behavior of "taking cover" delivers valuable data on CNS integration of sensory/motor functions, and the animal's processing of environment stimuli while under the influence of the test article.

Automated Locomotor Activity (LMA) Recording

Current guidelines specify that recordings of locomotor activity be automated, that the behavior of individual animals be observed, and that the automated recording session be long enough for the dependent measure to reach an asymptotic level in vehicle control animals. The automated data collection system provides measures of activity counts (horizontal plane, basic, and fine), rearing counts (vertical plane), and the total distance traveled by an animal for a longer duration of time than the open-field and cage-side observational periods (120 minutes).

The automated LMA system provides a different perspective of motor function when compared to the 18 observational units of the cage-side and open-field assays. Automated recording of animal behavior offers several advantages:

- Can be less labor-intensive than human intervention.
- Is compatible with the automated data recording demanded by GLP regulations (§58.61 to .63).
- Reduces human error and differences between individual observers.
- Can measure behavior in the dark, to include the full behavioral repertoire of the animal.

Cell and Gene Therapy in CNS Drug Development

In addition to small molecules and other advancements, gene therapy offers great potential in meeting medical needs for CNS diseases that currently have few, or no, treatment or prevention options. Gene therapy has the potential to cure, treat, or prevent diseases via the administration of biological medicinal products containing deoxyribonucleic acid (DNA) or ribonucleic acids (RNA), administered to regulate, repair, replace, add, or delete a genetic sequence.

Gene therapy holds a strong promise to becoming a safe and effective option for CNS diseases as the science and technology of this highly specialized and ground-breaking approach continues to advance.

ALTASCIENCES' CASE STUDIES – PRECLINICAL

Gene Therapy and Stem Cell Transplantation via MRI-Guided Intraparenchymal Delivery into Brain Regions (Cortical and Subcortical)

Abstract

This stem cell transplantation therapy required midline incision, unilateral or bilateral craniotomy, implantation of cannula guides into adult rhesus and cynomolgus macaques (~2.5 to 15 kg). The animals were prepared with Isoflurane anesthesia, and MRI-compatible stereotactic frame placement. Real-time MRI imaging allowed technicians to confirm delivery of the treatment to appropriate regions of the brain, and adjust flow rate, dosage, and other parameters as needed to deliver best results.

Two procedures are described below.

Method

Real-time MRI guided infusion and coverage.

Procedure 1

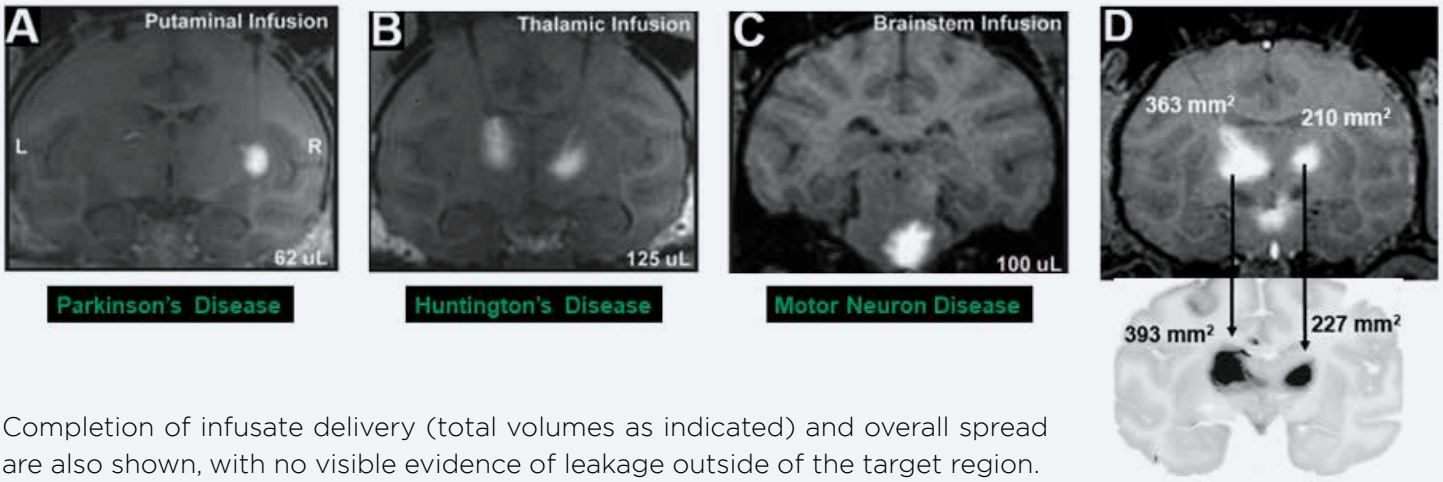
Pre-scanning was performed one week prior to scheduled dosing, to determine the volume to be distributed. An MRI clinical specialist was on-site for dosing.

An MRI-compatible cannula was advanced from the neocortical surface to both brain hemispheres, as both left and right hemispheres were deemed regions of interest. Following cannula tip placement within the cerebellar cortex, infusion was initiated. The infusate was visualized intraparenchymally using a paramagnetic contrast agent for real-time confirmation of test article delivery.

Serial MRI scans were acquired throughout administration to monitor distribution of the infusate within the targeted site (i.e., putamen, thalamus, etc.).

Volume was dependent on coverage. Coverage was monitored through serial MRI scans throughout infusion.

MRI images showing real-time monitoring of infusate into various target regions of interest:



Completion of infusate delivery (total volumes as indicated) and overall spread are also shown, with no visible evidence of leakage outside of the target region.

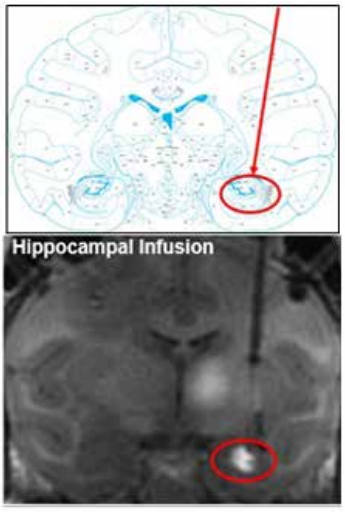
Procedure 2

In this procedure, we infused regions of interest deeper in the brain. Four adult cynomolgus macaques were involved. Three were infused with a vehicle containing gadolinium (Gd) as a contrast agent. One was infused with AAV5 vector encoding enhanced green fluorescent protein (eGFP) and mixed with Gd.

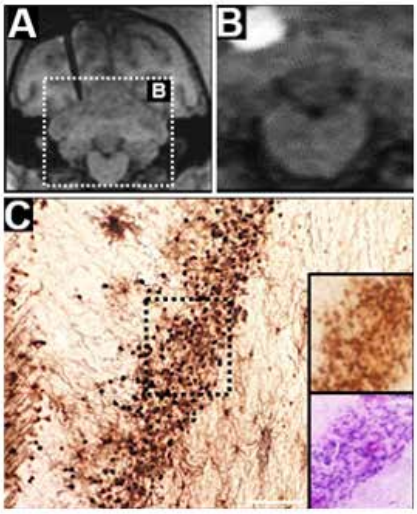
Hematoxylin and eosin staining and immunostaining against green fluorescent protein staining was performed six weeks post infusion.

Hippocampal and cerebellum delivery: images demonstrate looking at an atlas and confirming the delivery into the hippocampus without real-time MRI delivery.

Hippocampal Delivery



Cerebellum Delivery



Altasciences has robust experience and demonstrated expertise in MRI analysis of brain activity following infusion of gene and stem cell therapies.

These published articles detail the described procedures; preclinical work was conducted at Altasciences' Sacramento facility[2,3,4,5]:

Salegio EA, Kells AP, Richardson RM et al. Magnetic Resonance Imaging-Guided Delivery of Adeno-Associated Virus Type 2 to the Primate Brain for the Treatment of Lysosomal Storage Disorders. Hum Gene Ther. 2010 Sep; 21(9):1093-1103. http://doi.org/10.1089/hum.2010.040

Salegio EA, Samaranch L, Jenkins R et al. Safety Study of Adeno-Associated Virus Serotype 2-Mediated Human Acid Sphingomyelinase Expression in the Nonhuman Primate Brain. Hum Gene Ther. 2012 Aug; 23(8): 891–902. https://doi.org/10.1089/hum.2012.052

Salegio EA, Cmpagna V, Allan P et al. Targeted Delivery and Tolerability of MRI-Guided CED Infusion into the Cerebellum of Nonhuman Primates. Hum Gene Ther Methods. 2018 Aug; 29(4):169-176. http://doi.org/10.1089/hgtb.2018.049

Salegio EA, Cukrov M, Lortz R et al. Feasibility of Targeted Delivery of AAV5-GFP into the Cerebellum of Nonhuman Primates Following a Single Convection-Enhanced Delivery Infusion. Hum Gene Ther. 2022 Jan; 33(1).86-93. http://doi.org/10.1089/hum.2021.163

MANUFACTURING CNS-ACTIVE DRUGS FOR TRIALS AND COMMERCIALIZATION

Manufacturing drugs for effective CNS drug delivery is a challenging undertaking, and not only due to the protective nature of the BBB and BSCFB. Since drugs for CNS use will be required to cross the protective barriers, ensuring purity and consistency of dosage is of paramount importance. Adding to the complexity is a requirement to ensure the safety of pharmaceutical manufacturing operators and technicians, and limiting their exposure to the active ingredients during formulation and processing. Experienced pharmaceutical manufacturing partners with an excellent safety inspection record can navigate the specifics of CNS drug development.

The CNS drug field is vast, and includes numerous formulation options.

Liquid-Filled Capsules

Liquid-filled capsules (LFCs) are an ideal solution to many of the challenges involved in CNS drug formulation and manufacturing. This formulation option is effective for drugs that are:

- Poorly water soluble or with low bioavailability, like many CNS drugs.
- Highly potent, since there is a low risk of cross contamination or airborne exposure to operators due to closed system processing.
- Low dose, since they provide dosing uniformity as the active ingredient is dissolved in liquid.

LFCs are becoming a popular dosage form for early phase CNS clinical trials as they offer several advantages over other solid dosage forms, such as ease of scalability and manufacturing, faster absorption, and higher product stability. They also offer abuse-deterrent benefits since they make it harder to insufflate, inject, or alter the extended-release properties of potent drugs.

It is important to engage with a manufacturing partner who can expertly determine if your API is appropriate for an LFC dosage form, and who can efficiently manufacture the required clinical supply for your first-in-human (FIH) trials.

Nanotechnology Solutions

One promising new platform for developing CNS drugs is nanotechnology, which has shown great potential to overcome difficulties related to traditional drug delivery approaches.

Nanoengineered molecules can carry out specific functions, such as crossing the BBB, targeting specific cell or signaling pathways, responding to endogenous stimuli, and acting as a vehicle for gene delivery. A wide range of pharmaceutical nanocarriers have been developed, including liposomes, polymeric nanoparticles (PNPs), solid lipid nanoparticles (SLNs), micelles, and dendrimers.

Micelles

Micelles have a core-shell structural design with size ranging from 10 to 100 nm, consisting of an outer hydrophilic layer primarily composed of polyethylene glycol (PEG) and an inner hydrophobic core made up of molecules such as polycaprolactone, polypropylene glycols, phospholipids, and fatty acids. This combination allows for the loading of hydrophobic drugs. The external hydrophilic shell provides stability to micelles in an aqueous environment and prolongs their circulation time in the bloodstream. Micelles facilitate the delivery of low molecular mass drugs to the brain by enhancing the drug solubility and stability in plasma.

Micelles can be modified to enhance concentration of the loaded drug so they can cross the BBB more easily. One such modification that has proven effective in mice is attaching either polyclonal antibodies against brain-specific antigen, a2-glycoprotein, or insulin to target the receptor at the luminal side of the BBB. Another modification of the micelle system is direct conjugation of the drug molecule and targeting moiety to the amphiphilic segment.

Polymeric Nanoparticles

Polymeric nanoparticles (PNPs) range in size from 10 to 100 nm, and are composed of biodegradable and biocompatible polymers such as poly (alkyl cyanoacrylate) (PACA), polyesters such as poly (lactide) (PLA), poly (D,L-lactide-co-glycolic acid) (PLGA), and natural proteins, and polysaccharides with a size ranging from 10 to 100 nm. They consist of a core of dense polymer matrix to encapsulate the lipophilic drugs, and a hydrophilic corona to provide steric stability to nanoparticles (NPs).

The residence time of these NPs in systemic circulation can be increased by surface modification with physical adsorption or covalent binding of hydrophilic polymers such as PEGs and polysaccharides, which have demonstrated ability to cross the BBB and increase the brain concentration of drugs specifically targeted to the CNS.

Solid-Lipid Nanoparticles

Solid-lipid nanoparticles (SLNs) are an effective nano-delivery system. SLNs are aqueous colloidal nanocarriers, composed of physiological lipids (triglycerides, fatty acids, steroids, waxes, etc.), dispersed in water or an aqueous surfactant solution which solidifies upon cooling.

SLNs have several properties which make them excellent vehicles for CNS drug delivery—low intrinsic cytotoxicity, physical stability, shielding of labile drugs from degradation, and controlled release. Several strategies exist to increase the drug loading capacity and long-term stability of SLNs to increase penetration of the BBB and enhance drug delivery to the CNS.

Nanoemulsions

Nanoemulsions are oil-in-water (O/W) or water-in-oil (W/O) colloidal particulate systems composed of edible oils, surface-active agents (surfactants) and water, having a size range of 100 to 500 nm. Their properties address the challenges of low bioavailability, poor targetability, and penetrability across the BBB.

ALTASCIENCES' CASE STUDY – CDMO

Evaluating Milling Conditions for Scaling Up a Nanosuspension Drug Product

A client contracted Altasciences for our expertise and experience in scaling up the manufacturing of their nanosuspension drug product. Their goal was to have enough product to support Phase III clinical trials. The manufacturing conditions needed to be optimized to scale up to a 200 L theoretical yield and achieve target particle size through a series of 10 pilot batches. This study was proposed to best evaluate the milling conditions necessary to constantly and reliably manufacture their nanosuspension product.

Drug Development Phase: Phase III clinical studies

Class of Drug: Small molecule, BSC Class II, a synthetic neuroactive steroid that acts as a positive allosteric modulator of the gamma-aminobutyric acid (GABA)A receptor complex used for the treatment of seizures.

Route of Administration: Oral

Equipment: NETZSCH DeltaVita® 10000 milling system

Methods and Results

The equipment used was the NETZSCH DeltaVita® 10000 milling system, which has a 10 L milling chamber with a motorized agitator and 0.5 mm yttria-stabilized zirconia grinding media. The drug suspension was pumped through the chamber, reducing the drug compound's particle size. As this process was scaled up, the parameters needed to be optimized for the increase in drug volume.

Two initial runs were performed to establish the study's baseline process. From this baseline, three milling conditions were evaluated, focusing on two variables: media load and agitator speed. Media load ranged from 88% to 92%, and agitator speed went from 1,000 rpm to 1,100 rpm with the chiller set points at either -5 °C or 5 °C, for a total of 10 runs. The drug substance was milled at a concentration of 25% w/w until the target particle size was reached. The 25% w/w drug nanosuspensions were diluted to 5% w/w, bottled in primary packaging, and placed on stability.

Once completed, each run was release-tested for appearance, ID, assay, dissolution, particle size, pH, elemental impurities, and resuspendability. Eight of the 10 batches were placed on stability. The stability batches were tested at 0, 1, 3, and 6 months under standard ICH stability conditions.

The successful study outcome paved the way for a smooth scale-up of the nanosuspension by optimizing the milling speed, media load, and temperature, to provide a stable nanosuspension for Phase III clinical trials.

MAXIMIZING EARLY PHASE CLINICAL TRIALS OF CNS ACTIVE DRUGS

Phase I clinical trials for CNS drug development involve administration of potential therapeutics to healthy individuals with intact neurocircuitry. The availability of drug doses and concentrations, and pharmacological, clinical and/or toxicological data from animal models relevant for the human disease are powerful tools, assisting in the design of informative FIH protocols that minimize safety risks. High levels of inter-species data agreement can improve forecasting of effects in healthy normal volunteers and patients, and streamline the clinical study design process, while inter-species deviations in predictive characteristics can significantly alter the design and complexity of clinical studies.

A comprehensive understanding of the science, along with the principles of human behavior, is needed to support studies that can establish doses covering the therapeutic range, incorporate measurements predictive of clinical efficacy, and identify dose-related adverse effects.

Regulatory authorities provide general guidance for clinical trials involving INDs, including those for CNS disorders. The FDA guidance and EMA guidance include key considerations, such as:

1. **Study populations:** In Phase I, the drug is administered to healthy normal volunteers. The trial design and participant selection should consider the specific patient population that will ultimately be treated with the drug. It may be of value to include specific age groups, and participants with certain comorbidities/medication regimens, in early phase cohorts, as these factors could affect future phases of development.
2. **Study design:** The trial design, including the relevant endpoints, should be appropriate for the condition being studied and the stage of development of the drug. For CNS disorders, the study may need to include safety assessments related to CNS adverse events, measures of cognitive or behavioral outcomes, and specialized assessments, described in more detail in the next section of this article.
3. **Dose selection:** The dose of the investigational drug should be carefully selected based on pharmacokinetic and pharmacodynamic data, as well as safety considerations. Brain receptor occupancy/exposure can influence the dosing decisions, as receptor occupancy and exposure time determine the therapeutic effect of a drug. The exposure time is the duration of time that a drug molecule is present in the body at a concentration sufficient to produce a pharmacological effect. This parameter is influenced by a drug's ADME properties. Each drug is unique, and the specific measurement approach is based on appropriate preclinical findings, and the findings/observations from previous clinical trials in the same or similar drug classes.

4. **Biomarkers:** The trial may use biomarkers to help assess drug efficacy and safety, particularly for diseases with complex or unclear pathophysiology.
5. **Safety monitoring:** The trial should have a robust safety monitoring plan. Carefully selected Safety Review Committees (SRCs) and Data Safety Monitoring Boards (DSMBs) can ensure that all study-related decisions consider the complexity of the domain, and ensure subject safety. Specialized pharmacy capabilities are required to ensure that all potentially hazardous drugs are handled with utmost care. Test drugs should be kept in a secure and locked area, accessible only by appropriately trained pharmacy staff.
6. **Data analysis:** The trial should use appropriate statistical methods for analyzing the data, which may have complex data structures or require specialized methods for handling missing data or correlated outcomes. Expertise in pharmacodynamics, including behavioral and cognitive assessments and their analysis, is a key element of CNS drug development.

The FDA created the Office of Neuroscience in 2012, to promote and assist sponsors with CNS drug development. There are five divisions—Neurology I and II, Psychiatry, Addiction, and Pain. Contact information is available on their webpage.

Specialized Evaluations

Specialized evaluations drawing from neurosciences models (e.g., anxiety-like behaviors in mice, depressive behaviors associated with forced swimming in rodents, pain models), cognitive testing, evaluation of abuse potential or dependence, driving simulation, measures of neurological status associated to physiological reactivity (e.g., pupillometry), cardiac assessments (e.g., QT prolongation), CSF sampling, and proof of biochemical mechanisms (laboratory biomarkers) are some of the key elements of CNS drug development pathways.

Cognitive Tests

Examples of common cognitive tests include, but are not limited to:

- Choice reaction time
- Divided attention
- Digit vigilance
- Digit symbol substitution
- Spatial working memory
- Sternberg short-term memory
- Hopkins verbal memory
- Paired associates learning
- Questionnaires
- Visual analog scales (VAS)
- Rating scales
- Balance platform
- Finger tapping
- Simple reaction time

Abuse and Dependence Evaluations

Some CNS drugs carry higher risks of misuse, abuse, physical dependence, and possible overdose, and FDA guidance requires that NMEs that affect the CNS be evaluated for abuse and dependence potential. It is important to understand the risks associated with these molecules early in development, and ensure appropriate measures are taken to protect study participants, clinic staff and, ultimately, the patients benefiting from the therapeutic effects of these medications. Drugs that treat pain, depression, anxiety, attention disorders, and sleep disorders are considered high risk.

In general, it is recommended that the specific abuse-related studies be conducted only after the therapeutic dose range is determined. These data inform the appropriate labeling of the drug product and the FDA recommendations for drug scheduling under the CSA.

Cardiac Health Assessments

The FDA E14 guidance stipulates that all NMEs be evaluated for their cardiac effects. This requirement is particularly important for CNS drugs. Rigorous early QT assessments performed in FIH, SAD/MAD trials can provide highly relevant data that reduce the risks for the later stages of the drug development program, and may be used to waive a requirement for a dedicated QT study.

Driving Simulation

The *Evaluating Drug Effects on the Ability to Operate a Motor Vehicle* guidance issued by the FDA requires that new CNS drugs be evaluated for their potential to impair driving ability. Data should be collected throughout the program, beginning in Phase I trials, to inform the future decision on whether a dedicated driving trial is required. Adverse events of interest, such as somnolence and cognitive changes, should be analyzed throughout the program.

"A systematic effort to identify drugs that increase the risk of motor vehicle accidents is a critical component of assessing drug risk and designing strategies to reduce this risk... objective information about how a drug affects driving ability may be needed to enable safe use."

At Altasciences, we utilize an **in-house suite of 10 driving simulators** to meet this regulatory requirement.

Imaging Technologies

In combination with safety and tolerability data, non-invasive imaging techniques provide knowledge for informed decisions on novel CNS drug candidates. Non-invasive imaging technologies include:

- Electroencephalograms (EEG)
- Event-related potentials (ERP)
- Polysomnography (PSG)
- Nuclear molecular imaging
- Positron-emission tomography (PET)
- Single photon-emission computed tomography (SPECT)
- Functional and structural imaging
- Magnetic resonance imaging (MRI)
- Magnetic resonance spectroscopy

Early knowledge of important drug effects helps inform speedy decision-making and go/no-go decisions—if a program is to fail, it should fail early and fast, so that resources are not used in vain.

CSF Sample Collection

In Phase I CNS drug development, in addition to assessment of systemic exposure, critical PK data is generated via traditional standard blood/urine collections, although the need for CSF collected from healthy participants may also be required. The collection is typically done via lumbar puncture, also referred to as spinal tap for a single sample, and spinal catheterization in the subarachnoid space for continuous sampling. Small volumes of CSF are collected at each timepoint. As the procedure itself is high risk, and the adverse events associated with CSF withdrawal can be significant, collection timepoints are kept to a minimum. Collection is more challenging in certain patient populations, as well as with elderly participants. When conducting trials involving CSF collection, it is crucial to ensure appropriate expertise by the medical staff.

ALTASCIENCES' CASE STUDIES – CLINICAL

Case Study 1 – Driving Simulator Assessment

The study drug was a centrally acting, multifunctional serotonin agonist/antagonist that was developed for the treatment of hypoactive sexual desire disorder (HSDD) in premenopausal women. To achieve therapeutic efficiency and minimize the most common adverse effects (AEs) reported after dosing—which include dizziness (11.4%), somnolence (11.2%), nausea (10.4%) and fatigue (9.2%)—chronic bedtime oral dosing was necessary to ensure these AEs would not affect the ability to drive in the morning,

Altasciences conducted novel endpoint research to determine the extent of next-day impairment in cognition and alertness.

A randomized, double-blind, placebo-controlled, four-way crossover study was performed to evaluate the potential next-morning residual effects after bedtime dosing in 72 healthy premenopausal women. Treatment arms included a placebo administration group (acute and chronic), positive control zopiclone group (7.5 mg qhs; acute only), and two separate dosage groups of CNS study drug, which included 100 mg qhs (acute and chronic) or 200 mg qhs (acute after 100 mg chronic). Assessments were based on simulated driving, a Symbol Digit Coding Test (SDCT), and the Karolinska Sleepiness Scale (KSS).

Preliminary evaluation of next-day impairment during simulated driving revealed bedtime administration of the study drug, up to 200 mg did not impair next-day cognitive function or driving performance, while improving symptoms of HSDD. Women dosed with 100 mg qhs of the study drug before bed were found to have significantly ($p<0.01$) lower standard deviation of lateral position (SDLP) values compared to acute and chronically dosed placebo groups. No statistically significant differences were found between the 100 mg and 200 mg dose groups.

Altasciences was able to demonstrate that there was no impairment to driving the morning after nighttime dosing of the CNS study drug in premenopausal women. The study was described as "reassuring" by the FDA.

Case Study 2 – Consult this human abuse potential study conducted at Altasciences, published in the journal Sleep.

BIOANALYSIS FOR CNS DRUG DEVELOPMENT – PRECLINICAL TO PHASE IV

Bioanalysis of CNS therapies involves the measurement and analysis of drug levels, metabolites, and biomarkers in biological samples, such as blood, CSF, and brain tissue. This type of analysis is important in evaluating the efficacy and safety of CNS drugs, as well as understanding their pharmacokinetic and pharmacodynamic properties.

Bioanalysis of CNS therapies can be challenging due to the complexity of the CNS and the BBB, and the low concentrations of drugs and metabolites in biological samples can require sensitive and selective analytical methods for detection and quantification.

Various analytical techniques are used in bioanalysis of CNS therapies, including liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), and immunoassays. These techniques allow for the detection and quantification of drugs and metabolites at low concentrations, as well as the characterization of drug metabolism and pharmacokinetics.

Measures of Drug Pharmacokinetics in the CNS

The most common approach to generating PK data for nonclinical and clinical trials is drug sampling in CSF. Drug and metabolite concentration is often determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS), due to the technique's inherent ability to support high levels of sensitivity and specificity. Both small and large molecule biotherapeutics are amenable to LC-MS/MS, with pg/mL detection limits achievable. Advanced sample preparation processes can include solid-phase extraction, supported liquid extraction, and hybridization workflows, the latter applied to proteins and anti-sense oligonucleotides wherein highly selective complementary capture probes are leveraged to isolate the biotherapeutic of interest from a milieu of matrix components.

Precautionary measures are often required when processing CSF samples due to the high ionic strength of the matrix, facilitating non-specific binding of drug to the collection vessel. Such adsorption losses of analyte results in a negative bias for reported concentrations, particularly at low levels of circulating drug. To mitigate non-specific binding, several anti-adsorptive reagents are investigated during method development, and appropriate sample processing instructions are established to ensure accurate determination of drug concentration for each unique drug development program.

Concentrations of analyte in CSF, accurately determined by the analytical technique of choice, can be used as a surrogate measure of the extent to which a drug has crossed the BBB or BSCFB, and is available to the CNS. Generally, unbound CSF concentrations are used as surrogates for unbound brain tissue concentrations in animal models, based on the free drug hypothesis that protein-unbound drug moves passively from the plasma through the BBB and BCSFB into the brain and CSF. This hypothesis holds true for many CNS drugs, although there are significant exceptions.

In case of *in vivo* measurements made at a single timepoint, the concentration of drug in brain or CSF may be normalized to a simultaneously collected plasma concentration. This is a common method for estimating the extent of drug uptake into the CNS, and it allows for comparisons of uptake between drugs, as well as the rates of entry and elimination of the drug in plasma. However, CSF and brain compartments differ, which may confound the

results. Sparse serial sampling is one approach to avoid confoundment by characterizing the drug's full PK profile in the CSF and plasma and calculating the ratio of drug exposure in the two compartments by measuring the area under the concentration-time curve.

In vitro models of the BBB are used to determine the extent to which investigational agents cross into the brain. There are several validated models of the BBB from multiple species and, although there is no ideal cell line, the most widely used and well-characterized human cell line is the human immortalized endothelial cell line hCMEC/D3. hCMEC/D3 experiments can quantify drug permeability, identify relevant drug-efflux transporter interactions, rapidly screen drug candidates for CNS activity, and carry out initial PK studies. *In vitro* systems may have to undergo modification such as co-culture with other brain cells to replicate tight junctions of BBB.

In vivo imaging techniques, such as positron emission tomography (PET), are used to generate longitudinal data on drug disposition. PET is a non-invasive imaging technique that works by detecting radio-labelled ligands over a specified time period. It has been used to measure absolute spatial concentration of drug and determine PK parameters as well as target occupancy of several CNS-acting drugs.

All approaches and techniques have characteristics that make them more suited for one program than another. Discussion with qualified experts, and review of the data generation requirements, will allow for selection of the most appropriate quantification method for a specific CNS drug program.

Altasciences' CNS Center of Excellence

Altasciences has significant expertise in CNS-active drugs. We offer integrated end-to-end services from prototype formulation development and manufacturing to nonclinical and clinical studies, including bioanalysis for optimizing go/no-go decisions.

Our **pharmaceutical manufacturing** site can meet all your CNS drug development needs, from prototype formulation through commercialization. Our facilities are fully-equipped to handle **highly potent** compounds (drug Schedule I-V), as well as techniques such as **nanomilling,** and the manufacturing of almost every dosage form on the market, including **liquid-filled capsules**.

Altasciences' **bioanalytical capabilities** include support for preclinical and clinical studies (to Phase IV), with expertise in CSF and other matrices commonly used in CNS drug development. Our analysts, working with the **latest equipment**, are available 24/7 if needed, to ensure program timelines are respected.

We have supported numerous CNS **nonclinical** safety assessment programs for both small and large molecules, and have capabilities that include a full range of study types, in all species. We have particular expertise in cell and gene therapies and oligonucleotides, in epilepsy, amyotrophic lateral sclerosis (Lou Gehrig's disease), Huntington's disease, and Batten disease (among others).

In addition, for CNS-active drugs, we have specialization in many large and small animal models of disease, and assessment of various cognitive modalities using "trained" NHPs with the Cambridge Neuropsychological Test Automated Battery (CANTAB); neurophysiological biomarkers with EEG-implanted telemetry devices; and endpoint measurements for sleep and epilepsy, as well as pharmaco-EEG studies, among others. Our robust understanding of the regulatory guidelines ensures that your drug development program includes all the necessary elements to complete your IND.

Our state-of-the-art **clinical research** campuses were specifically designed for conducting trials that require multifaceted safety monitoring assessments of study participants, and are staffed 24/7 by Advanced Cardiac Life Support (ACLS) trained paramedics, safety officers, and nurses. All participants are assessed daily by an Investigator and under constant close supervision. Panic buttons are strategically placed throughout our clinics so that participants can alert clinical staff of emergencies. All clinical staff are certified in Basic Cardiac Life Support and trained in scenario-based responses. Our clinical facilities have controlled access throughout, and are in close proximity to major hospitals.

Participant safety is enhanced by our utilization of an Association of Accredited Human Research Protection Program (AAHRPP) IRB to oversee trial conduct.

Our clinical team is fully trained in the management and response to adverse events and dose side effects commonly seen from CNS and opioid IPs. We leverage our combined expertise to strategically develop an efficient design that will accomplish your regulatory post-marketing requirements in a robust and cost-effective manner.

On-site, specialized pharmacy services are provided by a team of full-time pharmacists and technicians, including dedicated, unblinded quality control staff for all IP preparation. Our state-of-the-art pharmacy employs multiple security features essential for completing complex clinical studies evaluating CNS or Scheduled IPs. Our multi-layered controlled access includes maintaining strict access controls limited to unblinded staff; ensuring secured access to IP via unique key card; 24/7 video surveillance monitoring; and biometric facial recognition security systems (Kansas facility). The clinic pharmacy is rated for and routinely works with Schedules I-V compounds as approved by the DEA. The pharmacy team is fully prepared to receive and procure the anticipated shipments of IP and compounding materials required (as necessary), including sourcing of products as required. The clinic pharmacy is equipped with sterile biosafety cabinets for IP preparation and a positive pressure clean room and negative pressure cleanroom for extemporaneous product preparation.

Our pharmacists have extensive knowledge and direct experience in preparing and dosing substance abuse, CNS, and HAP studies via oral, sublingual, intranasal, and parenteral routes. In addition, our pharmacy capabilities include over-encapsulation, manipulation, and usability processing of abuse-deterrent oral dosage forms and study blinding of referenced and comparator products. Upon the conclusion of the study, our pharmacy staff will ensure the proper return and/or destruction of IPs as per protocol guidance or site Standard Operating Procedure (SOP). We are in anticipation of what is needed for API analytical testing and capsule preparation prior to each cohorts dosing, and would also handle the necessary dosage strengths and matched placebos.

Altasciences' extensive clinical expertise in the evaluation of CNS drugs includes benzodiazepines, gabapentin, and opioids. We have designed, conducted, and reported on over 40 HAP studies in the past five years alone. We have direct, specific experience in conducting complex Phase I HAP crossover studies evaluating pharmacodynamics (PD) VAS assessments in addition to the safety, tolerability, and PK of CNS-active IPs, including the administration of Schedules I-V compounds.

Additionally, we have been engaged in clinical pharmacology studies in pain medication for almost 20 years. Our experience includes the completion of FIH PK studies to demonstrate equivalence between formulations, as well as studies on extended release and transdermal formulations. In the past 10 years, our experience has extended to the assessment of parameters for PK and PD on stand-alone studies and as part of a FIH protocol. The PD measures we have examined include abuse liability, cognition, biological biomarkers, as well as several different pain assessments in healthy normal volunteers and select patient populations. Our experience with pain models includes includes: heat probes, cold pressor, capsaicin, transcutaneous electrical nerve stimulation (TENS), barostat, staircase, Q-Tip sensory perception, and von Frey filaments.

Our medical network and strategic partnerships, with sites and key opinion leaders (KOLs) across Canada and the United States, allow Altasciences to accelerate enrollment of healthy normal volunteers and patient populations.

Our medical and clinical teams have extensive experience with CNS drugs at high doses and can manage psychiatric adverse events in our customized facilities. Altasciences is unmatched in conducting early phase clinical trials with CNS-active compounds, with special attention to consistent application of PD testing.

ALTASCIENCES' RESOURCES

Webinars:

Inside the Pharmacodynamic Toolbox

Navigating the Yellow Brick Road – Practical Approaches to Conducting Early Phase Clinical Trials with Psychedelics

Podcast – The Next Trip With Altasciences and DevelRx

The Many Facets of Early Phase Evaluation of Psychedelics in Psychiatry

The Strategic Use of Biomarkers and Cognitive Measures in Early Phase CNS-drug Studies

Navigating the Abuse Potential Evaluation of CNS-Active Drugs

Examining Cognition and Driving Ability in Clinical Pharmacology Studies

Navigating Early Phase CNS-Active Drug Development

Strategic Considerations for a Successful CNS Drug Development Pathway

Exploring Abuse-Deterrent Methods

Amphetamine to Zolpidem – Navigating the ABCs of CNS Drug Development

Posters

How Questionnaires, Models, and Tests of Cognition Can Accelerate the Development of CNS-Active Drugs

Methodological Considerations for the Human Abuse Potential Evaluation of Emerging Drug Therapies With Psychedelic Properties

The Altascientist

CNS, Psychedelics, and Other Schedule I Drugs

Complex Considerations for CNS Drugs

Blogs/Bulletins

New Generation Pain Relief Without Opioids

What it Takes — CNS Clinical Trials

Avoid Roadblocks in CNS Drug Formulation (CDMO)

Up Close and Personal With Dr. Denise Milovan, Neuroscientist, Scientific Manager, Biostatistics

Developing the Second Generation of Psychedelics and Their Analogs for Targeted Medical Use

Fact Sheet

Central Nervous System Clinical Trials

Videos

Quick Chat With Dr. Denise Milovan, Neuroscientist, Scientific Manager, Biostatistics

Quick Chat With Dr. Debra Kelsh, MD, Senior PI

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ABOUT ALTASCIENCES

Altasciences is an integrated drug development solution company offering pharmaceutical and biotechnology companies a proven, flexible approach to preclinical and clinical pharmacology studies, including formulation, manufacturing, and analytical services. For over 25 years, Altasciences has been partnering with sponsors to help support educated, faster, and more complete early drug development decisions. Altasciences' integrated, full-service solutions include preclinical safety testing, clinical pharmacology and proof of concept, bioanalysis, program management, medical writing, biostatistics, clinical monitoring, and data management, all customizable to specific sponsor requirements. Altasciences helps sponsors get better drugs to the people who need them, faster.