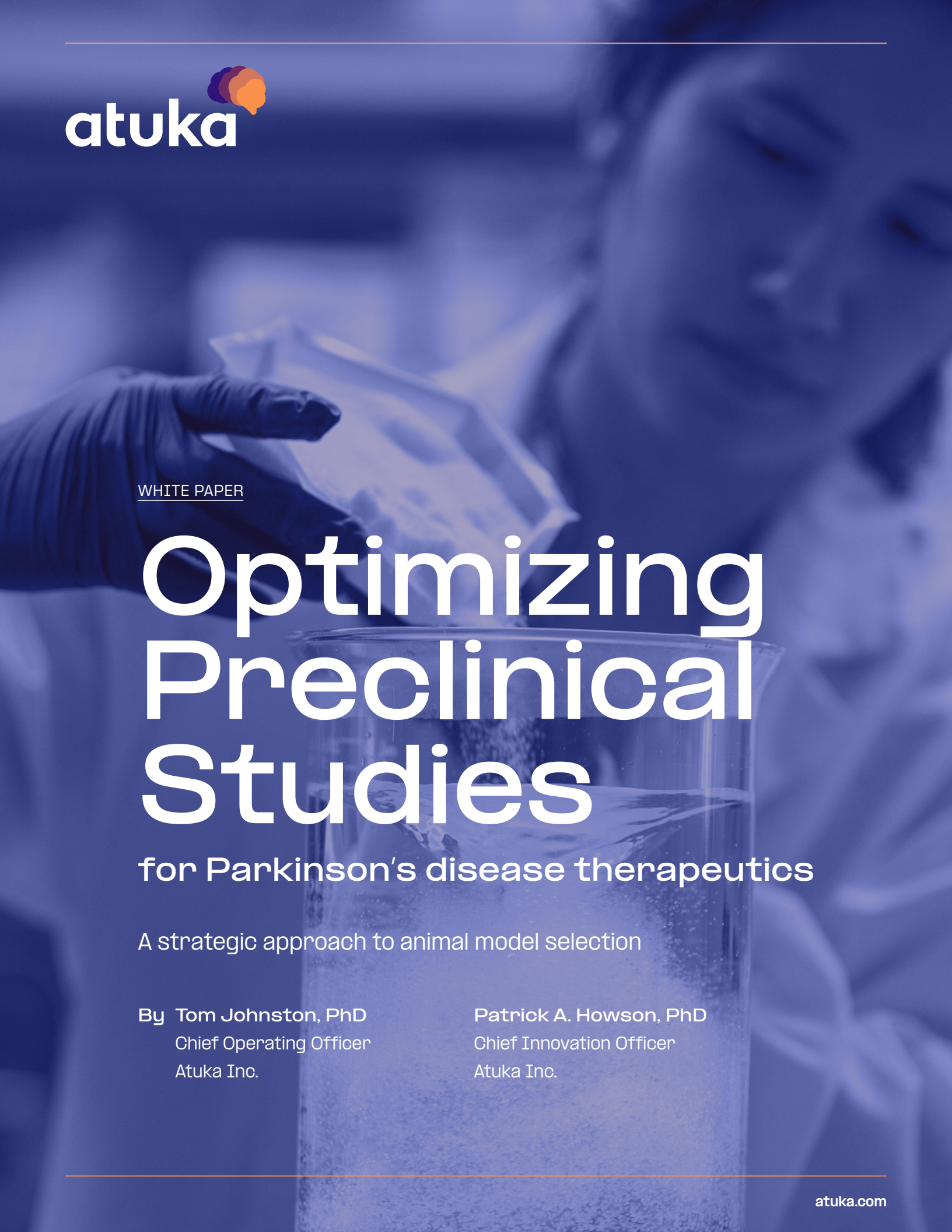

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WHITE PAPER

Optimizing Preclinical Studies

for Parkinson's disease therapeutics

A strategic approach to animal model selection

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When developing new therapies for Parkinson's disease, the choice of preclinical models can make or break the path to clinical success. Designing a preclinical package that properly assesses the pharmacology and efficacy of a new therapeutic, before it proceeds to costly and time–consuming clinical trials, demands careful planning, expertise, and understanding. No two therapeutics are identical, and the pathway to generating a body of evidence that provides a compelling rationale to proceed to clinical trial differs from candidate to candidate.

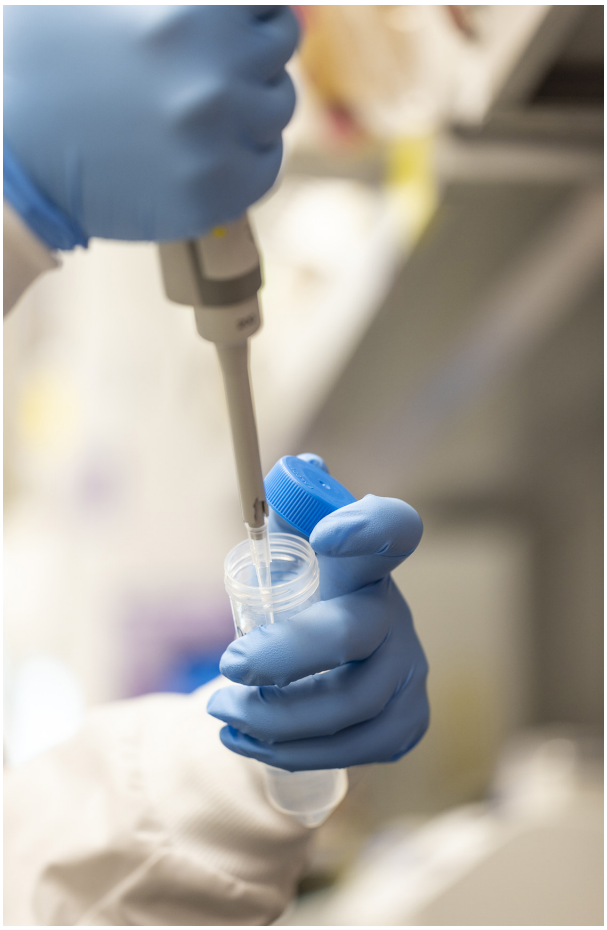
A reliable, high–quality preclinical package is designed to assess the potential benefit of a new therapeutic, and minimize translational risk before it proceeds to costly and time-consuming clinical trials. It's precisely in this assessment of efficacy, however, where too many preclinical studies come up short. Often, it's clear that a drug has some potential value in treating a disease, but not necessarily so clear how that value can be realized.

While the FDA assesses approvals for a therapeutic's moving into clinical trials on the basis of safety, toxicology, and CMC parameters, its Investigational New Drug (IND) application places less emphasis on the treatment's potential clinical benefit. This means that ensuring the preclinical efficacy package is providing high-quality data that supports the proposed design of a Phase II study is the responsibility of the company developing the therapeutic.

This document is a guide to the processes we use at Atuka to help our collaborators design the most impactful and robust studies for a preclinical efficacy package. While it is not possible to cover all scenarios, as each therapeutic is unique, questions common to most therapeutics are covered.

The process of compiling a preclinical efficacy package often involves preclinical efficacy testing performed *in silico* (e.g., computer simulations of biological systems), *in vitro* (e.g., in

isolated cells or biological molecules), or *in vivo* (e.g., in whole, living, animals). Preclinical efficacy evaluation is intended to build a body of evidence that supports, or rejects, progressing the potential therapeutic into clinical development.



A poorly planned and executed preclinical package leads to results that are sub-optimal for translating preclinical efficacy into clinical benefit. This can then make it necessary to perform additional preclinical studies to address knowledge gaps, delaying clinical development and incuring additional cost, or to commence clinical development with a high translational risk. These costs and delays can be prevented with the thoughtful early planning of a preclinical efficacy program that considers the potential clinical development program.

A well-designed preclinical study will inform the design of Phase II studies, de-risking them so that the likelihood of replicating preclinical efficacy results is maximized. However, procedures and endpoints used in preclinical studies sometimes cannot be practically incorporated into clinical studies and, in these cases, it is critical to understand the limits of what can be performed in clinical studies and use this information, in turn, to inform the design of pivotal preclinical efficacy studies. Preclinical efficacy studies like this that inform Phase II clinical study design, or are informed by Phase II clinical study design, are often called 'translational studies.' At Atuka, as is standard for both academic laboratories and Contract Research Organisations (CROs), we use animal models of neurological disorders to help generate preclinical efficacy packages. This process begins by taking known information on potential therapeutics to provide suggestions on what studies would help to define and de-risk future Phase II studies. We then use our *in vivo* animal models of Parkinson's disease to evaluate potential therapeutics and to generate data that informs the clinical development package. Studies can range

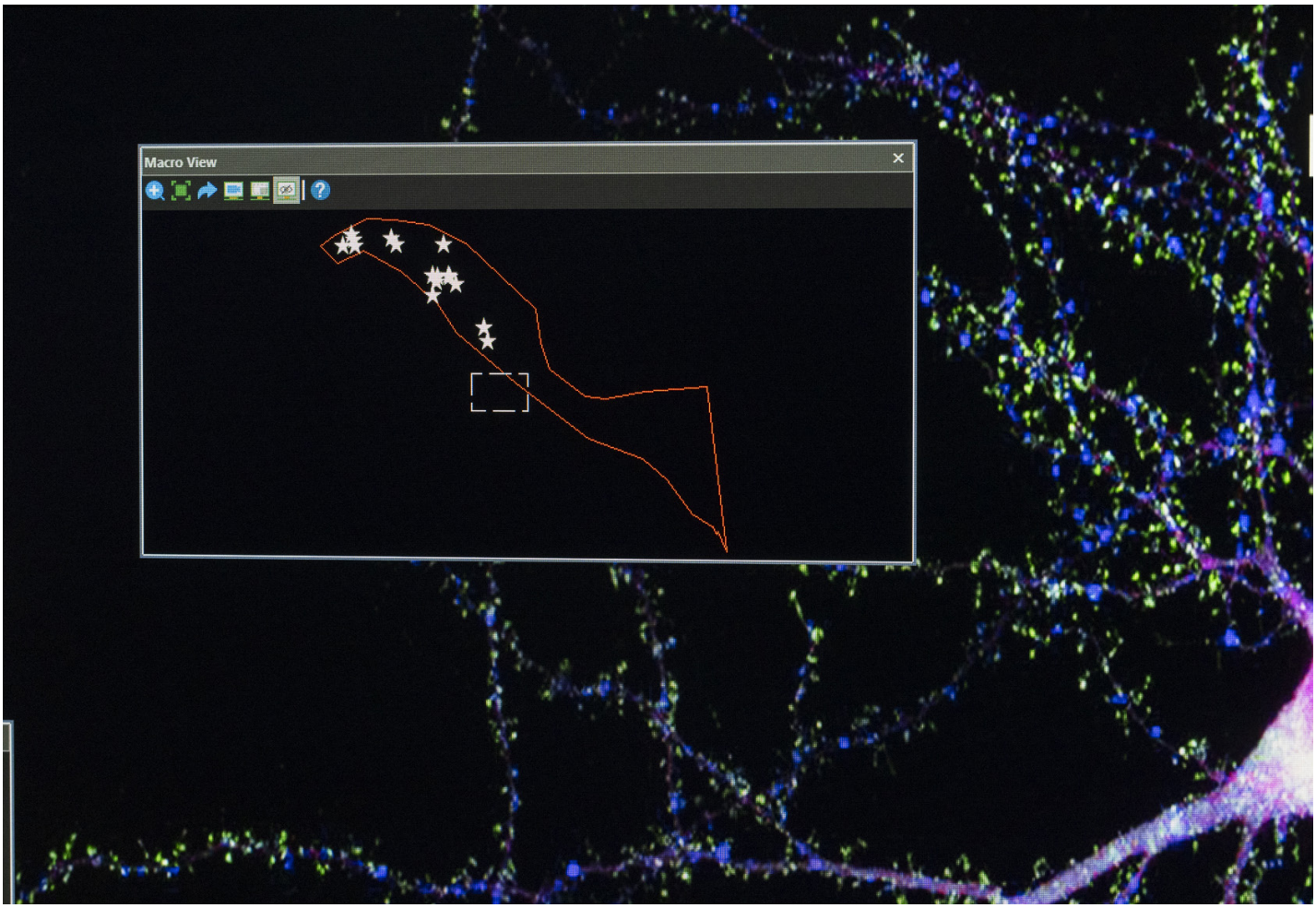
from investigative (pilot) studies, designed to provide high-quality data that allow formal efficacy studies to be designed, through to pivotal, translational studies that are designed to mimic Phase II clinical efficacy studies.

Our many years of using these models — in the evaluation of over 300 potential therapeutics for Parkinson's disease, over 30 of which have moved into clinical development — have led us to closely understand the strengths and limitations of each. No two therapeutics are identical, and the pathway to generating a body of evidence that supports the decision to commence, and inform, the design of Phase II clinical studies, differs from candidate to candidate. We work with our collaborators to provide solutions to their immediate needs and contextualize studies in the overall package of work needed to maximize the translational potential of their therapeutic approach. We regularly collaborate with leading clinicians and regulatory experts external to the organisation, particularly in the realm of disease modification, to refine our translational study design and interpretation to optimize the value of our data.

Getting the research plan right, and maximizing the value of our experience, means working closely with our collaborators until we have a comprehensive understanding of their therapeutic. Understanding numerous factors about the therapeutic, such as:

- the mechanism of action;
- whether it is disease modifying or symptomatic;
- the indication under development;
- whether the approach is small molecule, biologic, or device, and;
- whether the study is investigative or pivotal,

allows us to optimally use our experience in this area and provide the best advice that we can.



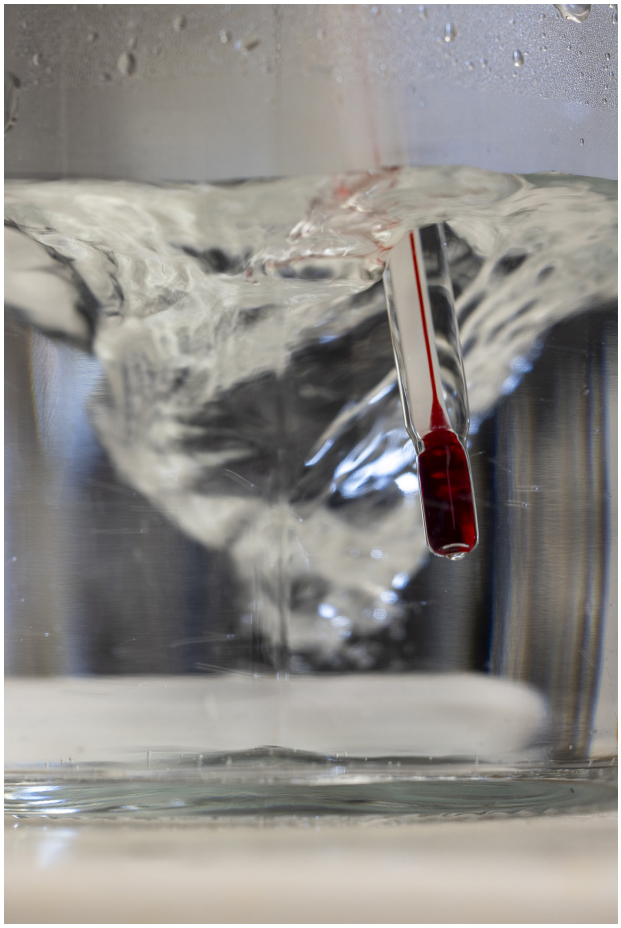
Selecting an appropriate animal model of Parkinson's disease

Individuals that develop Parkinson's disease will generally start off with mild symptoms. Over time, as the disease progresses, these symptoms will become more severe, and an individual will likely also develop additional symptoms. Although this reality is changing, currently Parkinson's is clinically diagnosed. This means that everyone diagnosed with the disease will already have motor impairments. However, the range of other symptoms they develop will vary between individuals. Given the complex symptomatology of people with Parkinson's, and that it evolves over time, it is unsurprising that there is not a "perfect" animal model of the disease. Instead, a suite of models, in different species, have been developed that reproduce one or more of its aspects. The types of models commonly used are:

- **Toxin–based models.** Neurotoxins, such as 6-OHDA, MPTP, and rotenone have been used to generate models of Parkinson's disease in rodents and primates. Numerous models based around neurotoxins exist and are generally used to produce a significant loss of dopaminergic neurons in the Substantia nigra and a behavioural impairment. Although these models can be used in many ways, they are most often used to assess symptomatic treatments.
- **Alpha synuclein based models.** There are three main types of alpha synuclein based models: AAV-based models, PFF-based models, and genetic models. These models drive pathology by the overexpression of alpha synuclein that, over time, leads to the development of a synucleinopathy. These models can be combined, for example, introducing alpha synuclein into transgenic models to produce models with distinctive characteristics such as more aggressive synuclein pathology. These models are often used to evaluate disease-modifying therapies (i.e., therapeutics aimed at slowing, halting, or reversing disease progression). They are also used to study the pathogenesis of Parkinson's disease in an animal system.
- **Non–alpha synuclein genetic models.** Numerous animal models have been generated that are based around known genetic mutations which, in humans, lead to the development of Parkinson's disease. Models targeting PARKIN, PINK1, DJ-1, and LRRK2 are examples of genetic models generated by modulating targets other than alpha synuclein. These models are used to better understand the pathways involved between the mutation and the development of Parkinson's disease, although they are also used to evaluate therapeutics designed to target dysfunction induced by these specific genetic mutations.

Historically, many of these models generate movement impairments, which are seen as correlates of the movement disorders that occur during Parkinson's disease. More recently, models have been developed that try to replicate the progressive nature of the disease and to model its non-motor aspects. Selection of an animal model of Parkinson's disease in which to evaluate a potential therapeutic depends on many factors and an appropriate choice can only be made when these factors are considered in combination. Some of the more important factors common to the evaluation of many potential therapeutics are discussed below. However, these should be seen only as a starting point and not an exhaustive list.

Stage of development of therapeutic



Many collaborators approach us when they already have a wealth of preclinical efficacy data. In these cases, the collaborator often wants to perform a single, pivotal study as a gating study to clinical studies. Such a study would often include endpoints that are translatable to the clinic, such as blood or CSF biomarkers, imaging endpoints, or using rating scales that have been back translated from scales used clinically.

These studies will often evaluate therapeutic effects in both male and female animals, be randomized and blinded, and be fully compliant with the knowledge-generating requirements detailed in Enhancing Quality in Preclinical Data (EQIPD), the Quality Management System we use at Atuka. Other collaborators reach out at a much earlier stage of therapeutic development, possibly with only *in vitro* data. We work with these collaborators to understand their specific needs and design a package of work that addresses them. This may involve investigating the mechanism of action of a therapeutic, determining doses that achieve target engagement, and developing bespoke models in which to evaluate their compounds. Such research is generally more investigative than a pivotal study, may use multiple models, may utilize a single sex, and may include much more post-hoc analysis of the data. Although investigative in nature, these studies are still performed to the quality requirements of EQIPD to ensure the data generated is robust, reliable, free from errors, and generates data that facilitates the design of a pivotal study.

Indication

Obviously, the indication for which a treatment is being developed is an important factor in deciding the most appropriate animal model to use. Within Parkinson's disease there are multiple therapeutic approaches such as disease modifying therapies, symptomatic therapies for motor impairments, symptomatic therapies for non-motor symptoms, and approaches to mitigate motor complications (e.g., L-DOPA sparing therapies and therapies to prevent the development of L-DOPA-induced dyskinesia). The choice of indication has a large bearing on the most appropriate model to use. For instance, if evaluating a compound that works by targeting alpha synuclein, then this needs to be evaluated in a model where pathology is driven by alpha synuclein, rather than a toxin-based model. Likewise, if evaluating a potential symptomatic therapy for treating L-DOPA induced dyskinesia, then a model that closely replicates the symptoms of L-DOPA induced dyskinesia would be needed, ideally MPTP-lesioned non-human primates chronically treated with L-DOPA.

Mechanism of action

While the indication that the therapeutic is being developed for is a crucial factor in model development, it is not the only consideration. Understanding the broad mechanism of action of a therapy also helps inform model selection. If an α-synuclein antibody is being developed to stop the spread of pathogenic α-synuclein, then it needs to be evaluated in a model of α-synuclein spread and not, for example, a toxin-based model of Parkinson's disease, or an α-synuclein-driven model that does not spread.

It is also important, when possible, to consider the molecular target of the therapeutic or the specific pathways that are engaged. Key questions might include:

- Is the molecular target present in rodents or only in non-human primates?
- Is the target or pathway modulated in the animal model in a similar manner to how it is modulated in people with Parkinson's disease?
- If a therapy targets the immune system, is there a need to use humanized mice or non-human primates to evaluate the model?

Carefully considering all the known information on the mechanism of action of a therapeutic, and the target or pathway it modulates, allows us to provide the best advice on the most appropriate model.

Type of therapeutic

The type of therapeutic under development may also inform model selection. For small molecules, there is a well understood development pathway, and mouse, rat, and non-human primate models have all been used to generate preclinical efficacy data to support moving into clinical development. However, development of biologics, especially gene therapy, is less well understood and, for products that have the potential to elicit an immune response, use of non-human primate models may be required.

Moreover, the large volume of non-human primate brains allows other aspects of biologic/gene therapy therapeutic development to be considered, such as biodistribution within the region of interest, and across the whole animal. For medical devices, such as brain–computer interfaces, non-human primates are often the species of choice due to their ability to be trained to perform complex cognitive tasks.

Pharmacokinetics and biodistribution

Many collaborators that we work with already understand the pharmacokinetic and biodistribution of their therapeutic. In these cases, a small pharmacokinetic study, often incorporated into the main efficacy study, might be all that is needed to ensure that the data generated by Atuka can bridge back to existing data. However, in other cases a collaborator has no existing pharmacokinetic data, or data that is in a species different from the most appropriate Parkinson's disease model. If collaborators have insufficient pharmacokinetic data, then we work with them to ensure that drug levels at the site of action will be high enough to produce target engagement. We can administer test items by a variety of routes so that even compounds with a poor pharmacokinetic profile can be assessed in efficacy studies.

Biodistribution of gene therapies is analogous to pharmacokinetic studies for small molecules. Our surgical techniques are such that in rodents we can provide complete coverage of the gene therapy in the region of interest. Biodistribution of gene therapies in non-human primates is more challenging, largely due to the larger volume of the brain and region of interest; these issues, however, can be overcome with pilot studies to determine the number of injections required to completely cover the brain region of interest.

Endpoints

The endpoints of a study are largely determined by the type of study performed. For instance, there is little value to incorporating endpoints to assess neurodegeneration of dopaminergic neurons in a study investigating a symptomatic therapy. We have a wide array of validated endpoints, ranging from rating scales that have been back translated from clinical rating scales (non-human primate studies only) to markers of target engagement (e.g., protein expression levels).

As well as existing assays, we routinely develop and validate novel assays for endpoints based on collaborators' requirements. Moreover, where possible, we encourage incorporating endpoints that are directly relevant to the design of Phase II studies, such as imaging endpoints, monkey parkinsonian rating scales, blood and CSF biomarkers of drug effect, and CANTAB (cognition). For investigative studies, we can use a primary endpoint to gate to other endpoints For example, if a test item shows a beneficial effect on striatal dopamine levels, then we progress to assessing additional endpoints of dopaminergic function such as dopamine transporter levels and the number of dopaminergic neurons. This approach helps control costs and provides maximal flexibility for our collaborators.

Partnering with Atuka

Development of a sub-optimal preclinical package is a surprisingly common occurrence.

Standard CROs may be able to offer animal models of Parkinson's disease similar to those that we provide. However, we have spent decades developing and understanding these models, and then using them to evaluate potential therapeutics. The average CRO simply does not have the same experience, knowledge, and understanding of these models, or how they can be best employed to generate a robust preclinical efficacy package. There are some academic laboratories that can rival our expertise with these models, and indeed some academics have spent much of their careers developing animal models of Parkinson's disease. These academic laboratories, however, generally do not understand the business of therapeutic development at the same level. Moreover, academic laboratories often do not have the quality systems in place to ensure that the data generated is robust, reliable, and can be used to inform clinical development packages.

We do not believe that evaluating potential therapeutics in an "off the peg" animal model of Parkinson's disease, as many CROs provide, is sufficient when developing a novel therapeutic. Our approach is to de-risk translation to Phase II clinical studies as much as possible. We believe that:

- a detailed knowledge of the disease;
- world-leading animal models of Parkinson's disease;
- an in-depth understanding of the therapeutic under development, ensuring that translational aspects are considered throughout the process, and;
- a Quality Management System designed to minimize biases and errors in the data generated

are necessary to produce a compelling preclinical efficacy package. In short, this means combining the in-depth knowledge of Parkinson's disease and animal models of Parkinson's disease found in academia with the quality, reproducibility, and reliability of a world-class CRO.

We pride ourselves on providing more than a standard CRO service. With each of our collaborators, we spend as much time as needed to fully understand their therapeutic and explain our models. These discussions will often involve multiple, PhD-level, Parkinson's disease researchers with years of drug development expertise, who provide expert opinions and suggestions on potential next steps. To put it plainly, no other organisation has evaluated as many potential therapeutics for Parkinson's disease as Atuka.

It may take many meetings before a collaborator decides upon a study design, and collaborators may pause this process to generate additional information before re-connecting with us. This process is critical to delivering what our collaborators most need from us. On multiple occasions, these discussions have led not to the placement of a study, but to collaboration on a grant for funding to progress the project. Over the last decade we have helped our collaborators secure over $20M USD to advance their therapeutic programs.

We understand that many of the organizations who approach us have only limited experience in outsourcing research. Therefore, it is important for clients to understand not just the services we provide, but the experts with whom they will be interacting with, and the overall process between a study's initiation and delivery of complete report.

Building personal connections with the Atuka team members managing our clients' studies means they know who to contact regarding any aspect of their study, facilitating fast and effective communication between all parties. Moreover, knowledge of the process helps our collaborators better understand the time required to complete the various aspects of any study. Our experienced team is happy to explain the processes underpinning a study and will always consult with you on important decisions.

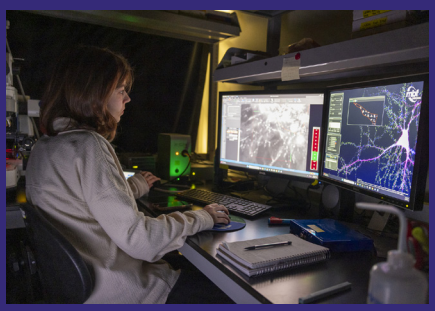
About the Authors

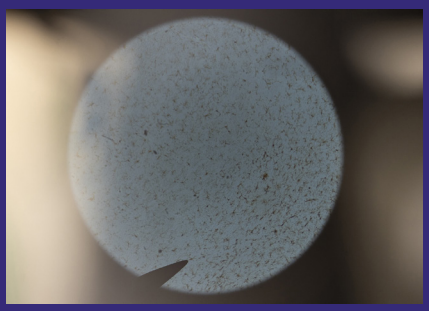
Dr. Tom Johnston's research interests focus on optimizing the translation of advances in fundamental Parkinson's disease research into novel therapeutic strategies. His Ph.D. work in behavioral neuropharmacology ignited a passion for developing and utilizing rodent and non-human primate models to study Parkinson's disease, particularly relating to motor symptoms and motor complications. More recent interests include the development of models to enhance the translation of putative disease-modifying therapies for PD and other neurodegenerative disorders. His work has yielded over 60 publications and earned him an *h*-index of 37. In addition to his scientific leadership, Tom oversees Atuka's facilities in Toronto and Suzhou, PRC and has served as the company's Chief Operations Officer since 2003.

Dr. Patrick Howson's primary research interest is in the development of disease-modifying therapies for neurodegenerative disorders. Patrick has a broad understanding of drug development through several years of working in virtual biotechs, where he has been responsible for research and development projects such as drug-screening programs, manufacture of GMP grade API, IND-enabling studies and Phase I and II clinical trials, including trials in Parkinson's disease. He uses this experience to help our partners develop preclinical programs suitable for their stage of development. Patrick is also experienced in the generation and management of intellectual property and is an inventor of several patents covering therapeutic approaches for Parkinson's disease. He has been with Atuka since 2013 and serves as the company's Chief Innovation Officer.





A cure for Parkinson's, faster, through the world's best preclinical neuroscience.

Atuka's lead scientists have dedicated their careers to furthering our understanding of Parkinson's disease, advancing novel therapeutics, and alleviating the burden of those suffering from neurological disorders.

For more than 20 years, we have collaborated with our partners to provide preclinical services that expand the frontiers of Parkinson's disease research, and help make new, life-changing therapeutics a reality. Our neuroscientists have extensive preclinical experience developing therapies for numerous indications—including Parkinson's, cognitive disorders, Alzheimer's, ALS and other movement disorders such as dystonia and dyskinesia—across multiple modalities, including small molecules and biologics.

Founded by Dr. Jonathan Brotchie in 2003, Atuka has been involved in the preclinical evaluation of more than 300 potential therapeutics, predominantly in Parkinson's disease, of which more than 30 have progressed to clinical trials—a level of experience without equal in our field globally.

Our lead scientists have collectively published more than 300 peer-reviewed, highly-cited papers, and individually possess h-indices ranging from 25 to 70.

Atuka has collaborated with over 90 organizations, including large pharmaceutical and biotech companies, charitable foundations, universities, and government agencies. Over the course of more than 400 preclinical projects, targeting more than 60 mechanisms of action, we have built an extremely rich understanding of Parkinson's disease, its causes, and potential treatments.

With offices and facilities in Toronto and Suzhou, our team is diverse both in background and expertise, bringing to every one of our partner engagements a spirit of close collaboration, along with a commitment to the highest ethical standards in scientific research.

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