WHITE PAPER

The Future of Gene Therapy

Development of gene therapies from a preclinical perspective

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The potential of gene therapies is finally being realized and these therapies can deliver life–changing treatments for a broad range of disorders.

The idea that DNA sequences could be introduced into patient's cells to cure genetic disorders was first conceived in the 1960s, but it was not until the 1980s that it was shown viruses could be used to insert genes into cells[1]. With the completion of the world's largest ever collaborative biological project, the Human Genome Project[2], in 2003, the basic tools were in place to identify genetic variants that cause, or increase the risk for, diseases.

Since the 1990s, there has been an explosion of research into gene therapies such that by 2023 there were over 4,000 publications retrieved by term 'gene therapy' in PubMed (Figure 1).

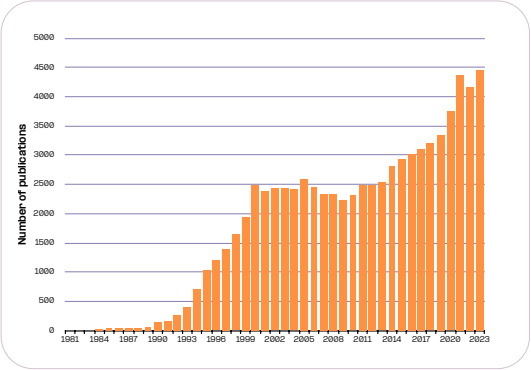

Fig 1 The number of publications by year using the term 'gene therapy' in PubMed. Search performed on 26th March, 2024.

As the field developed, two different ways of achieving gene therapy arose: ex vivo gene therapy (taking cells out of the body to correct the genetics before delivering back to the individual) and in vivo gene therapy (administering the genetic material directly to the individual, either systematically or targeted to a specific tissue of cell type). This paper focuses on in vivo gene therapies, in particular the current landscape and considerations for the preclinical development of novel gene therapies.

The first individual successfully treated by gene therapy was in 1990 when a 4-year old girl, Ashanthi de Silva, with severe combined immunodeficiency was successfully treated by delivery of a gene using a disabled virus that could not replicate. Now in her 30s, Ashanthi is active in the rare disease community. Although this success drove gene therapy research forward, setbacks have occurred, including gene therapy-induced cancers and fatal

immune responses. Undoubtedly, gene therapy had demonstrated its potential to treat incurable diseases, but understanding the risks involved with such a novel approach to treating disease was urgently needed to allow gene therapy to deliver its potential.

Improving the delivery of gene therapies

In the early 2010s, researchers began unraveling the problems produced by the viruses originally used to deliver gene therapies and started to produce delivery vectors that overcame these issues. Over the past 15 years, many gene delivery platforms have been developed (Figure 2) and it is an area of intense research. Currently, recombinant adeno associated virus (rAAV) based delivery of gene therapies is the most used delivery platform, accounting for approximately 30% of all in-vivo gene therapy clinical studies, with adenoviruses, retroviruses, and lentiviruses also being commonly used. It is worth noting that lentiviruses dominate the ex-vivo gene therapy landscape, accounting for ~50% of all ex-vivo gene therapies in development.

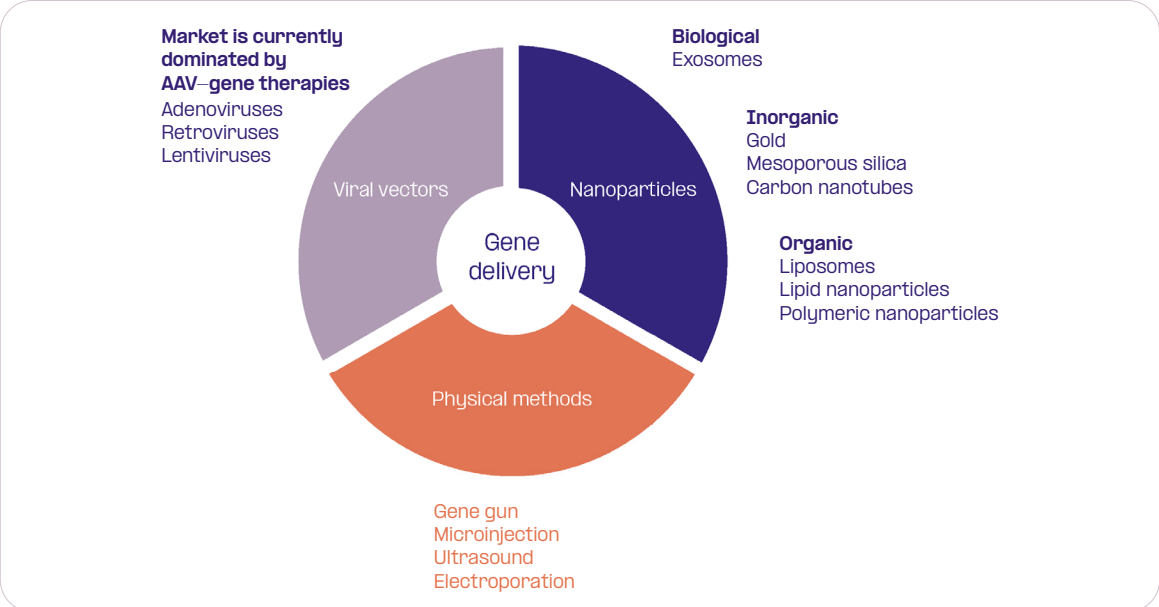

Fig 2 Summary of the different delivery platforms for gene therapy. Currently, gene delivery using rAAV, is the most common platform used by in vivo gene therapies.

Using rAAV as a delivery platform for in vivo gene therapies has been popularised by several major advantages that it possesses, including a good safety profile, ability to target specific organs, sustainable expression of transgene, and that the gene does not integrate into the treated individual's genome. However,

disadvantages of rAAV as a delivery platform include a low packaging capacity, susceptibility to antiviral humoral response (occurrence of anti-AAV neutralising antibodies), and co-infection with Adenovirus or Herpes Simplex can provoke an immune response.

Thus, although rAAV delivery of a transgene is not suitable for all in vivo gene therapies, it is often the preferred platform for targeting non-dividing cells, such as in the liver, nervous system, eyes, and skeletal muscle. Whilst research into overcoming some of the disadvantages to rAAV-based gene therapies is ongoing, such as addressing the occurrence of anti-AAV neutralising antibodies, there remain significant challenges with virally delivered gene therapies, including the cost and complexities of manufacturing large quantities of safe viral vectors.

Future developments in rAAV vectors

To-date, at least 12 naturally occurring AAV serotypes have been identified, and over 1,000 AAV variants within these serotypes have been detailed. These serotypes, and variants within serotypes, exhibit different preferences for cells or tissues, which is called tropism. These differences in tropism can be exploited to design vectors that preferentially transfect tissues or cells of interest. Modifying the vector, either by modifying the rAAV capsid sequence or the genomic DNA cargo, to produce a vector that can efficiently transfect desired cell types or tissues, is an area of intense research.

Capsid engineering and genomic modifications

Capsid engineering is a key strategy to produce a vector tailored for a specific disease. Four major approaches currently exist to produce a tailored capsid:

1. Identification of naturally occurring AAV variants.
2. Rational design of rAAVs. This approach uses structural changes to specific sites on rAAV capsids based on the understanding of rAAV structure and biology.
3. Directed evolution. This approach uses selective pressure to isolate capsids with desired properties. The use of random mutations or the addition of short-length surface peptides creates large capsid libraries which are then assessed in vivo, often in non-human primates, and capsids with desired properties identified. Further capsid libraries are then constructed based around the initial hits.
4. In silico and machine learning design. Computer-assisted rAAV engineering uses computational tools to enhance the design and optimization of rAAV.

In addition to modifying the capsid itself, alterations to the genomic DNA cargo can also change a vector's tropism. Thus, modifying the inverted terminal repeat (ITR) sequences, optimising the promotors, regulating transgene expression, post-transcriptional regulation, post-translational regulation, and modifying other cis-regulatory elements are all approaches that can be used to modify the tropism of a vector.

Specialised contract development and manufacturing organisations (CDMOs) are often employed to perform the development of novel capsids. However, due to species effects

between rodents and primates, it is important to assess the tropism of capsids in non-human primates (NHPs) early on in development.

Non–viral vectors

To reduce, or eliminate, some of the disadvantages of virally delivered vectors, researchers are developing non-viral vectors (Figure 2). In general, these non-viral vectors are cheaper to manufacture than viral vectors, can potentially deliver larger payloads, have a lower propensity for eliciting immune responses, and allow for repeated administrations. Whilst these are significant advantages over viral delivery platforms, the lack of ability to target specific organs or tissues, and that there is a lot less experience of working with them, means that virally based delivery platforms remain the most attractive gene therapy delivery platform.

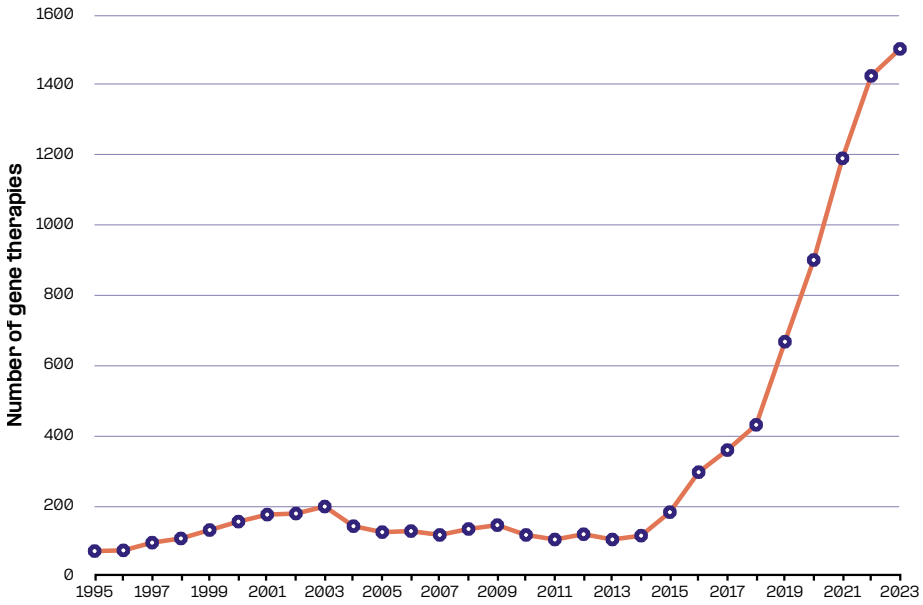

Fig 3 Number of gene therapies in preclinical development

Current market landscape

2023 was a landmark year for cell and gene therapies, with seven FDA approvals[3], accounting for 10% of all new therapeutic approvals[4]. This trend is expected to accelerate in 2024 with up to 17 cell and gene therapy approvals anticipated in 2024. In the longer term, the cell and gene therapy market is expected to grow from USD 9Bn in 2023 to USD 45Bn in 2032[5]. Within this area, AAV-delivered gene therapies currently dominate, accounting for 45% of market share in 2022[6] and expected to peak at USD 22Bn in 2029. Thus, for at least the next decade, AAV-gene therapies will dominate the gene therapy market and the rest of this paper focuses on challenges that must be addressed when developing an AAV-based gene therapy.

In 2023, as well as the seven FDA approvals, there were >2000 gene therapies in development, with the majority (74%) in preclinical development. The increase in the number of gene therapies entering preclinical development increased dramatically from 2015 onwards driving a need for Contract Research Organisations that can provide support to companies developing gene therapies.

Preclinical gene therapies in development are dominated by anti-cancer therapies (54%). Neurological gene therapies are the second largest class, accounting for 13% of all gene therapies in preclinical development (Figure 4). Currently, there are almost 200 preclinical gene therapies targeting neurological disorders and the large number of therapies in this area are likely due to a number of factors including the difficulty in treating neurological disorders with small molecules, that neuronal cells do not divide, thus a single administration of a gene therapy can provide a long lasting benefit, the large number of neurological disorders with a genetic basis, and the large number of neurological orphan diseases. Together, these factors provide a strong scientific and commercial rationale for developing gene therapies for neurological disorders and further growth in this area is expected.

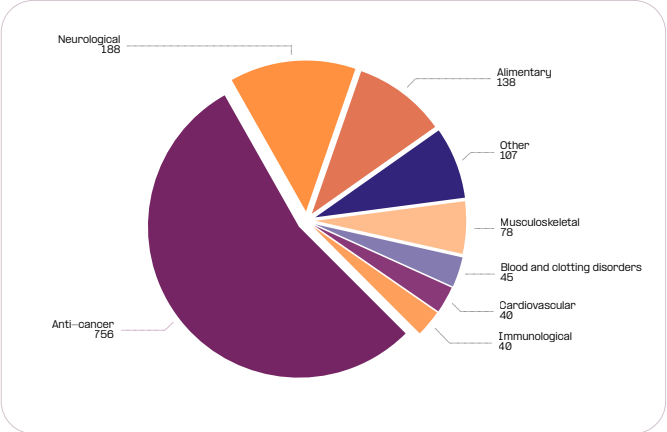

Fig 4 Preclinical gene therapies in development by therapeutic area

Current preclinical development considerations

The preclinical development issues that need to be considered when developing an in vivo gene therapy are similar irrespective of the delivery vector, although the delivery vector will alter the likelihood of any given potential issue occurring. The major current preclinical items that should be considered when developing a gene therapy are:

1. Types of preclinical studies to perform
2. Species to use in preclinical studies
3. Dose translation from animals to humans
4. Immunogenicity
5. Genomic risks

Types of preclinical studies to perform

Preclinical studies help identify, characterise, and mitigate any potential toxicities that a gene therapy may possess. They will also help clinical study design, including defining safe doses and regimens. Preclinical study design will vary depending on the gene therapy under development, such as local or systemic delivery, function of the transgene product, and what disorder the therapy is intended to treat.

There are three main types of preclinical studies that need to be performed:

1. Pharmacodynamic studies to help define doses associated with efficacy and/ or target engagement.
2. Biodistribution studies to help define distribution and transduction profiles of the gene therapy.
3. Toxicology studies to investigate the safety of the gene therapy. These studies support First-in-Human studies or may focus on other areas such as developmental and reproductive testing.

These studies can potentially be combined into a single study. Thus, pharmacodynamic and biodistribution studies could be combined to evaluate distribution and transduction profiles across organs, how these profiles correlate with pharmacodynamic measures in those tissues, and how, ultimately, these profiles translate into efficacy. There is no regulatory expectation that these studies are conducted to Good Laboratory Practice (GLP), thus it may be more efficient to combine these studies together, before performing GLP toxicology studies, than, for example, combining biodistribution and toxicology studies.

Prior to preclinical toxicology studies, understanding the biodistribution and pharmacodynamics of the gene therapy are important. Different gene therapies will have different distribution and transduction profiles, which will depend on many factors, such as the vector, route of administration, disease, and transgene product. In addition, shedding profiles can start to be generated if replication-competent viruses are used as the vector. It should also be noted that, for a gene therapy, the biodistribution of the gene product is analogous to pharmacokinetic studies of small molecules. Thus, a good understanding of both the biodistribution and pharmacodynamic attributes of a gene therapy will allow the minimum efficacious dose and the dose that produces a maximal effect to be defined and the distribution and transduction profiles

associated with these doses. This information will then inform dose selection for IND-enabling toxicology studies.

Toxicology studies for gene therapies need to capture potential acute, chronic, and/or delayed onset toxicities. Different gene therapies will have potentially very different toxicities that may develop over different timeframes, thus protocols for GLP toxicology studies need to be developed on a case-by-case basis. For instance, the justification for study duration will be partly based on persistence of the vector and length of time the transgene is expressed. A good understanding of the biodistribution and pharmacodynamics of the gene therapy will therefore guide the toxicology studies. Importantly, biodistribution studies will inform whether developmental and reproductive toxicology studies are needed; a biodistribution study showing a lack of expression and activity in germline cells may mean that further developmental and reproductive toxicology studies are not needed.

Species selection

Animal model dose-response data can act as a guide, but may not directly translate, to human studies. Thus, species selection needs to consider the ability to predict human dose-related response of both tissue uptake of the delivery vector and biological activity of the gene product. Moreover, the impact of the disease pathology should also be considered, as this can affect both uptake and expression. Ultimately, species selection should be aimed at developing an understanding of the dose-related pharmacodynamic and toxicity profile to enable selection of a safe clinical starting dose.

Regarding IND-enabling studies, unless there is existing relevant data from humans, data to select the species to use should include a comparison to non-human primates (NHPs). If there are distinct differences in tissue uptake of the vector or biological activity of the gene product between NHPs and the other species, then either NHPs should be used, or careful consideration should be given as to how these species differences will be factored into dose selection for clinical trials.

Data from human studies using a similar vector, route of administration, and dose can also be used to justify a relevant animal species, as can in vitro data comparing species differences in cellular tropism and transgene expression, although caution should be taken if exclusively relying on this information.

Generally, a single species for toxicology programs is acceptable, so long as the species that most likely predicts the human dose-related pharmacology and toxicology response is used.

Dose translation to humans

Dose selection for First-in Human gene therapy studies is similar to dose selection for other medicines, with the caveat that, for gene therapies, translation from animals to humans is poorly understood. For ethical reasons, First-in Human gene therapy studies are performed in people with the disorder being treated, not in healthy volunteers. Moreover, due to the development of an immune response to viral vectors, most virus-based gene therapies can only be dosed once, e.g. dose escalation studies within an individual are not possible and thus a potentially therapeutic dose should be selected. These considerations do not apply to gene therapies delivered by non-viral platforms.

Due to the poorly understood translation between animals and humans, the preclinical toxicology studies should aim to generate a good therapeutic window, i.e. a range beyond the efficacious window predicted by preclinical efficacy studies. Thus, once the therapeutic dose range is established in efficacy studies, the doses selected for toxicological studies should bracket the therapeutic dose range with the highest dose either the maximum dose that can be administered or one that provides a high multiple over the anticipated maximum efficacious dose.

Immunogenicity

An assessment of immunogenicity is typically included in toxicology studies. The intent of this is to understand the immune response to the transgene-derived product. Most commonly, the human transgene-derived product is evaluated in animals, which may induce a response in the animal to a human protein. Thus, the relevance of this to a human risk assessment is somewhat limited.

Genomic risks

There is always a potential genomic risk when developing a gene therapy. Two main classes of risk have emerged, those posed by viral vectors and those posed by direct genomic modification (e.g. CRISPR). As viral delivery of gene products is currently the most

used gene therapy technology, only risks posed by viral vectors will be considered further.

The site of viral integration can result in cellular dysregulation and potentially transformation. The risk of a gene therapy producing such an event is dependent on several factors, such as the vector, promotor, transgene, and number of copies within a cell. For replication competent vectors that possess the potential for genomic integration, the insertional mutagenesis risk increases with dose. Even for vectors that are non integrating, such as AAV, there remains a small chance of integration occurring. For instance, AAV viral integration may occur at 0.1-0.5% integrations per AAV infectious unit and AAV-associated insertional mutagenesis has been reported in mice. However, to-date no confirmed associations between exposure to an AAV-based gene therapy and insertional tumorigenesis in humans or non-rodent models have been reported.

A fractured regulatory landscape

Authority	Documents
European Medicine Agency (EU)	13
Food and Drug Administration (US)	16
Japan	1
Brazil	1
Russia	1
South Korea	2
China	2

Table 1 Number of national guidance documents relating to the development of gene therapies.

Currently, there are numerous national guidelines covering the development of gene therapies. However, there is only one The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline (ICH S12 – Nonclinical biodistribution considerations for gene therapy products). Work was started on other gene therapy ICH guidelines but work on them was stopped in 2011. These draft guidelines subsequently became European Medicine Agency guidance documents.

Therefore, apart from the biodistribution studies, the development pathway for a gene therapy will be dependent not only on the product being developed but also the territories that it is being developed for. The guidance is largely fragmented because developing gene therapies is a new field and there simply has not been enough time, or experience, for different agencies to coalesce around harmonised guidelines. Moreover, gene therapy is a fast-developing field, with new technologies, such as direct genome editing and non-viral delivery systems, constantly evolving and being improved upon.

Nevertheless, there are some key preclinical topics that have matured over the last 20 years, with considerable preclinical and clinical experience now available, and these topics may start the process of being harmonised in the near future. Such topics may include species selection, aspects of study design, addressing genomic integration risks, and developmental and reproductive toxicology studies.

Future challenges for next generation gene therapies

Gene therapy is one of the fastest growing areas for therapeutic development and the field will continue to evolve over time. Issues that hamper gene therapy development today are likely to be resolved whilst new, currently unknown, issues will arise. Predicting how the field will evolve is fraught with difficulty. Current challenges that next-generation gene therapies may overcome include:

Improved AAV capsid biodistribution

Researchers are constantly devising new strategies to allow better targeting of AAV vectors, for instance, developing vectors that can easily cross the primate blood-brain barrier or AAV vectors that target organs other than the liver effectively.

Enabling repeat dosing of virally delivered gene therapies

The inability to re-dose a viral vector, due to the emergence of host immunity, can limit the effectiveness of a gene therapy. For instance, in fast-dividing cells the effectiveness of transgene expression may be diluted over time, but host immunity could prevent redosing. Similarly, if a person already has immunity to the vector, as many people already have to AAVs, then they may not be able to receive the gene therapy treatment. This issue may be resolved in several ways, including suppressing the immune response, removing the immune response, or using a non-viral delivery platform. However, when this issue is resolved, repeat dosing becomes possible and so preclinical testing will also need to evolve to assess the risks associated with multiple dosing strategies.

Better preclinical assessment of genome editing technologies

Compared to conventional gene therapies, strategies that directly edit the genome are in their infancy. New molecular tools are continually evolving to better assess the human health risk of genome editing, with more sensitive methods to detect off-target editing and genotoxicity risks in development. As these technologies advance, and are better understood, it is likely that the preclinical studies required to support gene editing clinical studies will also evolve.

About the Author

Dr. Patrick Howson is Atuka's Chief Innovation Officer, and has been with the company since 2013. His primary research interest is 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 including 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 clients develop preclinical programs suitable for their stage of development. Patrick also is experienced in the generation and management of intellectual property and is an inventor on several patents covering therapeutic approaches for Parkinson's disease.

Within Atuka, Patrick uses his experience to help define the team's scientific priorities and construct the strategies necessary to achieve them. He also leads Atuka's grant-writing activities and has helped secure more than $20 million of non-dilutive funding for the company and its clients.

Alongside his role at Atuka, he is a founder and CEO of Junaxo Inc., a drug development company focused on novel therapies for Parkinson's disease. Previously, Patrick was Head of Preclinical Sciences at Phytopharm plc, a U.K.-based, FTSE-listed, pharmaceutical company.

He holds a BSc (Hons.) in neuroscience from the University of Manchester, UK and a Ph.D. in neurobiology from the University of Bristol, U.K. (1999).

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Atuka—your partner to accelerate gene therapy development

At Atuka we have decades of experience when it comes to understanding complex biological issues and working with our collaborators to find bespoke solutions to their problems. Atuka was founded on an in-depth understanding of the pathophysiology of Parkinson's disease, and we have utilised this knowledge to construct gold-standard models of Parkinson's disease in rodents and non-human primates.

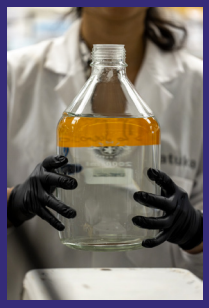
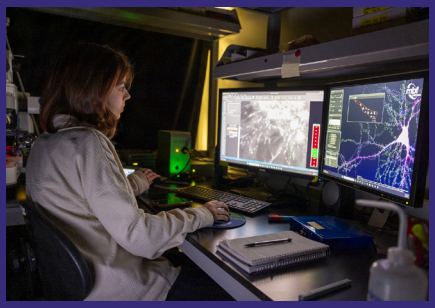
More recently, we have leveraged our expertise in rodent and NHP neuroanatomy and neurosurgery to become experts at delivering pharmacodynamic and biodistribution studies in the gene therapy area. Our access to a plentiful supply of NHPs also allows us to be your partner in evaluating the tropism of newly developed vectors.

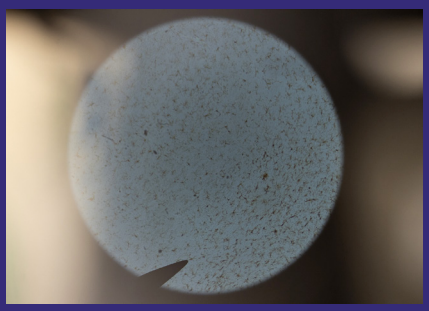
We understand that all gene therapies are unique and that different gene therapies require different solutions for them to progress smoothly into the clinic. At Atuka, we pride ourselves on creating individual solutions for our collaborators and would be delighted to discuss your gene therapy development requirements.

- **20 biodistribution studies performed supporting regulatory submissions**
- **Rodent and non–human primate studies**
- **Access to large numbers of NHPs of both sexes and various ages**
- **Wide range of routes of administration – PO, IV, IM, SC, IT, ICM**
- **Direct injection into parenchyma – surgical delivery to discrete brain regions**
- **Neurosurgical expertise**
- **In–life assessments and biofluid collections**
- **In–life imaging**
- **Hisological services – non–GLP toxicology assessments**
- **Tissue collection including sub–brain regions**
- **Behavioural and post–mortem endpoints to assess pharmacodynamics**
- **Neutralising antibody analysis**





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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