AWARD NUMBER: W81XWH-22-1-1043

TITLE: Development of a Non-Human Primate Model for the Identification and Development of Novel Therapeutics for Dystonia

PRINCIPAL INVESTIGATOR: Dr. Jonathan Brotchie, Ph.D.

CONTRACTING ORGANIZATION: Atuka Inc., Toronto, ON, Canada

REPORT DATE: October 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
October 2023	Annual	30Sep2022-29Sep2023

4. TITLE AND SUBTITLE

Development of a Non-Human Primate Model for the Identification and Development of Novel Therapeutics for Dystonia

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-22-1-1043

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Dr Jonathan Brotchie
Dr James Koprich
Dr Patrick Howson

E-Mail: J.Brotchie@atuka.com

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Atuka Inc.
100 King Street West, Suite 5600
Toronto, ON M5X 1C9
Canada

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

During the research period we have developed a quantitative assay (Western blot) to measure torsinA in macaque in the deep cerebellar nuclei and cerebellar tissue and have also synthesized the AAV vector that we will use to knockdown torsinA and demonstrated that it efficiently knocks down torsinA *in vitro*. Therefore, we successfully developed the tools that we require to progress the project.

We have gained IACUC approval for the non-human primate (NHP) studies and purchased 12 macaques. By injecting gadolinium followed by MRI scans, we have shown that we can successfully target the deep cerebellar nuclei. All 12 NHPs have now been injected with AAV vector, the animals left 2 months, and behavioral data collected over this period. Animals have now been killed and deep cerebellar nuclei tissue and cerebellar tissue successfully collected. This tissue is currently being analyzed to determine the degree of torsinA knockdown. The behaviour of the NHPs is also being analyzed.

The project is currently on track with the agreed Statement of Work and data on the degree of torsinA knockdown will be available by month 14 of the project.

15. SUBJECT TERMS					
Dystonia, torsinA, knockdown, non-human primate model, AAV					
16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRDC
a. REPORT Unclassified	**b. ABSTRACT** Unclassified	**c. THIS PAGE** Unclassified	Unclassified	26	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. Introduction

Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research. [remove red before submitting]

The overall aim of the project is to develop a non-human primate (NHP) model of dystonia. We hypothesize that knockdown of torsinA in the cerebellar deep nuclei and Purkinje cells will cause the NHPs to develop dystonia. In humans, a loss of function mutation in torsinA leads to the development of dystonia. The project has two specific aims:

1. To develop the first NHP model of dystonia based around knockdown of a gene which, when mutated, is known to cause dystonia in people.
2. To demonstrate the predictive validity of the models by establishing the pharmacological effect of several dystonia treatments on dystonia expression in our NHP model.

This report details the progress made on this project in Year 1 of the project, which has focused on developing the required tools needed to develop the model.

2. Keywords

Provide a brief list of keywords (limit to 20 words).

Dystonia, torsinA, knockdown, non-human primate model, AAV

3. Accomplishments

The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

- **What were the major goals of the project?**
    - *List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project identify these dates and show actual completion dates or the percentage of completion.*

For this reporting period (Year 1) the major goals of the project were:

Milestone 1: Development of a quantitative assay to measure torsinA (months 0-2)

Milestone 2: Quantification of torsinA protein level in cynomolgus macaque cerebellum (months 3-4)

Milestone 3: Synthesis of AAV vectors that are required to complete the project (months 0-4)

Milestone 4: Demonstration that target coverage can be achieved and optimising the injection parameters (months 0-8)

Milestone 5: Demonstration of knockdown of torsinA to a level expected to produce dystonia in cynomolgus macaques (months 9-14)

- **What was accomplished under these goals?**

    - *For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*

The major activities of this reporting period were focused around developing the tools and techniques required to demonstrate torsinA knockdown in the deep cerebellar nuclei of macaques, purchasing and acclimatising a cohort of macaques, and injection of different titres of the vector into the deep cerebellar nuclei. This has all been achieved and the cohort of macaques has now been killed and the brain samples collected. Analysis of this tissue to assess the degree of knockdown of torsinA is currently ongoing with completion due in month 14. The project is currently on schedule to the timeline presented in the Statement of Work.

Development of the AAV vector

Generation and selection of torsinA targeting plamids

TorsinA-targeting miR and shRNAs were prepared and evaluated in HEK293 cells. The plasmids generated were:

1	miRTor1A	#748 pAM/CBA-mCherry-miRTor1Ax3-WPRE-bGHpA
2	miRNeg	#749 pAM/CBA-mCherry-miRNegx3-WPRE-bGHpA
3	shTor1A(1)	#750 pAM/U6-shTor1A(1)-CBA-mCherry-WPRE-bGHpA
4	shTor1A(2)	#751 pAM/U6-shTor1A(2)-CBA-mCherry-WPRE-bGHpA
5	shNeg	#752 pAM/U6-shNeg-CBA-mCherry-WPRE-bGHpA
6	Tor1A	pcDNA3.1 Tor1A-DYK(FLAG)-bGHpA

Plasmids 1, 3, and 4 were designed to knockdown macaque torsinA. Plasmids 2 and 5 are the appropriate negative control plasmids for plasmids 1, 3, and 4. Plasmid 6 was used to express macaque torsinA in cells.

Calcium phosphate transfection: Human embryonic kidney 293 cells (HEK293) were maintained in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen) supplemented with 10% (v/v) foetal bovine serum (FBS), 25 mM HEPES, 0.5 mM L-glutamine, 44 mM NaHCO3, 0.1 mM non-essential amino acids, and 1 mM sodium pyruvate. For immunocytochemistry, cells were plated into collagen coated 24-well plates and co-transfected with 0.2 µg Tor1A, and either 0.2 µg (1:1 ratio), 0.4 µg (1:2 ratio) or 0.8 µg (1:4 ratio) of miRTor1A, shTor1A(1) or shTor1A(2) plasmid. Co-transfection with miRNeg and shNeg plasmids at the same ratio were included as controls for Torsin1A expression using anti-FLAG immunocytochemistry.

Growth media was changed to IMDM (supplemented with 5% FBS) approximately 18-24 h after plating and 2-3 h prior to transfection. A calcium phosphate-DNA precipitate was prepared by mixing plasmid DNA in 0.3M CaCl2 and HeBS buffer (50 mM HEPES, 280 mM NaCl, 1.5 nM Na2HPO4, pH 7.05) together at an optimised 1:0.58 ratio with vortexing, followed by a 5 min incubation at room temperature to allow the DNA and calcium phosphate to co-precipitate. The transfection mixture was applied to cells and 5 h after transfection the media was replaced with complete DMEM.

Immunocytochemistry: mCherry fluorescence was imaged using the EVOS M5000 imaging system (ThermoFisher) just prior to cell fixation. Transfected cells were fixed with 4% (w/v) paraformaldehyde in 0.1M phosphate buffer 48 h post-transfection for 15 min. Cells were washed twice with PBS and endogenous peroxidases quenched by incubation in 1% H_2O_2 (v/v) in 100% (v/v) methanol for 2 min. Cells were washed with PBS containing 0.2% (v/v) Triton X-100 (PBS-Triton) and 200 µL of primary antibody, Mouse anti-FLAG (1:500, F1804, Sigma-Aldrich), diluted in phosphate buffered saline (PBS) containing 0.2% (v/v) Triton-X100 and 10% (v/v) horse serum was applied and incubated overnight at room temperature. The biotinylated donkey anti-mouse secondary antibody (1:1000, Jackson Laboratory) was then applied for 2.5 h at room temperature before incubation in ExtrAvidin Peroxidase (1:500, Sigma Aldrich) for 2 h at room temperature. Cells were washed with PBS containing 0.2% (v/v) Triton-X100 between each incubation step. Immunoreactivity was visualized by incubation with diaminobenzidine and images captured using 10x and 20x objectives on a Nikon Eclipse TE 2000-U microscope.

Results: The expression of mCherry from all Tor1A silencing constructs and negative control constructs was confirmed following fluorescent microscopy (Figure 1). As expected, mCherry fluorescence increased with increasing transfection ratios. Lower levels of mCherry expression were observed for both miR expressing constructs due to the lack of the WPRE and polyA on the mature mCherry mRNA following miRNA processing. Both miRNeg and shNeg appeared to express lower levels of mCherry compared to the equivalent Tor1A targeting plasmids.

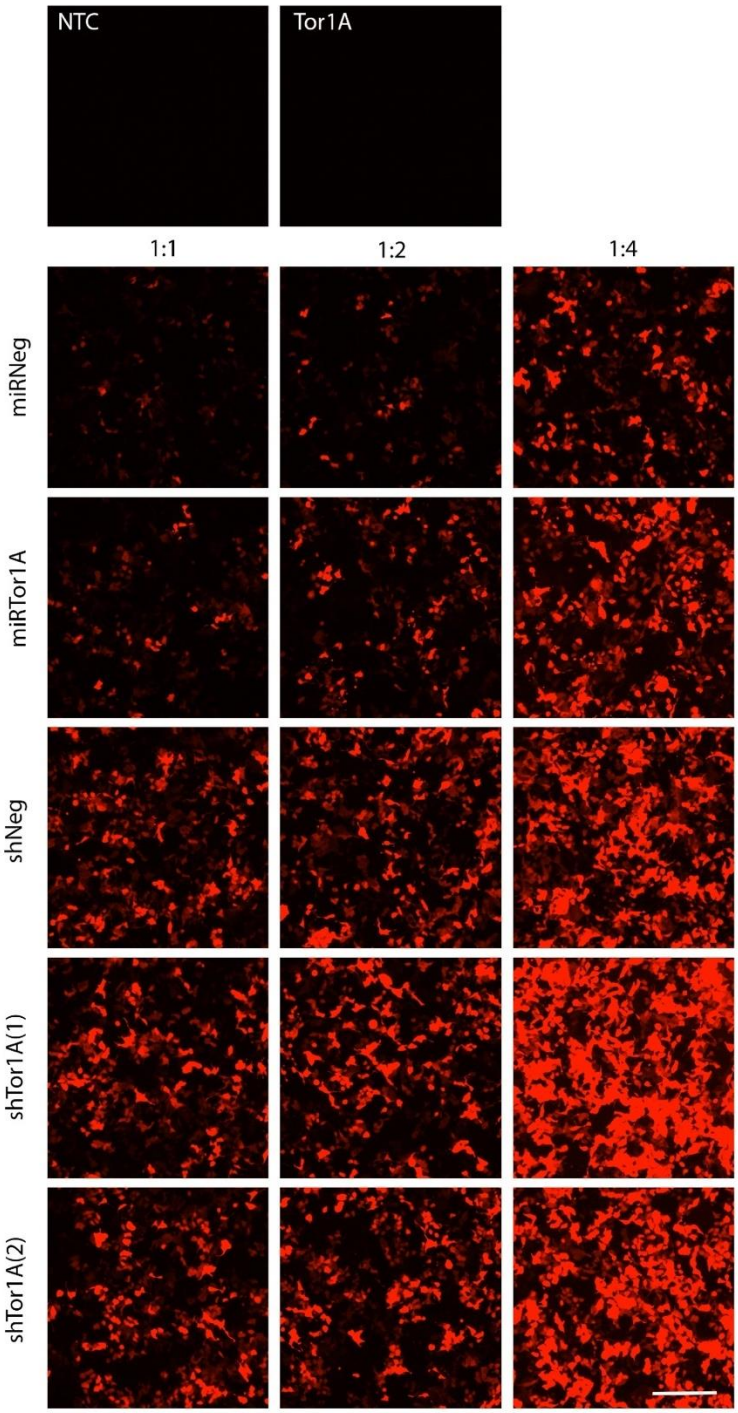

Figure 1. Evaluation of mCherry expression from microRNA (miR) and short hairpin RNA (sh) expression constructs. mCherry fluorescence in Tor1A co-transfected HEK293 cells 48 h post-transfection. Transfection ratios of 1:1, 1:2 and 1:4 Tor1A:silencing plasmid were tested. No mCherry fluorescence was observed in cells expressing Tor1A alone, and in non transfected cells (NTC). Scale bar, 250 µm.

Anti-FLAG immunocytochemistry confirmed expression of FLAG epitope tagged Tor1A (Figures 2-5). Of the three Tor1A-targeting constructs evaluated, miRTor1A appeared to provide the most effective silencing of Tor1A expression. Transient co-transfection of HEK293 cells with plasmids expressing Tor1A and miRTor1A at a 1:1 ratio resulted in nearly complete loss of FLAG immunoreactivity 48 h post-transfection (Figure 2 and 5). This pattern was similar for the higher 1:2 (Figure 3) and 1:4 (Figure 4 and 5) transfection ratios tested.

Silencing efficiency was lower for the two shTor1A constructs (Figure 2-5). Transient co-transfection of HEK293 cells with plasmids expressing Tor1A and shTor1A(2) at a 1:1 ratio resulted in lower levels of FLAG immunoreactivity when compared to co-transfection with shTor1A(1) 48 h post-transfection (Figure 2). This pattern was also observed when higher ratios of 1:2 and 1:4 were used (Figures 2-5). Increasing the ratio of silencing plasmid to 1:2 and 1:4 appeared to increase the silencing efficiency of shTor1A(2). However, a 1:4 co-transfection ratio with Tor1A(1) appeared to be less effective in reducing levels of FLAG immunoreactive cells, when compared to shNeg (Figure 4).

A reduction in FLAG immunoreactivity was also observed at the highest concentration of shNeg (Figure 4) when compared to transfection with Tor1A alone or miRNeg co-transfection. This could suggest that at higher ratios there might be non-specific effects on Tor1A expression from shRNA constructs, possibly as a result of high levels of mCherry expression or other shRNA-mediated effects.

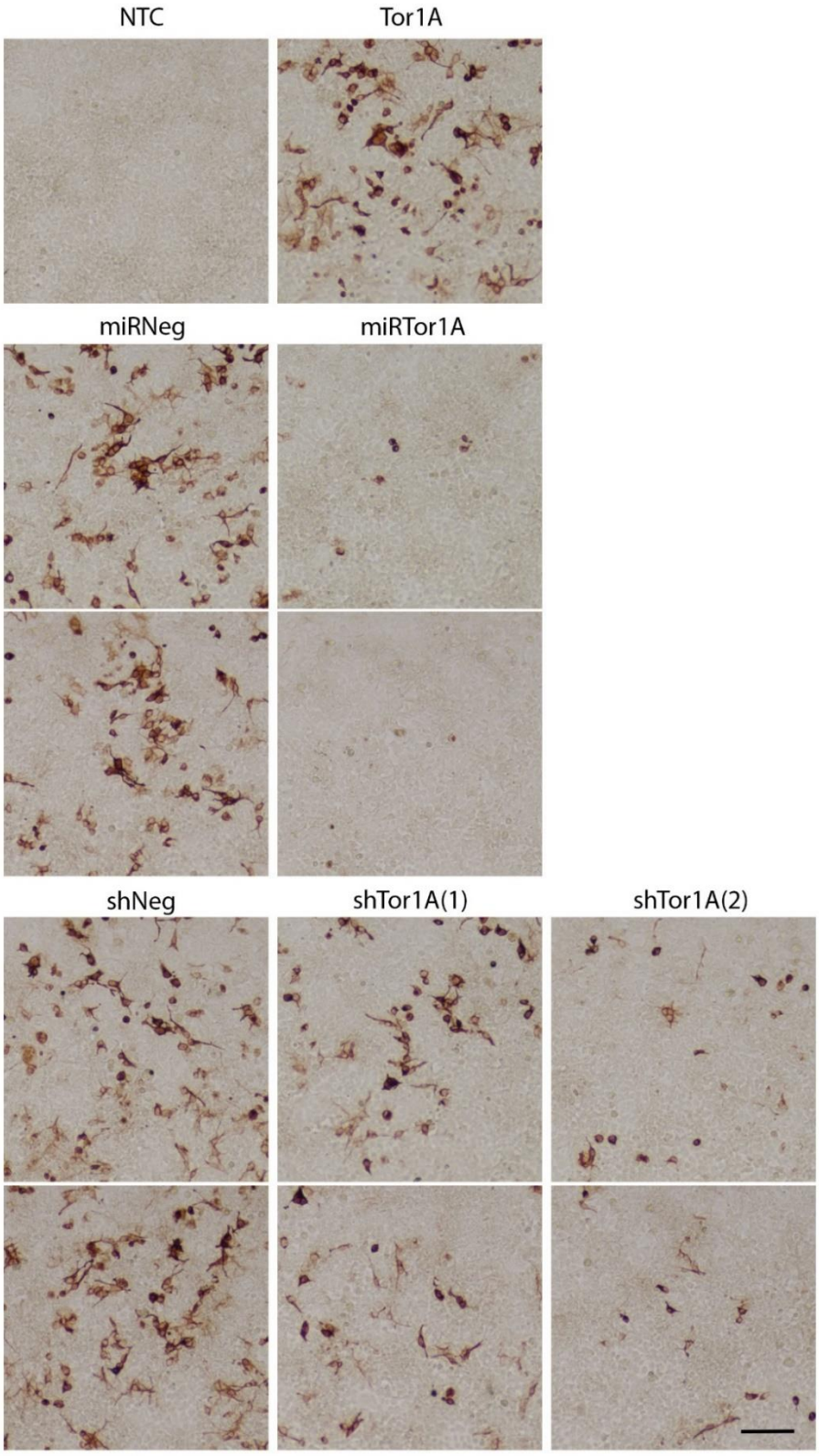

Fig 2. Evaluation of Tor1A silencing in HEK293 cells co-transfected with a 1:1 ratio of Tor1A:silencing plasmid based on FLAG immunoreactivity 48 h post-transfection. Control levels of FLAG immunoreactivity are shown in cells co-transfected with miRNeg and shNeg plasmid or Tor1A alone. Images are shown for two separate wells. No FLAG immunoreactivity was observed in non transfected cells (NTC). Scale bar, 250 μm.

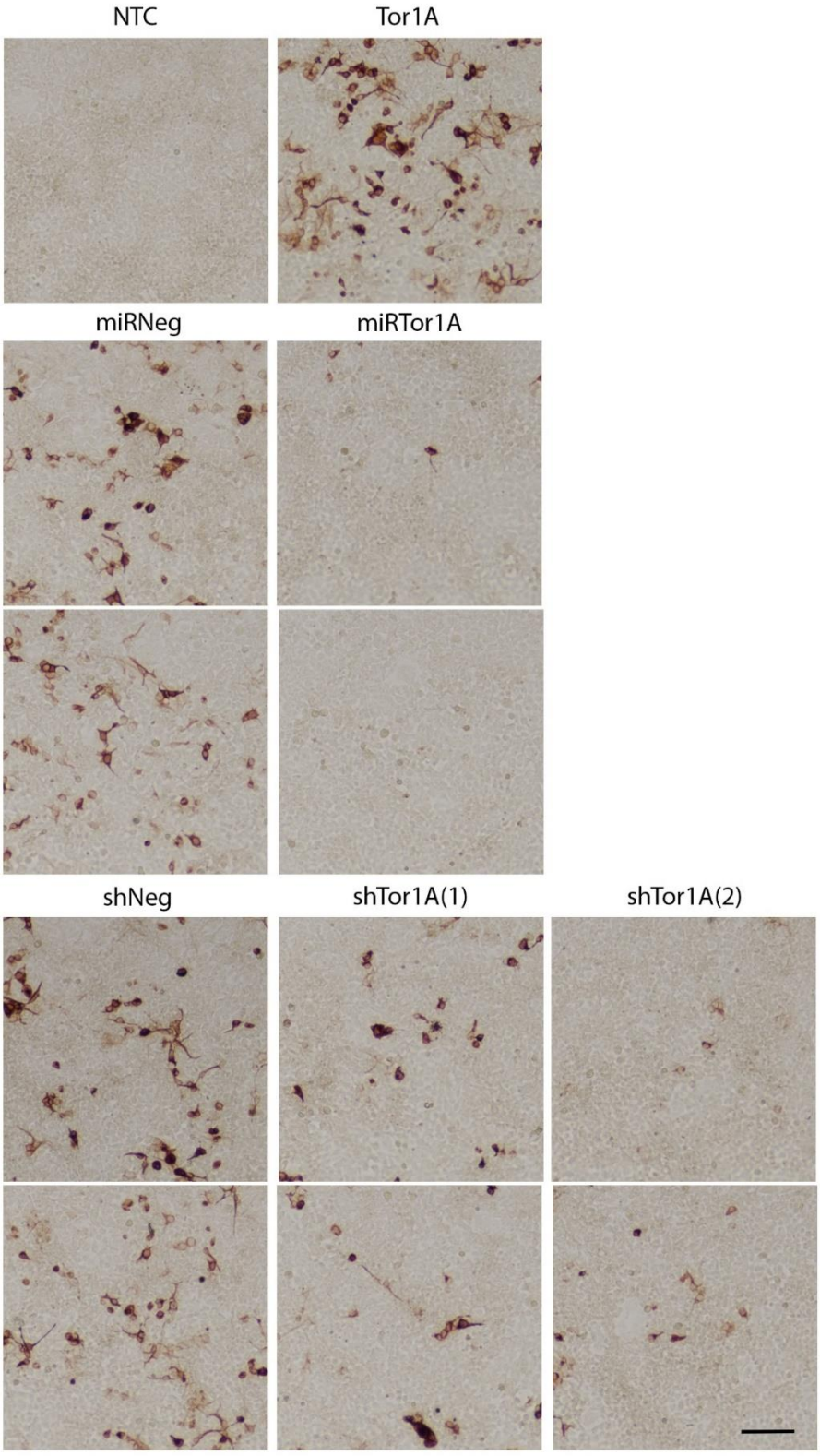

Fig 3. Evaluation of Tor1A silencing in HEK293 cells co-transfected with a 1:2 ratio of Tor1A:silencing plasmid based on FLAG immunoreactivity 48 h post-transfection. Control levels of FLAG immunoreactivity are shown in cells co-transfected with miRNeg and shNeg plasmid or Tor1A alone. Images are shown for two separate wells. No FLAG immunoreactivity was observed in non transfected cells (NTC). Scale bar, 250 μm.

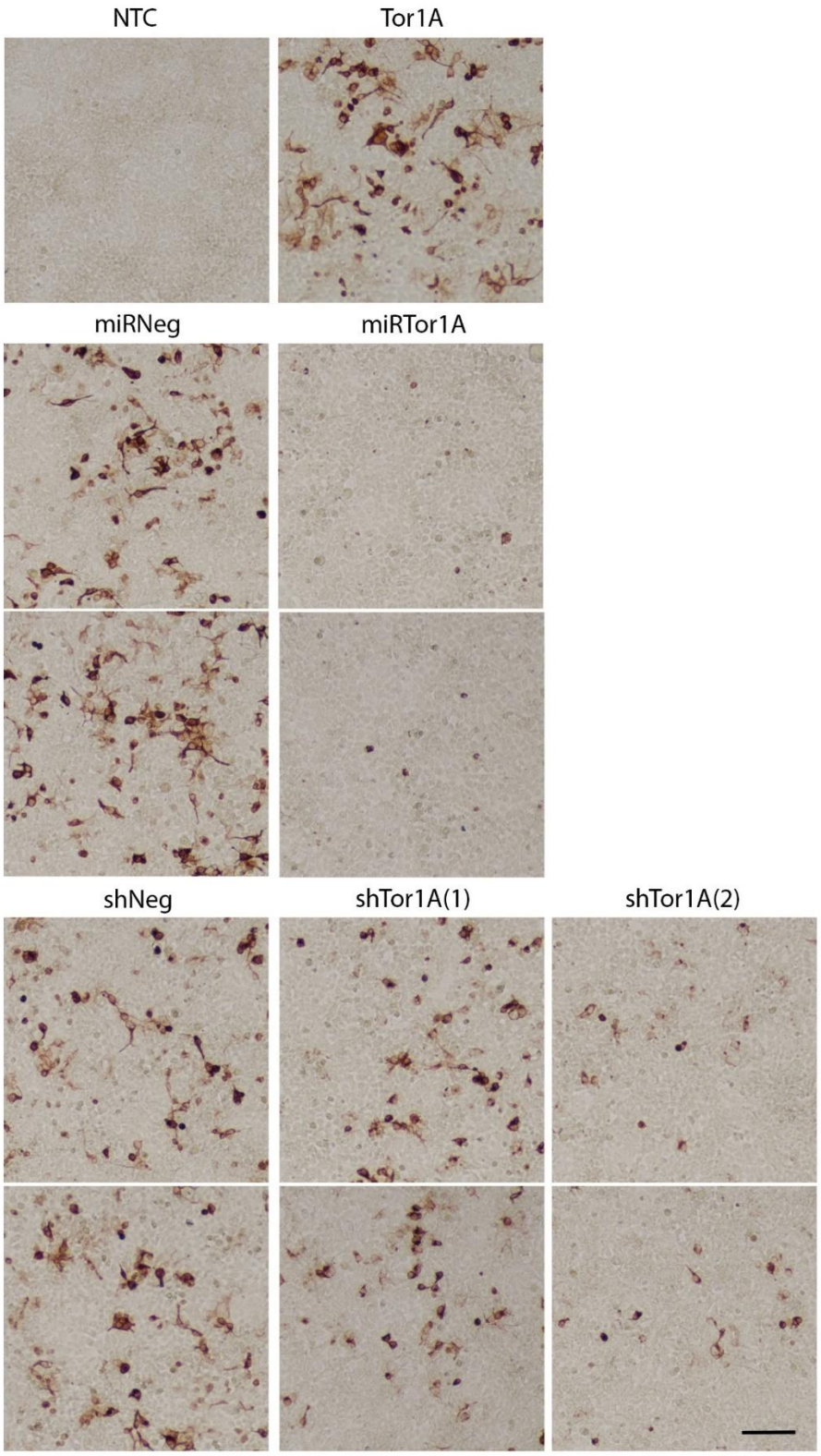

Fig 4. Evaluation of Tor1A silencing in HEK293 cells co-transfected with a 1:4 ratio of Tor1A:silencing plasmid based on FLAG immunoreactivity 48 h post-transfection. Control levels of FLAG immunoreactivity are shown in cells co-transfected with miRNeg and shNeg plasmid or Tor1A alone. Images are shown for two separate wells. No FLAG immunoreactivity was observed in non-transfected cells (NTC). Scale bar, 250 μm.

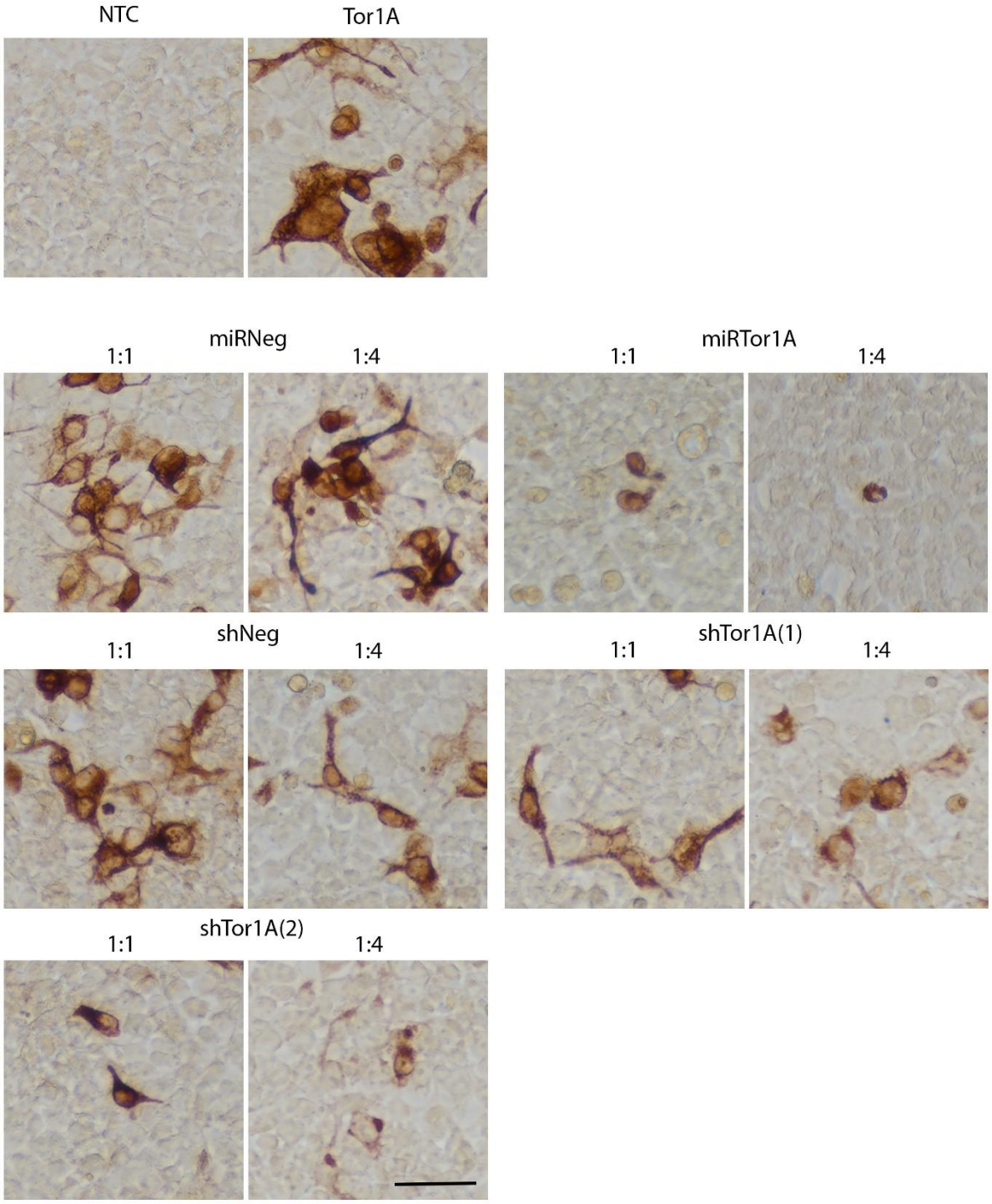

Fig 5. Higher magnification images of FLAG immunoreactivity in HEK293 cells co-transfected with a 1:1 and 1:4 ratio of Tor1A:silencing plasmid 48 h post-transfection. Control levels of FLAG immunoreactivity are shown in cells co-transfected with miRNeg and shNeg plasmid or Tor1A alone. No FLAG immunoreactivity was observed in non transfected cells (NTC). Scale bar, 100 μm.

Summary: On the basis of these results, it was concluded that (i) all plasmids could transfect HEK293 cells, and (ii) that the miRTor1A plasmid produced a more complete knockdown of torsinA than either of the shRNA plasmids. Thus, the miRTor1A plasmid was selected for the AAV vector.

Selection of the AAV capsid

The vector produced was AAV9-Retro-CBA-mCherry-miTorAx3-WPRE-BGH-pA (AAV-miTorA). The retro AAV9 capsid was selected as it is known that this vector can be retrogradely transported, which is necessary to allow knockdown of torsinA in Purkinje cells following injection into the deep cerebellar nuclei. The cytomegalovirus (CMV) enhancer/chicken β-actin (CBA) promoter consists of the chicken β-actin promoter hybridized with the CMV immediate early enhancer sequence and is highly efficient at transducing most tissue types. The Woodchuck post-transcriptional regulatory element (WPRE) and the presence of a bovine growth hormone (BGH) polyadenylation sequence ensures high transcription following transduction. The plasmid contained in this vector was the miRTor1A plasmid as outlined above.

Development of the Western blot assay to assess torsinA levels

A Western blot assay was developed to evaluate the expression of torsinA in the cerebellum of cynomolgus macaques. Running conditions, antibody selections and dilutions were optimized to produce a protein band predicted to be TorsinA (37 kDa). Negative (SF9 cells) and positive controls (A549 cells) were used to ensure that the protein band of interest was being recognized by the primary antibody and that non-specific bands did not interfere with signal. We were also able to show a protein dose response using macaque cerebellum tissue (20 and 50 µg of protein).

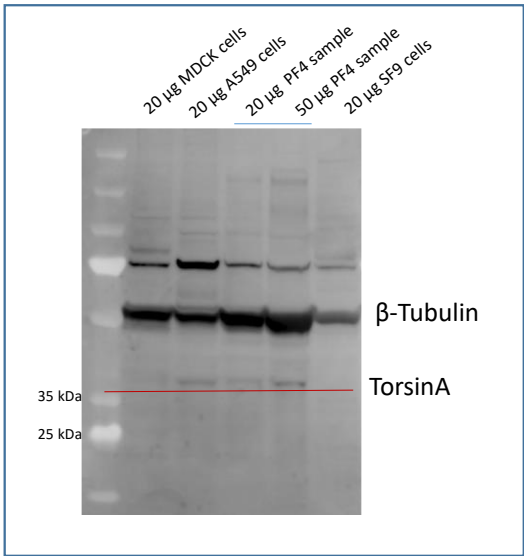

Fig 6. Western blot of TorsinA protein in macaque cerebellar samples. Torsin1A was detected in macaque cerebellum at 2 protein concentrations of tissue. The predicted molecular weight of torsinA is (37kDa).

Summary: On the basis of these results, it was concluded that (i) we can detect Torsin1A protein using Western blot and importantly that we can detect it at various concentrations in macaque brain cerebellar samples. This assay

will be used in subsequent experiments to determine whether the AAV9-miRTor1A can knockdown Torsin1A levels in the deep cerebellar nucl (DCN).

Targeting of the deep cerebellar nuclei

Two macaques received MRI imaging from which to derive surgical coordinates to target the DCN (comprising of the dentate nucleus, interposed nucleus, and the fastigial nucleus). The MRI contrast reagent gadolinium (Gd) was injected into the cerebellum with the goal of determining a volume that would cover all cerebellar nuclei using a single injection. The volume and placement of the injection allowed us to determine injection and targeting parameters to use in the AAV injection experiments. Following three independent injections we were able to determine the optimal volume of injection and placement of the delivery cannula within the cerebellum to allow for distribution of Gd (surrogate for AAV injection) to cover the intended target. Figure 7 shows one of these injections and demonstrates that with slight adjustment of coordinates in the medial, ventral and anterior directions, that coverage of the DCN could be achieved in future AAV injection experiments. Figure 7 shows that >70% of the DCN was covered using the parameters chosen.

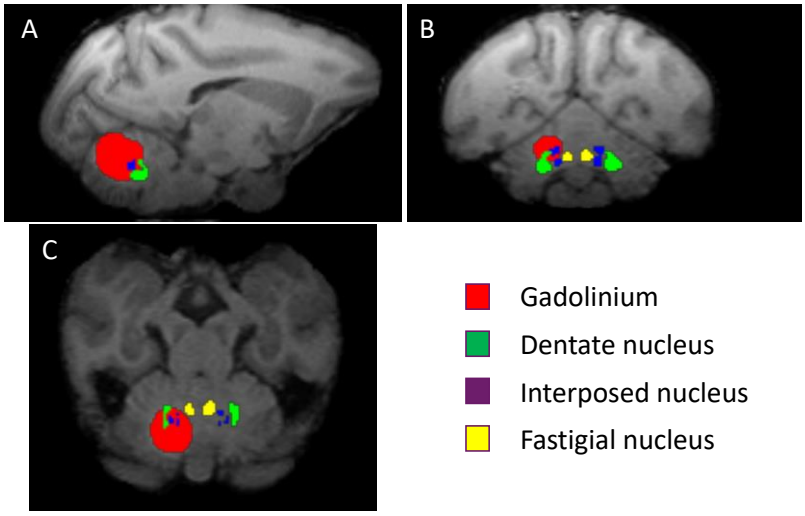

Fig 7. Targeting of the deep cerebellar nuclei with gadolinium. Gadolinium was injected into the deep cerebellar nuclei (DCN) to optimize injection parameters for AAV injections in a subsequent experiment. Following surgical injection of gadolinium, animals were MRI-imaged to reveal the distribution of the injectate and reviewed in three orientations, sagittal (A), coronal (B) and horizontal (C). Calculations were made to produce percent volume of distribution of gadolinium coverage of the intended target (DCN).

Summary: On the basis of these results, it was concluded that (i) target coordinates for DCN could be reliably determined and (ii) the volume of injectate would distribute sufficiently to cover the DCN in subsequent AAV injection experiments to knock down Torsin1A.

Preparation of macaque cohort and surgical delivery of AAV vector (experiment ongoing)

Twelve macaques received MRI imaging and based on these images surgical coordinates were derived using the parameters established in the previously mentioned gadolinium injection experiments. Three different concentrations of AAV-miTorA (5 x 10^{10} vg/ml, 5 x 10^{11} vg/ml, 5 x $10^{12)}$ vg/ml) or AAV-control were each independently injected into 6 hemispheres. Thus, each group had n=6 hemispheres. Animals were allowed 8 weeks for the transgene to be produced and act. Animals were then euthanized and brains harvested to procure punches of cerebellar tissue in the region of the DCN and purkinjie layer. Punches have been prepared for Western blot analysis to reveal protein levels of Torsin1A (assay shown in Figure 6).

- **What opportunities for training and professional development has the project provided?**
    - *If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."*
    - *Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.*

Nothing to Report

- **How were the results disseminated to communities of interest?**
    - *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
    - *Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.*

To-date, there is nothing to report to communities of interest. However, once we have shown that we can successfully knockdown torsinA (month 14) we will reach out to disease-specific charities to start disseminating the information.

- **What do you plan to do during the next reporting period to accomplish the goals?**
    - *If this is the final report, state "Nothing to Report."*
    - *Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

In the next year of the project (Year 2) we will:

- Kill the 12 macaques that have received different titres of AAV9-Retro-CBA-mCherry-miTorAx3-WPRE-BGH-pA (AAV-miTorA) and evaluate knockdown of torsinA in deep cerebellar nuclei tissue and Purkinje cells (complete by month 14 of the project)
- Purchase and acclimatize 12 female cynomolgus macaques (complete by month 18)
- MRIs on all 12 macaques to allow targeting of deep cerebellar nuclei (complete by month 18)
- Collect baseline behavioral and imaging data (complete by month 18)
- Bilaterally inject the selected titre of AAV-miTorA into the deep cerebellar nuclei of all 12 macaques (complete by month 19)
- Gather and analyze behavioral and imaging data up to 18 weeks post-surgery (complete by month 25)

Therefore, in the next reporting period (Year 2) we will have demonstrated knockdown of torsinA, prepared the cohort of animals that have torsinA knocked-down to a level that is likely to produce dystonia, and be collecting behavioral and imaging data on this cohort of animals.

4. Impact

Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

- **What was the impact on the development of the principal discipline(s) of the project?**
    - *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
    - *Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).*

Nothing to Report

- **What was the impact on other disciplines?**
    - *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
    - *Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.*

Nothing to Report

- **What was the impact on technology transfer?**
    - *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
    - *Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:*
        - *transfer of results to entities in government or industry;*
        - *instances where the research has led to the initiation of a start-up company; or*
        - *adoption of new practices.*

We have demonstrated that we can successfully inject directly into the deep cerebellar nuclei. This has expanded the number of central nervous tissues that we directly inject into and thus has helped to extend our gene therapy offerings. Atuka already offers contract services to support the development of gene therapies.

- **What was the impact on society beyond science and technology?**
    - *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
    - *Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:*
        - *improving public knowledge, attitudes, skills, and abilities;*
        - *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
        - *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. Changes/Problems

The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

- *Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.*

In the grant application, we stated that we would develop a retroAAV9-U6-torsinA-shRNA-CMV-GFP. However, during development of the vector, we discovered that miRTor1A was more effective at knocking down torsinA than shTor1A. Therefore, the final vector we developed was AAV9-Retro-CBA-mCherry-miTorAx3-WPRE-BGH-pA (AAV-miTorA), as it was determined that this vector will provide the most efficient knockdown of torsin A.

This change, development of a more efficient vector, has no impact on the deliverables of the project.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

  - *Describe problems or delays encountered during the reporting period and actions or plans to resolve them.*

No problems or delays have been encountered during the reporting period.

- **Changes that had a significant impact on expenditures**

  - *Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.*

The price of macaques increased substantially between writing the grant and purchasing the macaques. This additional cost has been covered by Atuka and thus the overall budget has not been affected.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

  - *Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.*

Nothing to Report

- **Significant changes in use or care of human subjects**

Nothing to Report

- **Significant changes in use or care of vertebrate animals.**

Nothing to Report

- **Significant changes in use of biohazards and/or select agents**

Nothing to Report

6. Products

List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

We have developed a new AAV vector, AAV9-Retro-CBA-mCherry-miTorAx3-WPRE-BGH-pA (AAV-miTorA).

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

Nothing to Report

  - **Journal publications.** *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

  - **Books or other non-periodical, one-time publications.** *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g.,*

book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to Report

- **Other publications, conference papers, and presentations.** *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing the report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

We have demonstrated that we can directly inject into the deep cerebellar nuclei of macaques. Whilst this has been done previously, we believe that we are the only Contract Research Organisation that can perform this service. This technique strengthens our already existing gene therapy services. We often present an overview of our gene therapy services, at meetings and to potential clients, and we are also planning to present the data at a suitable conference.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*
  - *biospecimen collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to Report

7. Participants & Other Collaborating Organizations

- **What individuals have worked on the project?**

  - *Provide the following information for: (1) PDs/Pis; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."*

Name:	*Jonathan Brotchie*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0003-2337-0816*
Nearest person month worked:	*2*
Contribution to Project:	*Dr Brotchie has reviewed all the protocols, IACUC submissions, and data generated. Dr Brotchie has also chaired project meetings throughout the year*
Funding Support:	*Atuka Inc.*
Name:	*James Koprich*
Project Role:	*Post doctoral associate*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-9909-5991*
Nearest person month worked:	*3*
Contribution to Project:	*Dr Koprich wrote the protocol, managed the Western blot development, performed the imaging work, and performed the AAV-surgeries*
Funding Support:	*Atuka Inc.* *Michael J Fox Foundation*
Name:	*Su Jin*
Project Role:	*China site PI*

Researcher Identifier (e.g. ORCID ID):	-
Nearest person month worked:	*6*
Contribution to Project:	*Mr Su Jin submitted the local IACUC applications, was responsible for the purchasing and animal husbandry aspects of the work, provided support to Dr Koprich for the imaging and surgeries, and is responsible for collecting the behaviour data*
Funding Support:	*Atuka Inc.*
Name:	*Chaohuan Yan*
Project Role:	*Technician*
Researcher Identifier (e.g. ORCID ID):	-
Nearest person month worked:	*2*
Contribution to Project:	*Mr Yan provided day to day support to Mr Su Jin for the animal husbandry, surgery preparation, and collection of behavioural data.*
Funding Support:	*Atuka Inc.*
Name:	*Patrick Howson*
Project Role:	*Post doctoral associate*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-5310-0844*

Nearest person month worked:	*1*
Contribution to Project:	*Dr Howson prepared the IACUC application and managed the synthesis of the AAV vector performed by GeneDetect*
Funding Support:	*Atuka Inc.*

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
    - *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
    - *If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.*

Nothing to Report

- **What other organizations were involved as partners?**
    - *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
    - *Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.*
      Provide the following information for each partnership:
        - **Organization Name:**
        - **Location of Organization:** *(if foreign location list country)*
        - **Partner's contribution to the project** *(identify one or more)*
            - **Financial support;**

- **In-kind support** *(e.g., partner makes software, computers, equipment, etc., available to project staff)*;
- **Facilities** *(e.g., project staff use the partner's facilities for project activities)*;
- **Collaboration** *(e.g., partner's staff work with project staff on the project)*;
- **Personnel exchanges** *(e.g., project staff and/or partner's staff use each other's facilities, work at each other's site)*; and
- **Other**.

Nothing to Report

8. Special Reporting Requirements

- **COLLABORATIVE AWARDS:** *For collaborative awards, independent reports are required from **BOTH** the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to https://ebrap.org for each unique award.*

Not Applicable

- **QUAD CHARTS:** *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

Not applicable

9. Appendices

*Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc. Reminder: Pages shall be consecutively numbered throughout the report. **DO NOT RENUMBER PAGES IN THE APPENDICES.***

No appendices