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TITLE: Developing Gene Silencing for the Study and Treatment of Dystonia

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CONTRACTING ORGANIZATION: The Children's Hospital of Philadelphia

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14. ABSTRACT
Dystonia is a debilitating neurological disease with no cure. In dystonia, there are involuntary muscle contractions that cause abnormal twisting postures. DYT1 dystonia is an autosomal dominant disease with onset of dystonia during childhood. The most common early onset inherited dystonia, DYT1 is caused by a common deletion of a single amino acid (ΔE) in torsinA. There is abundant evidence suggesting that suppressing expression of torsinA(ΔE) through gene silencing techniques would be beneficial. We have already achieved this goal in cultured cells through RNA interference (RNAi) and antisense oligonucleotides (ASO).

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1. INTRODUCTION

Dystonia is a debilitating neurological disease with no cure that is characterized by involuntary muscle contractions that cause abnormal twisting postures. DYT1 dystonia, an autosomal dominant disease, the most common early onset inherited dystonia. DYT1 is caused by a common deletion of a single amino acid (ΔE) in torsinA. Abundant evidence suggests that suppressing expression of torsinA(ΔE) through gene silencing would be beneficial. Our overall hypothesis is suppressing expression of torsinA(ΔE) through RNA interference (RNAi) or antisense oligos (ASO) will be a safe an effective treatment for DYT1 dystonia. In innovative proposal, we use a rat model of DYT1 to test the efficacy and safety of viral (AAV)-mediated RNAi or intraventricular delivery of ASO in vivo. Key questions on dystonia research that we will address are whether the DYT1 phenotype is reversible and what is the neuroanatomical substrate that causes motor dysfunction in dystonia.

2. KEYWORDS

adeno-associated virus (AAV); antisense oligonucleotide (ASO); RNA interference (RNAi); torsinA; DYT1; dystonia; gene silencing; cerebellum; striatum; therapy;

3. ACCOMPLISHMENTS

We will list the major goals of the project as stated in the approved SOW for months 1-12 (this reporting period) and will described the status of those goals. Those milestones that are not taking place between months 1-12 are not listed. Overall, the project is progressing as planned with about a 1-2 month delay due to the time required for animal care protocol approval and for smaller litters of animals than expected.

What were the major goals of the project?

Task 1. Regulatory review and approval processes for animal studies (2-4 months): completed at month 5.

Task 2. Dose-finding experiments with ASO in DYT1 knock in rats.

2a. Animal cohort 1:

1) Generation of animal cohort 1 (months 1-4). We will generate 2 month-old rats (18 DYT1 and 18 WT): completed by month 5.

2) Baseline behavior (month 4): completed by month 5.

3) Infusion of ASO during 2 weeks (month 4-5): completed by month 6.

4) Post-infusion behavioral testing/sacrifice (month 5): completed by month 6

5) Molecular and histological analyses (months 8-14): ongoing analysis. Protein lysates and mRNA preps have been obtained and are ready for analysis. Tissue for histological analysis has been fixed and archived. About 50% of this task is completed, and we anticipate completing this by month 14 as proposed with a potential 1 month delay..

2b. Animal cohort 2:

1) Generation of animal cohort 2 (months 2-5). We will generate 2 month-old rats (18 DYT1 and 18 WT): completed by month 6.

2) Baseline behavior (month 5): completed by month 6.

3) Infusion of ASO during 2 weeks (month 5-6): completed by month 7.

4) Post-infusion behavioral testing/sacrifice (month 6): completed by month 7.

5) Molecular and histological analyses (months 8-14): ongoing analysis. Protein lysates and mRNA preps have been obtained and are ready for analysis. Tissue for histological analysis has been fixed and archived. About 50% of this task is completed, and we anticipate completing this by month 14 as proposed with a potential 1 month delay..

2c. Animal cohort 3:

1) Generation of animal cohort 3 (months 3-6). We will generate 2 month-old rats (18 DYT1 and 18 WT): completed by month 8.

2) Baseline behavior (month 6): completed by month 8.

3) Infusion of ASO during 2 weeks (month 6-7): completed by month 9.

4) Post-infusion behavioral testing/sacrifice (month 7): completed by month 9.

5) Molecular and histological analyses (months 8-14): ongoing analysis. Protein lysates and mRNA preps have been obtained and are ready for analysis. Tissue for histological analysis has been fixed and archived. About 50% of this task is completed, and we anticipate completing this by month 14 as proposed with a potential 1 month delay.

2d. Animal cohort 4:

1) Generation of animal cohort 4 (months 4-7). We will generate 2 month-old rats (18 DYT1 and 18 WT): 50% completed by month 10. The remainder 50% completed at month 12.

2) Baseline behavior (month 7): 50% completed by month 10. The remainder 50% completed at month 12.

3) Infusion of ASO during 2 weeks (month 7-8): 50% of animals completed by month 11. The remainder 50% expected to be completed by month 13.

4) Post-infusion behavioral testing/sacrifice (month 8): 50% of animals completed by month 11. The remainder 50% expected to be completed by month 13.

5) Molecular and histological analyses (months 8-14) ongoing analysis. Protein lysates and mRNA preps have been obtained and are ready for analysis. Tissue for histological analysis has been fixed and archived. About 50% of this task is completed, and we anticipate completing this by month 14 as proposed with a potential 1-2 month delay.

Task 3. Therapeutic trial with ASO in DYT1 rats.

3a. Animal cohort 1:

1) Generation of animal cohort 1 (months 6-18). We will generate 12 month-old male rats (16 DYT1 and 16 WT): cohort generated and in the process of aging to the target age of 12 months as planned.

3b. Animal cohort 2:

1) Generation of animal cohort 2 (months 7-19). We will generate 12 month-old male rats: cohort generated and in the process of aging to the target age of 12 months as planned.

What was accomplished of these goals?

As briefly described under the previous heading, the bulk of the experiments planned for year 1 (task 2) are based on infusing different doses of the ASOs into the lateral ventricle of 2-month-old DYT1 and control rats to identify efficacy and toxicity thresholds. At this age, most torsinA(ΔE) rats don't exhibit motor dysfunction. Behavioral evaluation was completed at baseline. Subsequently, an osmotic pump with ASO (active or missense control) was inserted in the mid-scapular subcutaneous space and its tip placed into the lateral ventricle. We used 3 different doses (50, 100 or 200 µg/day). The infusion lasted for 14 days. The rats underwent repeated behavioral testing after the infusion and were sacrificed. Their brains were extracted for mRNA, protein and histological analyses. To determine silencing efficacy, we are measuring

levels of the target mRNA and protein (torsinA) with RTPCR and wester blotting. Those experiments are ongoing. All the animals for the 100 and 200 µg/day have completed the protocol, have been sacrificed and protein, mRNA and fixed brain collected for analysis. Half of the animals of the 50 µg/day group have also completed the protocol. The remainder 50% will receive the infusion and complete the protocol by month 13. Therefore, all molecular and histological analyses will be completed by month 15-16 (initially planned to be completed by month 14, therefore with a 1-2 month delay as initially explained).

For silencing efficacy we will measure expression levels of torsinA by RTPCR and western blotting. To detect toxicity we have completed behavioral analysis at baseline and after the infusion and we will evaluate for histological markers of glial reaction and neuronal loss. Final analysis of behavioral data has not been finalized as the investigators are still blinded to animal genotypes. Once the last group of rats ins sacrificed, genotypes will be unblended and all data will be subject to statistical analysis for evidence of genotype or treatment-based effects, or a genotype-treatment interaction.

Thus, upon the completion of the entire set of experiments included in Task 2 (planned for month 14) we expect to have identified a dose for the therapeutic efficacy studies planned in Task 3 in older rats once they have developed a motor phenotype (by age 11 months) to assess reversibility. The cohort of animals for Task 3 has been generated and is currently being aged to the target 12 months of age for these experiments.

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

The project is progressing as planned. Task 2 should be fully completed by the beginning of the next reporting period (by month 15-16). Therefore, the main bulk of the next reporting period (months 13-24) will be to complete Task 3. The animal cohorts needed have been generated and are aging. The goal of those experiments is to determine if the safe and efficacious dose of the ASO identified in Task 2 reverses the motor phenotype in 12 month old DYT1 rats once developed after a 1 month infusion, and if this benefit is reversible after a washout period. In addition, during the next reporting period (months 13-24), we will generate the AAV vector and the animal cohorts that will be used for Task 5 in the final reporting period (months 25-36).

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS.

Changes in approach and reasons for change

None.

Actual or anticipated problems or delays and actions or plans to resolve them.

None.

Changes that had a significant impact on expenditures.

None.

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

None

6. PRODUCTS.

Publications, conference papers, and presentations.

None.

Website(s) or other Internet site(s)

None.

Technologies or techniques

None.

Other Products

None.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS.

What individuals have worked on the project?

Name: Pedro Gonzalez-Alegre
Project Role: PI
Nearest person month worked: 3
Contribution to Project: No change.

Name: Beverly Davidson
Project Role: Co-Investigator
Nearest person month worked: 1
Contribution to Project: No change.

Name: Genevieve Beauvais
Project Role: Postdoctoral Associate
Nearest person month worked: 12
Contribution to Project: No change.

Name: Jaime Watson
Project Role: Research Technician II
Nearest person month worked: 12
Contribution to Project: Ms. Watson assists Dr. Beauvais in colony maintenance, animal genotyping, surgeries, behavioral, histological and molecular assessments.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Dr. Gonzalez-Alegre has no changes on support. The following changes apply to Dr. Beverly Davidson (Co-Investigator):

New Grants

NIH/NIA R01 AG047644; Holtzman,D. (PI) 8/15/14-4/30/19 .6 cal/months
Novel Strategies and Mechanisms to Target APOE and Alzheimer's Disease

National Ataxia Foundation; Davidson (PI) 1/1/15-12/31/16 .24 cal months
Translating RNAi Therapy for Spinocerebellar Ataxia 1 (SCA1) to the Clinic

R56NS093392; Hughes (PI) 7/1/15-6/03/16 1.2 months
Mechanisms of RRAS Regulation of Huntington Toxicity & Turnover

R56NS090390; Thompson (PI) 9/30/14-8/31/15 .96 cal months
Neuroregulatoy Mechanisms of PIAS1 and Implications for Huntington's Disease

Spark Therapeutics; Davidson (PI) 2/1/15-6/30/18 .24 cal months
Next generation gene therapy

WaVe Life Sciences; Davidson (PI) 4/1/15-7/30/15 .24 cal months
Allele-selective knockdown of mHTT using ASOs

TERMINATED

NIH/NHLBI P01 HL78810 Ertl (PI) 7/1/14-6/30/15 1.2 cal/months
Immune Responses to AAV-mediated FIX Gene Transfer Core B Vector Core
(Davidson, PI)

RAGS Charitable Foundation; Davidson (PI) 2/1/10-8/31/15 .6 cal months
Accessing the Utility of AAV2/5 and AAV2/1for Transduction of Cerebellar Neurons in NHP Brains

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS.

None

9. APPENDICES.

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