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TITLE: Early Treatment of Language Impairment in Young Children with Autism Spectrum Disorder with Leucovorin Calcium

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CONTRACTING ORGANIZATION: Phoenix Children's Hospital, Phoenix, AZ

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13. SUPPLEMENTARY NOTES

14. ABSTRACT Autism Spectrum Disorder (ASD) has an enormous impact on individuals, families and society, yet there is no FDA approved treatment that addresses underlying ASD pathophysiology and/or core deficits. However, a growing body of evidence indicates that leucovorin, a form of folate (vit B9) that bypasses physiological blocks in folate metabolism found in ASD, can significantly improve verbal communication as well as other symptoms associated with ASD. Our recent double blind placebo controlled (DBPC) trial published in **Molecular Psychiatry**, found that leucovorin significantly improved verbal communication in children with ASD with a medium-to-large effect size especially in the subset positive for the folate receptor autoantibody (FRAA+). However, the effect on social communication measures was mixed. We hypothesize that leucovorin could have a definitive positive impact on social communication if treatment is initiated beginning around 2-3 years of age, when neuroplasticity is greater and social communication is being established. We will also study the neuronal mechanisms underlying leucovorin's improvement in social communication / language. We propose a multisite 12-week DBPC trial with 12-week open-label extension of leucovorin in 2½-5 year old children with ASD who are FRAA+. Our primary outcome will be the Brief Observation of Social Communication Change, a sensitive, validated, direct assessment of change in social communication developed by C. Lord at the Center for Autism and the Developing Brain. We will also measure changes in

15. SUBJECT TERMS
Autism Spectrum Disorder; leucovorin; social communication; verbal communication.

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1. INTRODUCTION:

Autism Spectrum Disorder (ASD) has an enormous impact on individuals, families and society, yet there is no FDA approved treatment that addresses underlying ASD pathophysiology and/or core deficits. However, a growing body of evidence indicates that leucovorin, a form of folate (vit B9) that bypasses physiological blocks in folate metabolism found in ASD, can significantly improve verbal communication as well as other symptoms associated with ASD. Our recent double-blind placebo controlled (DBPC) trial published in **Molecular Psychiatry**, found that leucovorin significantly improved verbal communication in children with ASD with a medium-to-large effect size especially in the subset positive for the folate receptor autoantibody (FRAA+). However, the effect on social communication measures was mixed. We hypothesize that leucovorin could have a definitive positive impact on social communication if treatment is initiated beginning around 2-3 years of age, when neuroplasticity is greater and social communication is being established. We will also study the neuronal mechanisms underlying leucovorin's improvement in social communication / language. We propose a multisite 12-week DBPC trial with 12-week open-label extension of leucovorin in 2½-5-year-old children with ASD who are FRAA+. Our primary outcome will be the Brief Observation of Social Communication Change, a sensitive, validated, direct assessment of change in social communication developed by C. Lord at the Center for Autism and the Developing Brain. We will also measure changes in neuronal activation and connectivity using non-invasive neuroimaging: magnetoencephalography and near infra-red spectroscopy. If leucovorin can be shown to improve the core deficit of social communication in ASD, the potential ***positive impact*** will be significant, laying the groundwork for a 'precision medicine' approach in which FRAA screening identifies children likely to benefit from leucovorin treatment.

2. KEYWORDS:

Autism Spectrum Disorder; leucovorin; social communication; verbal communication.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: Major Task 1: Subtask 1:

1. Develop data sharing agreements (DSAs) between SUNY and PCH – Complete
2. Prepare REDCap Database System - Complete
3. Refine eligibility criteria, exclusion criteria, screening protocol – Complete
4. Request use of Investigational New Drug (IND) application filed for L-Leucovorin – Complete
5. Finalize consent form & human subjects protocol – Complete
6. Coordinate with Sites for IRB protocol submission – Reliance Agreement through SmartIRB Complete
7. Submit Protocol to IRB – Complete
8. Submit IND to FDA -- Complete

Specific Aim 1: Major Task 1: Subtask 2:

1. Acquire psychological testing materials and set up outcome procedures - Complete
2. Train psychometrician/testers to research level of reliability – Complete and On Going

Specific Aim 1: Major Task 2: Coordinate Clinic Trial:

1. Advertise and recruit potential participants – Complete
2. Screen Participants– Ongoing
3. Evaluate Psychometrician Rated Measures: – Ongoing
4. Monitor fidelity of Psychometrician Measures– Ongoing
5. Evaluate Clinical Rated Measures: – Ongoing
6. Track and Score Behavioral Questionnaires– Ongoing
7. Distribute Treatment– Ongoing
8. Check Compliance– Ongoing
9. Enter Data in Database– Ongoing

Specific Aim 1: Major Task 3: Analyze Data and Complete Report

1. Check Research Files for Completeness -- Ongoing
2. Check Database for Entry Errors-- Ongoing
3. Assemble Final Recruitment Reports -- Pending: Requires Complete Clinical Trial Data Collection
4. Analyze Clinical Trial Data-- Pending: Requires Complete Clinical Trial Data Collection
5. Write and Submit Study Findings-- Pending: Requires Complete Clinical Trial Data Collection

Specific Aim 2: Major Task 1: Prepare for Clinical Trial

1. Develop material transfer agreements (MTAs) between SUNY and PCH - Done
2. Prepare Laboratories for Sample Processing – Done

Specific Aim 2: Major Task 2: Biomarkers for Clinical Trials

1. Obtain and Process Samples for Biomarkers– Complete
2. Process Samples for Biomarkers– Complete
3. Enter Data in Database– Complete

Specific Aim 2: Major Task 3: Analyze Data and Complete Report

1. Check Research Files for Completeness-- Complete
2. Check Database for Entry Errors-- Complete
3. Analyze Biomarker Trial Data-- Pending: Requires Completed Clinical Trial Data Collection
4. Write and Submit Study Findings-- Pending: Requires Completed Clinical Trial Data Collection

What was accomplished under these goals?

Above are described the major activities and specific objectives completed. We have completed all of the regulatory and preparatory steps to initiate the trial and enrolled our first participant in January 2021. We are continuing to enroll participants on a regular basis.

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

We are regularly involved in both training and professional development. We have regular training session as a group on the outcome measures usually several times per week, with those with knowledge of the outcome measure providing the training and those not proficient with the instruments undergoing professional development. This is occurring in several settings including in-person at each site and across sites through Zoom meetings.

How were the results disseminated to communities of interest?

We hare recently published an article pertaining to our work.

1. Frye, R.E. Rossignol, D.A., Scahill, L., McDougle, C.J., Huberman, H., Quadros, E.V. Treatment of Folate Metabolism Abnormalities in Autism Spectrum Disorder. Seminars in Pediatric Neurology, 2020 Oct;35:100835. doi: 10.1016/j.spen.2020.100835. Epub 2020 Jun 25. PMID: 32892962; PMCID: PMC7477301.
2. Rossignol DA, Frye RE. Cerebral Folate Deficiency, Folate Receptor Alpha Autoantibodies and Leucovorin (Folinic Acid) Treatment in Autism Spectrum Disorders: A Systematic Review and Meta-Analysis. J Pers Med. 2021 Nov 3;11(11):1141. doi: 10.3390/jpm11111141. Erratum in: J Pers Med. 2022 Apr 29;12(5): PMID: 34834493; PMCID: PMC8622150.

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

1. Continue to enroll participants in the trial and publish any new results that have been finalized.
2. Validate and perform the folate receptor alpha autoantibody assay in the Frye laboratory

4. IMPACT:

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

This project will provide a better understanding of the treatment and underlying biology associated with ASD

What was the impact on other disciplines?

This project will provide a better understanding of the treatment and underlying biology associated with nervous system development and other neurodevelopmental disorders besides ASD.

What was the impact on technology transfer?

None

What was the impact on society beyond science and technology?

Improving the lives of children with ASD will no doubt have a broad positive effect on society as the cost and resource currently required to care for such children is significant.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

1. We are now working directly with the manufacturer to develop the drug.
2. The PI is now the IND sponsor.
3. We will perform the folate receptor alpha autoantibody assay in the Frye laboratory

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

Cox Biosciences is no longer viable to develop the drug product commercially and failed to submit the IND to the FDA. Thus, we are now working directly with the manufacturer to develop the drug. The PI has submitted the IND and is the sponsor of the study rather than Cox Biosciences. The IND has been approved by the FDA. In addition, COVID provided many challenges to recruitment which have now been resolved. Lastly, Dr Quadros is retiring and has stopped performing the folate receptor alpha autoantibody assay in his laboratory. Thus, the Frye laboratory will start performing the assay. In the meantime, the commercial vendor for this assay, Illiad Neuroscience / Vascular Strategies, is performing the assay at a reduce costs (compare to the commercial test).

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.

Delays in filing the IND has led to a delay in approval. COVID resulting in slow initial recruitment. We will carry forward money unspent because of this delay so there will not be any overall impact on the overall budget of the project.

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

Significant changes in use or care of human subjects

None

Significant changes in use or care of vertebrate animals

None

Significant changes in use of biohazards and/or select agents

None

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

1. Frye, R.E. Rossignol, D.A., Scahill, L., McDougle, C.J., Huberman, H., Quadros, E.V. Treatment of Folate Metabolism Abnormalities in Autism Spectrum Disorder. Seminars in Pediatric Neurology, 2020 Oct;35:100835. doi: 10.1016/j.spen.2020.100835. Epub 2020 Jun 25. PMID: 32892962; PMCID: PMC7477301.
2. Rossignol DA, Frye RE. Cerebral Folate Deficiency, Folate Receptor Alpha Autoantibodies and Leucovorin (Folinic Acid) Treatment in Autism Spectrum Disorders: A Systematic Review and Meta-Analysis. J Pers Med. 2021 Nov

Books or other non-periodical, one-time publications.

None

Other publications, conference papers and presentations.

None

- **Website(s) or other Internet site(s)**

None

- **Technologies or techniques**

None

- **Inventions, patent applications, and/or licenses**

None

- **Other Products**

None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Richard Frye, MD, PhD
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID): 0000-0003-4442-2937
Nearest person month worked: 1.0
Contribution to Project: Dr. Frye is an Professor of Child Health at the University of Arizona College of Medicine and Chief of Neurodevelopmental Disorders and Director of the Autism and Fragile X Programs at the Barrow Neurological Institute at Phoenix Children's Hospital. Dr. Frye is a Child Neurologist with fellowship training in both Behavioral Neurology and Psychology. He has become a national leader in the medical evaluation and treatment of children with neurodevelopmental disorders, particularly children with autism spectrum disorder (ASD) and published multiple papers on abnormalities in folate metabolism and mitochondrial function as well as seizure treatment in children with ASD, including recent papers in Molecular Psychiatry. As Principal Investigator, Dr. Frye will oversee the project progress from startup to data analysis. Dr. Frye will be responsible for assuring the success, startup, and organization of the project, obtaining regulatory approval, facilitating and overseeing recruitment and retention of participants, monitoring of the ongoing trial, assuring timely and accurate follow-up with families, data analysis and interpretation, and writing publications and reports. He will act as the treating clinician in this study.

Name: Sallie McLees
Project Role: Research Coordinator
Researcher Identifier (e.g. ORCID ID): NA
Nearest person month worked: 1.0
Contribution to Project: Planned overall project, worked w team refining, developing and piloting baseline / outcome measures and procedures.

Name: Harris Huberman
Project role: Site Co-PI
Researcher identifier:
Nearest person month worked: 0.15
Contribution to project: Planned overall project, worked w team refining, developing and piloting baseline / outcome measures and procedures.

Name: Edward Quadros
Project role: Site Co-PI
Start date: 3/1/19
Nearest person month worked: 0.05
Contribution to project: Planned overall project and laboratory procedures, conducted initial lab assays for initial subjects

Name: Daniel Mishan
Project role: Co-Investigator
Start date: 2/14/20
Nearest person month worked: 0.36
Contribution to project: Participated in overall project planning, helped with regulatory and IRB aspects of project, conducted recruitment screening, baseline / outcome measures and procedures for enrolled subjects and laboratory processing of subject samples

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

The PI has received the following additional awards but these will not impact the PIs effort on the current award

2020	**Mitochondrial disease and dysfunction in neurological and neurodevelopmental disorders: Measurement of mitochondrial function in fresh human brain.** Innovative Neuroscience for Kids (INK) Foundation, **Principal Investigator**
2020-2022	**Autism Learning Health Network**, Autism Speaks, **Site Principal Investigator**
2020-2021	**Changes in Mitochondrial Function in Children with Autism and Severe Gi Disease Treated with MTT**. The Brain Foundation. **Principal Investigator**.
2020-2021	**Changes in Mitochondrial Function in Children Treated with MTT**. N of One Foundation. **Principal Investigator**.
2020-2023	**Resting State Functional MRI to Locate Seizure Onset Zone and Pathological Neurocognitive Networks in TSC**. Department of Defense. **Co-Investigator**
2020-2022	**Molecular Studies to Identify Mechanisms that Underlie Symptom Improvement in Microbiota Transfer Therapy Patients**. Department of Defense. **Consultant**
2020-2021	**Mitochondrial disease and dysfunction in neurological and neurodevelopmental disorders: Measurement of mitochondrial function in fresh human brain.** Innovative Neuroscience for Kids (INK) Foundation, **Principal Investigator**
2021-2026	**The FXCRC FORWARD Registry and Database. Study to Explore Early Development (SEED) Follow-up Study.** Centers for Disease Control and Prevention (CDC)
2021-2024	**Health Information Technology to Support Autism Spectrum Disorders (ASD) Risk Assessment for Early Diagnosis**, National Institute of Mental Health, **Co-Investigator**
2021-2022	**High-Frequency Oscillation as a Biomarker of Mitochondrial Dysfunction associated with Epilepsy in Autism**. The Brain Foundation. **Principal Investigator**.
2021-2022	**Autism with Neuro-developmental Regression Associated Mitochondrial Dysfunction: Further Development of In Vitro Models and Pathways to Treatment**. The Brain Foundation. **Principal Investigator**.
2022-2023	**End the Diagnostic Odyssey Grant.** 3billion. PI
2022-2027	**Efficacy of pharmacologic management of ADHD in children and youth with autism spectrum disorder**. PCORI. Site-PI

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: