SPONSORED RESEARCH AGREEMENT

THIS SPONSORED RESEARCH AGREEMENT (the "**Agreement**") is entered as of February 14, 2022 (the "**Effective Date**"), between The Regents of the University of California, Davis located at Office of Research, Sponsored Programs, 1850 Research Park Drive, Suite 300, Davis, CA 95618-6153 a non-profit, educational institution ("**University**"), and SynGAP Research Fund, located at 1270 Lincoln Avenue, Palo Alto, CA 94301, a 501(c)(3) non-profit profit foundation (the "**Sponsor**," and together with University, the "**Parties**").

RECITALS

WHEREAS, Sponsor is involved in the funding of research; and

WHEREAS, University has research facilities and expertise in the investigation and study of SYNGAP1-related NSID ("SYNGAP1").

NOW, THEREFORE, the parties agree as follows:

1. Sponsored Research

University agrees to undertake and perform a research project in the field of SYNGAP1 in accordance with the scope of work described in the research proposal attached as Exhibit A, as amended from time to time by the mutual written consent of the Principal Investigator and the Sponsor (the "**Research**"). The Parties may only amend this agreement in writing signed by an authorized representative of each Party.

University agrees to use reasonable efforts to perform the Research and will furnish the research staff, technical know-how, equipment, instruments, supplies and facilities necessary to carry out the Research. University agrees that all work done on the Research will be duly recorded and evidenced in laboratory notebooks maintained by the Principal Investigator and the persons working on the Research at his direction in accordance with customary laboratory practices. The Sponsor shall provide funding to University to support the performance of the Research, as described in Section 4. The Sponsor acknowledges that University makes no expressed or implied warranties for the Research.

2. Principal Investigator

The Research will be supervised by Dr. Kyle Anderson, a Professor in the department of Infectious Disease at University ("**Dr. Joseph Anderson**"), as the Principal Investigator of the Research. All the work conducted in performing the Research shall be under the direct supervision of Dr. Anderson. If for any reason Dr. Anderson is unable to continue to serve as Principal Investigator—whether due to his death, his incapacitation, or for any other reason— and a replacement Principal Investigator cannot be agreed to by the University and the Sponsor within thirty (30) days of such inability of Dr. Anderson to continue to serve as Principal Investigator, Sponsor shall have the right to terminate this Agreement without further liability therefore, and no additional payments by Sponsor to University shall be made. Notwithstanding any language to the contrary, Sponsor shall pay for any work already completed as well as for any uncancellable obligations.

AHC

3. Period of Performance

The Research will be conducted during the period March 1, 2022 through February 28, 2024 and may be extended by written agreement of the parties.

4. Payment for Research

In exchange for performing the Sponsored Research described herein, Sponsor agrees to pay University a total payment amount of **US $128,000** ("**Budget**"), which payment includes all expense and other related direct costs due by Sponsor to University, but not any associated indirect cost, as consideration for the services provided by University and Investigator under this Agreement. No other amounts shall be due or payable by Sponsor to University or Investigator for performance of the Research. Sponsor does not cover or otherwise pay University overhead or indirect costs. University contributes to this project the sum of **US$75,796**, shown in Exhibit B, as cost-sharing for the indirect cost of the project, at the federally negotiated rate for performing research at the University that Sponsor is not covering.

5. Payment Schedule

(a) The Sponsor shall make payments to University, for the amounts owed under the Budget as per Exhibit B. Payments will be credited according to the payment schedule upon submission from the Principal Investigator of a detailed progress report that shows the project advancement and success, including, without limitation, a detailed analysis of whether milestones have been achieved, and if they have not been achieved, what further work will be needed in order to achieve them. Sponsor reserves the right to withhold future milestone payments in case of discrepancies in the use of funds and if the specific aims are not adequately addressed. Once resolved, milestone payments shall be made per Exhibit B. Sponsor does not cover Research Institute overhead or indirect costs.

(b) Wire transfers shall be made payable to University in accordance with the following wire instructions:

Account number:	1233714115
Active ACH Blocks/Filters on file	YES
Routing number ACH/EFT	121000358
Routing number DOM. WIRES	026009593
SWIFT Code INTL WIRES	OFOAU53N
Account Name:	University of California Davis Regents of University of California Main Depository Account
Account Address:	One Shields Ave. Davis, CA, 95616

(c) A final financial accounting of all costs incurred and all funds received by University, together with a wire transfer for the amount of the unexpended balance, if any, shall be submitted to Sponsor within thirty (30) days following the expiration or any earlier termination of this Agreement.

AHC

6. Term and Termination

Unless earlier terminated, the Agreement shall expire upon the complete satisfaction of the final milestone set forth in the Budget as described in Exhibit B. This Sponsor may terminate this agreement for any reason or no reason upon 30 calendar days written notice to the University; provided, that the Sponsor shall remain liable for all payments due under the Budget through the date of termination of the Agreement, but only to the extent University uses reasonable efforts to minimize the Sponsor's payment obligations after University's receipt of such notice of termination.

7. Disclosure of Inventions and Research Results

University shall provide to the Sponsor a complete written disclosure for each and every invention or other discovery, whether or not patentable, first conceived or reduced to practice in the performance of the Research work funded under this Agreement (an "**Invention**"), promptly after each such Invention is made. University shall inform the Sponsor of the results of the Research performed as set forth in Section 12 below in email format, and shall provide the Sponsor confidential copies of the results and raw data of the Research. As used herein, "raw data" means information and data in the notebook pages kept by the Principal Investigator and the persons working at his direction on the Research, including, without limitation, all materials such as films, printouts, and photographs that generate such information or data and are typically included in such notebooks, all to the extent concerning work relating to and/or arising from the Research. The intent of the Parties is to ensure that the results of research performed under this Agreement will be available to interested researchers in non-profit organizations/foundations, and in public institutions of higher education, for further non-commercial research purposes. University shall also provide detailed written summaries at the end of each milestone of the Research as set forth in Section 1. Sponsor shall be entitled to ask questions and provide feedback to such written summaries and request further clarification and/or explanation, as the case may be. Sponsor shall be free to use all such results and information for its own internal purposes, but shall not disclose any such results and information to any third party.

8. Patents and Inventions

(a) Any Inventions made during the course of the Research solely by the University shall belong to University ("**University Inventions**"). University shall have the right to obtain patent protection in the United States and foreign countries for any such Inventions, at its expense, unless otherwise agreed upon by the parties. In the event University decides not to seek to obtain patent protection for a University Invention, Sponsor shall have the right to file a patent application for such Invention in University's name and obtain the same rights as it would have obtained if University had filed the patent application. University Inventions shall be deemed Confidential Information of the University.

(b) Any Invention made during the course of the Research jointly by the University and Sponsor shall belong jointly to University and Sponsor ("**Joint Inventions**"). Title to all patents issued thereon shall be jointly held, all expenses incurred in obtaining such patents thereunder shall be jointly shared, and each party shall have the unrestricted right to exploit such Joint Inventions, without the duty of accounting to or seeking consent from the other party. University shall have the first right to obtain patent protection in the United States and foreign countries for any such Joint Inventions. With respect to any Joint Invention, where University elects not to file a patent application in any country or not share equally in the expense thereof, Sponsor shall have the right to file such application at its own expense and shall have full control over the prosecution and maintenance thereof even though title to any patent issued shall be jointly held. With respect to any Joint Invention, where Sponsor elects not to share equally in the expense

AHC

thereof, University shall have the right to file such application at its own expense and shall have full control over the prosecution and maintenance thereof even though title to any patent issued shall be jointly held.

(c) In consideration for the support provided by Sponsor under this Agreement:

(i) University shall grant Sponsor a nonexclusive, royalty-free worldwide license, with the right to sublicense, to use and practice under University's rights in University Inventions and any and all patent applications, patents and other rights claiming or covering such University Inventions for research and development purposes only.

(ii) University shall also grant similar non-commercial license(s) as stated above, to interested researchers in non-profit organizations/foundations, and in public institutions of higher education for further research.

(iii) For the purpose of commercialization of University Inventions, and/or Joint Inventions, University and Sponsor shall cooperate in identifying potential licensee(s) acceptable to both. Once such potential licensee(s) is/are identified, Sponsor and University shall collaborate in the process of discussions and negotiations of the commercial license terms, all with the primary intent of ensuring appropriate availability and use of the inventions for the medically effected patient population, and public benefit, in the areas of rare diseases, with the emphasis in the rare disease subject of this Agreement. The intent is not to license such inventions to the most financially beneficial partner.

(iv) In the event that University grants a license to another party to commercialize University's rights in University Inventions and/or Joint Inventions, such license shall include provisions that obligate the licensee to commercialize the invention in a commercially reasonable diligent manner, pursuant to, for example, appropriate diligence requirements and milestones agreeable to both Sponsor and University. University, as the lead licensor, shall monitor performance by the licensee, and keep Sponsor fully informed of all developments on a timely basis. Unless otherwise agreed with Sponsor, University agrees that if it, its designee or licensee has not taken effective steps to arrange for practical or commercial application (e.g., through a license agreement or other reasonable terms) of the invention within two years from the date of issuance of a patent, or another clear determination of commercial value, or such other term that is commercially reasonable under the circumstances, and the University or other titleholder cannot show commercially reasonable cause acceptable to Sponsor why it should retain rights in and title to the invention for any further period of time, then Sponsor shall have the right to require: (1) assignment of the patent or other intellectual property rights to Sponsor; (2) cancel any outstanding exclusive license agreement; (3) grant a license under such patent or intellectual property right on terms that are reasonable in the circumstances; and/or (4) any other reasonable disposition of rights in the invention.

(v) In the event that University grants a license to another party to commercialize University's rights in University Inventions and/or Joint Inventions, Sponsor shall share in any monies received by the University, following accounting for all of University's out-of-pocket expenses associated with such license(s). University shall not enter into any agreement that will derogate Sponsor's right to share in such monies and shall notify Sponsor promptly and in writing of or any license, lease, sale, or other agreement concerning University Inventions, and/or University's ownership rights in any Joint Inventions that is intended to generate revenue. Sponsor's right to share in monies shall include, without limitation, the sharing of licensing fees, royalties, or any other income derived from such inventions. Sponsor's participation in such revenues generated shall be on a pro rata basis, based on Sponsor's portion of funding support

AHC

for the research which led to the University Invention. The parties shall work together to develop the details of a reasonable formula.

(d) With regard to all tangible research property ("**TRP**") arising from this Research, which does not include patents created, e.g. biological materials, animal models, Induced Pluripotent Stem Cell (iPSC) lines for SYNGAP1, such TRP must be made available to Sponsor and any and all researchers in in non-profit organizations/foundations, and public institutions of higher education, for the purposes of continued research prior to any publication by University. Any mouse model created as part of Research, for example, shall be deposited by University in a repository, such as The Jackson Laboratory (www.jax.org), and/or to the University's nationally recognized, and federally funded Mouse Biology Program (https://mbp.mousebiology.org/), should Mouse Biology Program be interested in such mouse models, that will make the model available to the general research community. Fibroblasts from patient derived biopsies and iPSC lines shall be deposited by University in the SYNGAP1 repository at Coriell Institute sponsored by the United States National Institute of General Medical Sciences. All costs associated with depositing TRP in said repositories shall be covered by the Sponsor.

(e) University agrees that it shall not grant to any third parties any rights, interests, or options in or to the Inventions or TRP or any patent or other intellectual property right thereto unless and until Sponsor is notified of University's intentions, and Sponsor and University collectively agree for the University to so proceed.

9. Confidentiality; Publication; Sharing

(a) Confidentiality. Sponsor and University acknowledge that each party may receive technical information of the other party in written form and marked as being confidential ("**Confidential Information**") during the term of this Agreement. Each party hereto agrees that during the term of this Agreement and for a five (5) year period thereafter, to use the same degree of care it uses to protect its own confidential information, but in no event less than reasonable care, to not disclose any Confidential Information that is disclosed or submitted by the other party. Each party hereto agrees: (i) not to use Confidential Information of the other party except for purpose of conducting activities properly in accordance with the Research Plan; and (ii) not to disclose Confidential Information of the other party to third parties without the express written permission of the other party; *provided, however,* that each party shall not be prevented from disclosing Confidential Information of the other party to its employees, officers, agents and affiliates requiring access thereto for the purposes of this Agreement provided each such employee, officer, agent or affiliate is bound by nondisclosure obligations at least as restrictive as the obligations in this Section 9, or to use or disclose such Confidential Information in connection with the exercise of its rights hereunder. The parties will take all steps necessary to ensure that its employees and agents comply with the terms and conditions of this Agreement.

(b) Exceptions. The obligations of this Section shall not pertain to any information which, to the extent that the party receiving Confidential Information of the other party can establish by competent written proof:

(i) at the time of disclosure is in the public domain;

(ii) after disclosure, becomes part of the public domain by publication or otherwise, except by breach of receiving party's obligation of confidentiality with respect thereto;

(iii) was in party's possession at the time of disclosure, or was subsequently and independently developed by receiving party's employees, officers, independent contractors or affiliates who had no knowledge of or access to the Confidential Information; or

(iv) is received by the receiving party from a third party without breach of an obligation of confidentiality with respect thereto.

If a court order, law or regulation requires the receiving party to disclose Confidential Information, the receiving party may disclose such Confidential Information, *provided* that the receiving party shall give notice to the disclosing parties for that party to make a reasonable effort to obtain a protective order requiring that the Confidential Information be disclosed only to the extent required by, and for the purposes of, such order, law or regulation.

(c) Publication. University shall have the right, at its discretion, to release information or to publish any material resulting from the Research, subject to the following. University shall furnish the Sponsor with the latest draft for review and comment only of any proposed publication thirty (30) days in advance of the submitting such publication to the publisher. University agrees, upon the Sponsor's request, to delay any such publication an additional thirty (30) days in order for University or the Sponsor, as applicable, to prepare and file patent applications to protect the potential patentability of any Invention described therein. Such delay shall not, however, be imposed on the filing of any student presentation, student thesis, or dissertation, or the preparation of a vitae for a job or postdoctoral position, or oral or written presentation at a meeting or retreat in the Department. Students and postdoctoral fellows are free to discuss their research.

(d) Sponsorship. University shall acknowledge Sponsor in any publication for its sponsorship of the Research in a form reasonably acceptable to Sponsor.

(e) Sharing. Principal Investigator will be expected to join and participate in conference calls with Sponsor's Science Team which includes Sponsor's core labs working in the field of SYNGAP1 Research. Principal Investigator shall make all reasonable efforts to attend at least one SYNGAP1 conference as disclosed by Sponsor, and at Sponsor's cost, during the period of the Research term. The goal of the conference is for all SYNGAP1 scientists globally to meet and participate in discussions to further progress in the field of SYNGAP1 research. Principal Investigator's willingness to join discussions and collaborate with a global group of scientists working to cure SYNGAP1 Syndrome is a critical and material condition upon which Sponsor is willing to enter into this Agreement.

10. Consultation

Selected personnel of Sponsor, designated by the Sponsor to University, shall have the right to confer with the Principal Investigator and his associates for such reasonable periods and at such times as are mutually agreeable—such frequency shall be no less than bi-weekly.

11. Publicity

Neither party shall use the name of the other in connection with any product, promotional literature, or advertising material without the prior written permission of the other party, which permission shall not be unreasonably withheld. This restriction shall not apply to materials used by the Sponsor solely for financing purposes or to documents available to the public that identify the existence of the Agreement.

12. Reports and Updates

Principal Investigator and/or the Postdoctoral Representative assigned to this study will meet with Sponsor via conference call once a month to furnish updates. At the end of each milestone and when reasonably requested during the term of this Agreement, University shall furnish the Sponsor a report summarizing the work conducted under the Research during that

AHC

period and the significant results, discoveries, and/or data resulting from such work. In addition, University shall furnish Sponsor a yearly annual report summarizing the results for the year and setting forth a research plan for the Research to be conducted in the coming year. A final report setting forth the accomplishments and significant research findings of the Research for the entire term of the Agreement shall be prepared by University and submitted to the Sponsor within ninety (90) days of the expiration of the Agreement. At the request of the Sponsor, University shall provide financial accounting of the status of the funds received from the Sponsor, with reports submitted at the end of each milestone, as well as with the final report described above.

13. Assignment

Neither party shall assign this Agreement to another without the prior written consent of the other party. Such successor shall expressly assume in writing the obligation to perform in accordance with the terms and conditions of this Agreement. Any other purported assignment shall be void.

14. Exclusivity; Independent Inquiry

University and the Principal Investigator agree that the Principal Investigator and any other University employees, students or post-doctoral fellows supported under the funding provided by the Sponsor hereunder shall not conduct any research or other investigations covered by the Research plan, as appended as Exhibit A, except pursuant to this Agreement.

15. Reserved.

16. Notices

Any notice required or permitted under the Agreement shall be in writing sent or delivered to the appropriate address listed below, and shall deemed effective (a) when received, if by courier, (b) on the fourteenth business day following mailing, if sent by first-class mail, prepaid, or (c) upon receipt, if hand delivered:

To Sponsor:

SynGAP Research Fund
1270 Lincoln Avenue
Palo Alto, CA 94301
Attention: Michael Graglia

to University:

Attn: Executive Director, Research Administration
Office of Research, Sponsored Programs
1850 Research Park Dr., Suite 300
Davis. CA 95618-6153

17. Reform of Agreement

If any provision of this Agreement is, becomes or is deemed invalid, illegal or unenforceable in the applicable law of either party, such provision shall be deemed amended to conform to applicable laws so as to be valid and enforceable; or if it cannot be so amended without materially altering the intention of the parties, it shall be stricken, and the remainder of this Agreement shall remain in full force and effect. This Agreement, including all exhibits and attachments, constitutes

AHC

the sole agreement between the parties with respect to its subject matter. The parties may only amend it in writing signed by an authorized representative of each party.

18. Counterparts

This Agreement may be executed in any number of counterparts, each of which shall be an original, but all of which together shall constitute one instrument.

IN WITNESS WHEREOF, University and Sponsor entered into this Agreement effective as of the date first hereinabove written.

SynGAP Research Fund

By: _____
Michael Graglia
Managing Director

The Regents of the University of California, Davis

By: Ahmad Hakim-Elahi, Ph.D., J.D. Digitally signed by Ahmad Hakim-Elahi, Ph.D., J.D. Date: 2022.02.15 18:27:21 -08'00'
Ahmad Hakim-Elahi, Ph.D., J.D.
Executive Director, Research Administration

I have read this agreement and acknowledge my obligations hereunder:

BY: _____
Joseph Anderson, Principal Investigator

AHE

Exhibit A
Scope of Work

Feasibility of a stem cell gene therapy approach for the treatment of SYNGAP1-related NSID

PI: Dr. Joseph Anderson, PhD, MAS
Institution: University of California Davis Medical Center
Address: 2921 Stockton Blvd. Room 1300
Sacramento, CA 95817
jsanderson@ucdavis.edu

ABSTRACT

SYNGAP1-related NSID is a genetic neurodevelopmental disorder caused by an insufficient level of SynGAP protein. Due to the lack of normal levels of SynGAP, neuronal synapses do not function properly and develop too early which presents with a wide array of clinical phenotypes including intellectual disabilities, decreased motor skills, epilepsy, speech abnormalities, and sleep and behavioral disorders. Currently there is no cure for SYNGAP1-related NSID and only palliative care is available. Therefore, new and innovative therapies need to be developed where functional SynGAP can be delivered to affected neurons.

Hematopoietic stem cell gene therapy for SYNGAP1-related NSID is a potential therapeutic approach for the treatment of patients as the cells of the hematopoietic system are found throughout the body, including in the central nervous system as brain microglia. If genetically modified to express functional SynGAP, these microglia, once engrafted in the brains of transplanted patients, could deliver protein to the affected neurons and possibly restore function or halt disease progression.

In our current proposal, we will develop a stem cell gene therapy for SYNGAP1-related NSID by modifying human hematopoietic stem cells with a lentiviral vector expressing functional SynGAP. We will also develop an immunodeficient and SynGAP deficient mouse model to evaluate the therapeutic efficacy of our approach. This mouse model will allow us to transplant human cells without concern for rejection. These proof-of-concept studies will allow us to determine if this approach can potentially help SYNGAP patients in future clinical trials.

OBJECTIVE: The overall goal of this project is to evaluate the feasibility of a stem cell gene therapy approach for SYNGAP1-related NSID. We will develop a therapeutic candidate which includes the transduction of human hematopoietic stem and progenitor cells (HSPC) with a lentiviral vector expressing functional SynGAP. These cells will subsequently be derived into mature immune cells to express the therapeutic protein throughout the body including in the brain thus delivering SynGAP to affected neurons. We will also develop an immunodeficient mouse model of SYNGAP1-related NSID to evaluate the in vivo efficacy of our stem cell gene therapy approach.

HYPOTHESIS: Human CD34+ HSPC transduced with a SYNGAP1-expressing lentiviral vector will effectively deliver functional SynGAP to affected cells and reverse and/or halt disease progression. Engraftment of the vector transduced cells and expression of SynGAP will be observed in the brains of transplanted mice.

BACKGROUND: SYNGAP1-related NSID is a genetic neurodevelopmental disorder caused by an insufficient level of SynGAP protein. Most cases are due to de novo mutations which occur in the SYNGAP1 gene (1-4). Due to the lack of normal levels of SynGAP protein, neuronal synapses do not function properly and develop too early which presents with a wide array of clinical phenotypes including intellectual disabilities, decreased motor skills, epilepsy, speech abnormalities, and sleep and behavioral disorders (1-4). Currently there is no cure for SYNGAP1-related NSID and treatments are typically individualized depending on what clinical phenotypes are displayed (1-2). These include physical and speech therapy, rehabilitation, behavioral therapy, and anti-seizure medications. Therefore, novel therapies need to be developed which allow for the delivery of functional SynGAP to affected neurons.

A stem cell gene therapy approach to treat SYNGAP1-realted NSID has great potential in offering a life-long and one-time treatment to affected individuals. By genetically modifying a patient's own HSPC with a functional copy of the SYNGAP1 gene, constitutive and systemic expression of SynGAP could be achieved. Patient CD34+ HSPC would be transduced with the SYNGAP1 lentiviral vector and directly infused intravenously. The engrafted genetically modified HSPC would then produce immune cell progeny expressing this functional version of SynGAP throughout the body including in the central nervous system. Myeloid cells would engraft in the brains of transplanted patients as microglia and these cells would deliver functional SynGAP to affected neurons through a process called cross-correction.

This approach has been successfully demonstrated in other monogenic diseases including metachromatic leukodystrophy (MLD), adrenoleukodystrophy (ALD), mucopolysaccharidosis I (MPSI), Tay-Sachs/Sandhoff disease, and Angelman syndrome (AS) (5-13). The success of this approach in numerous human clinical trials and preclinical studies leads us to believe that this approach of cross-correction can benefit other monogenic diseases including those affecting the CNS. This is the main basis and hypothesis for the development of our therapeutic candidate. Immune system cells derived from the genetically modified and transplanted HSPC migrate into the brain and establish long-term residence as microglia (14-15). Through cross-correction, SynGAP will be secreted by the SYNGAP1 lentiviral vector modified cells of the hematopoietic lineage and taken up by neurons and other enzyme deficient cells in the CNS via the mannose-6-phosphate receptor where SynGAP can then provide its normal function.

Our lab has proven that this approach can effectively be used for both lysosomal storage disorders and also for neurological diseases. In our previous work in Tay-Sachs and Sandhoff disease we have demonstrated significant improvement in both motor and behavioral skills in an immunodeficient Sandhoff disease mouse model transplanted with human CD34+ HSPC transduced with a HexA/HexB expressing lentiviral vector (5). An extension of life span was also observed. This work is currently in the IND-enabling study phase. To further exploit this strategy, we applied this therapeutic approach to a neurological disease, Angelman syndrome, and demonstrated both prevention and reversal of clinical symptoms in an immunodeficient Angelman syndrome mouse model transplanted with human CD34+ HSPC transduced with a Ube3a expressing lentiviral vector (6). Expression of Ube3a was detected in the brains of these mice which would not normally have any Ube3a present (6). This work is also currently in the IND-enabling study phase. Our recent work in Angelman syndrome demonstrated that this therapeutic strategy could be

utilized not only for lysosomal storage disorders but also for neurological diseases. As myeloid cells engraft in the brains of transplanted mice and in future human patients as microglia, the hematopoietic system can successfully deliver these therapeutic proteins to affected neurons. Therefore, if additional SynGAP protein was delivered throughout the body, including in the brain to affected neurons, a prevention and possible reversal of clinical symptoms could be achieved.

Potential advantages of the proposed stem cell gene therapy approach for SYNGAP1-related NSID:

1. Autologous HSPC are derived from each patient and thus less likely to cause complicating immune responses that require life-long immune suppression
2. Genetically engineered HSPC-derived cells overexpressing SYNGAP1 are capable of secreting functional enzyme in large quantities.
3. Genetically engineered HSPC-derived cells infiltrate the CNS and potentially all tissues in the body where they work as enzyme donors. This approach is likely to address CNS and peripheral tissue pathology and thus work as a whole-body therapy.
4. Constitutive long-term expression of SynGAP from HSPC-derived cells will likely allow for a one-time treatment.
5. Neurons are not transduced directly, minimizing the potential for vector-mediated neurotoxicity.
6. Lack of intracranial administration required by other vector systems including AAV and nucleic acid modifying enzymes including TALES and CRISPR systems.
7. Lack of reliance on specific correction of each individual neuron as microglia supply the therapeutic proteins.

Lentiviral vectors and transduction of hematopoietic stem cells:

Lentiviral vectors are an effective means of delivering the SYNGAP1 transgene into HSPC due to their ability to stably transduce both dividing and non-dividing cells (16). They also provide long-term expression of the introduced genes due to integration in chromosomal regions that are less prone to gene silencing in differentiated cells (16-20). The proven track record of lentiviral vectors in HSPC gene therapeutic approaches highlights its potential use for neurological diseases as we have successfully demonstrated for Angelman syndrome.

Immunodeficient and SYNGAP1-deficient humanized mouse model:

For translation of our research to human clinical trials, we utilize disease-specific immunodeficient mouse models where human CD34+ HSPC can be used to evaluate therapeutic efficacy. This allows us to use the exact therapeutic candidate in our preclinical and IND-enabling studies as we would use in future human clinical trials. In our current proposal, we will develop and use an immunodeficient SYNGAP1-/+ mouse model to evaluate the efficacy of the human CD34+ HSPC transduced with the SYNGAP1 lentiviral vector. We have previously been successful in developing these mouse models for both Sandhoff disease and Angelman syndrome.

PRELIMINARY DATA: As described above, a stem cell gene therapy approach for monogenic enzyme deficiencies, including those affecting the CNS, have been successful in both clinical trials and in preclinical studies. In establishing whether the same success can be developed for SYNGAP1-realted NSID, the following preliminary data is being presented.

The preliminary data presented here is from our previous work on Angelman syndrome and the lentiviral vector expression of a modified form of Ube3a in human CD34+ HSPC transplanted into an immunodeficient and Ube3a-deficient mouse model. The data presented includes significant prevention and reversal of disease phenotypes in Ube3a-deficient mice and the expression of Ube3a in the brains of mice transplanted with Ube3a vector transduced human CD34+ HSPC.

Prevention and reversal of Angelman syndrome phenotypes in Ube3a-deficient mice:

To demonstrate our ability to develop stem cell gene therapy candidates for monogenic neurological diseases, our previous work in immunodeficient and Ube3a-deficient double knockout mice for the treatment of Angelman syndrome are displayed below. A novel mouse model created by our group, an immunodeficient Ube3a-deficient double knockout mouse model was made so that we could evaluate the

in vivo efficacy of our Ube3a-expressing lentiviral vector transduced human CD34+ HSPC (6). We successfully created this mouse model and demonstrated engraftment of human CD34+ HSPC at levels of 20-65% human CD45+ in the peripheral blood as determined by flow cytometry. These levels of human cell engraftment are similar to other immunodeficient mouse models including the NOD-SCID-IL2rg-/- (NSG) and NOD-RAG1-/-IL2rg-/- (NRG) mice (21-22). We next evaluated the in vivo efficacy of the Ube3a vector transduced human CD34+ HSPC to either prevent or reverse motor and behavioral deficits observed in the mouse model. Mice were transplanted as either 2-5 day old pups to evaluate the prevention of disease phenotypes or as 8 week old mice to evaluate the reversal of disease phenotypes. Assays included open field, beam walking, rotarod, gait analysis, novel object recognition, and EEG which are specific for Angelman syndrome phenotypes.

As displayed in Figure 1, significant prevention of Angelman syndrome phenotypes were observed in immunodeficient Ube3a-deficient mice transplanted at 2-5 days old with Ube3a vector transduced human CD34+ HSPC (Ube3a-HET). This was in comparison to mice left nontransplanted (HET) or transplanted with nontransduced (NT-HET) human CD34+ HSPC.

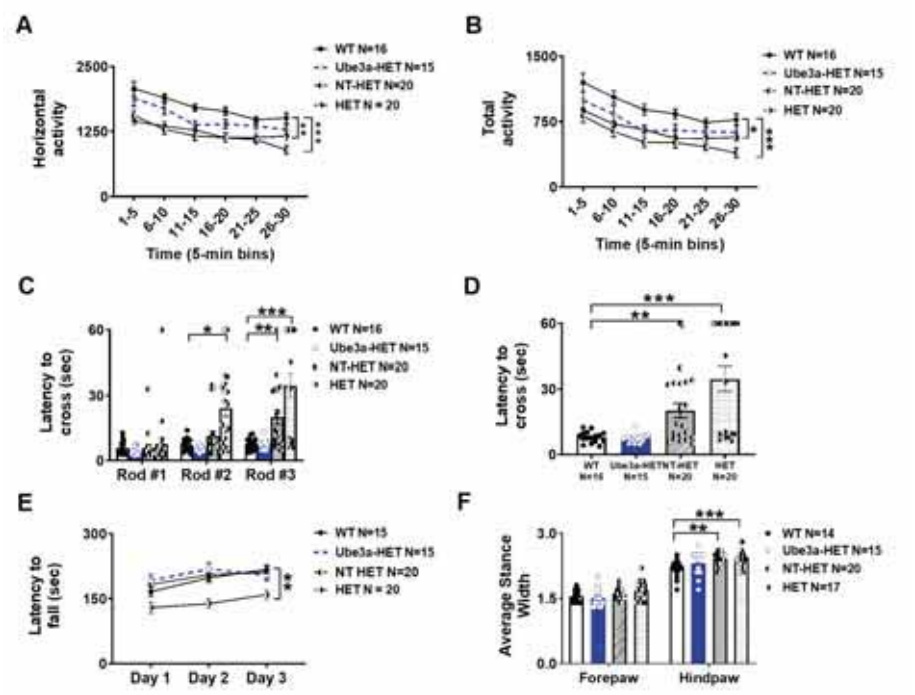

Figure 1. Locomotor ability, balance, motor coordination, and gait from Ube3a vector transduced HSPC transplanted neonates: Rigorous assessment of motor translational phenotypes using four standard motor and behavioral tests in treated and untreated Ube3a mat-/pat+ IL2rg-/y mice that were irradiated and transplanted as neonates with either nontransduced (NT-HET (open bar) or the Ube3a vector transduced (Ube3a-HET (hatched bar)) human CD34+ HSPC. Eight weeks post-transplant, mice were subjected to (A,B) open field locomotion, (C,D) balance beam, (E) rotarod, and (F) DigiGait treadmill walking. In all tests, Ube3a-deficient mice transplanted with the Ube3a vector-transduced human CD34+ HSPC (Ube3a-HET) exhibited wildtype values of performance. Data are expressed as standard error of mean. *p<0.05, ** p<0.001, *** p<0.0001.

After successfully demonstrating prevention of Angelman syndrome phenotypes in transplanted mice, we next wanted to evaluate whether the Ube3a vector transduced cells could reverse and/or halt the progression of Angelman syndrome in transplanted adult mice. As displayed in Figure 2, successful reversal of Angelman syndrome phenotypes were observed in adult immunodeficient Ube3a-deficient mice transplanted with Ube3a vector transduced human CD34+ HSPC compared to control mice.

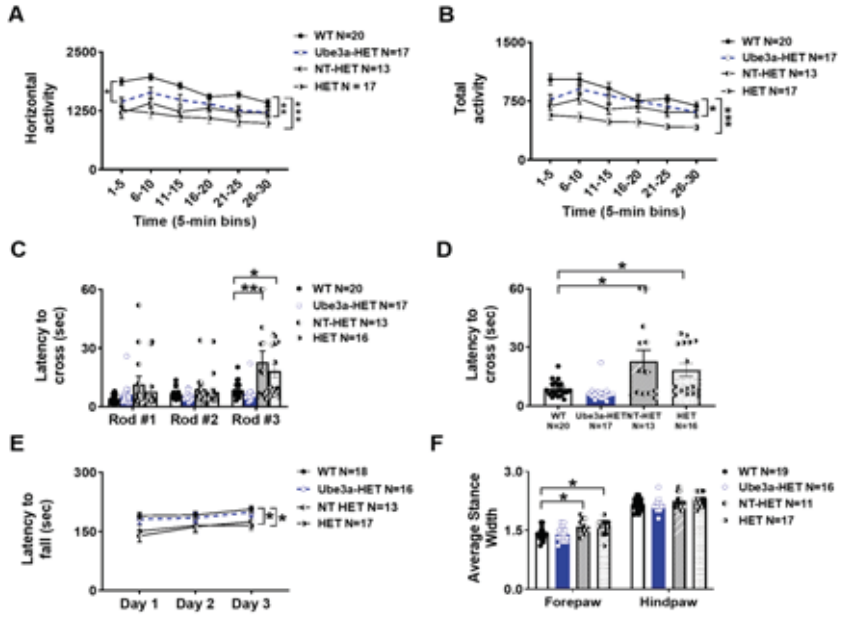

Figure 2. Locomotor ability, balance, motor coordination and gait from Ube3a lentivector transduced HSPC transplanted adults: Rigorous assessment of motor translational phenotypes using four standard motor and behavioral tests in treated and untreated (HET) Ube3a mat-/pat+ IL2rg-/y mice transplanted as adults with either nontransduced (NT-HET (open bar) or the Ube3a lentivector transduced (Ube3a-HET (hatched bar)) human CD34+ HSPC. Eight-week old mice were conditioned with busulfan and transplanted via intravenous injection. Six weeks later mice were subjected to (A,B) open field locomotion, (C,D) balance beam, (E) rotarod and (F) DigiGait treadmill walking. In all tests, Ube3a-deficient mice transplanted with the Ube3a vector-transduced human CD34+ HSPC (Ube3a-HET) exhibited wildtype values of performance. Data are expressed as standard error of mean (SEM). * p<0.05, ** p<0.001, *** p<0.0001.

Next we wanted to determine if Ube3a was being expressed in the brains of mice transplanted with the Ube3a vector transduced cells. Immunohistochemistry was performed with a Ube3a specific antibody. As displayed in Figure 3, successful detection of Ube3a was observed in the brains of Ube3a vector transduced cell transplanted mice (Ube3a-HET). This was in comparison to nontransplanted (HET) and NT cell transplanted mice (NT-HET).

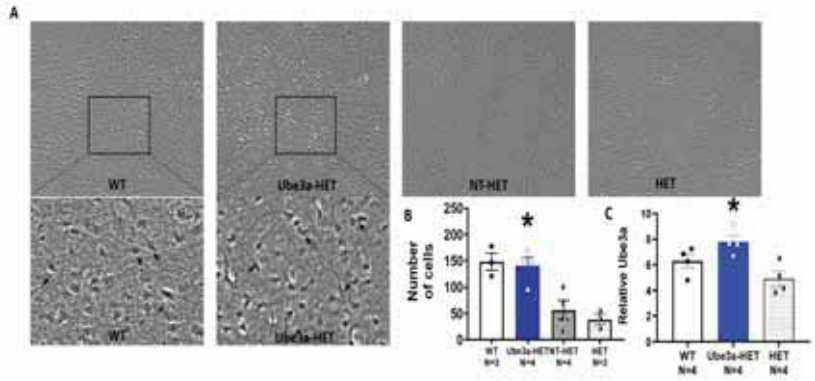

Figure 3. Immunohistochemical analysis of UBE3A expression in cortex of the mouse brain. Six weeks posttransplant, mice were assessed for UBE3A expression using DAB as a chromogen. A) IHC images showing expression of UBE3A in the mouse cortex of nontransplanted WT mice, those transplanted with Ube3a vector transduced (Ube3a-HET) human CD34+ HSPC, those transplanted with nontransduced human CD34+ HSPC (NT-HET), and non-transplanted HET mice (HET). B) A significant increase in the UBE3A positive cells, similar to the WT level, was observed in transplanted Ube3a-HET compared to NT-HET and HET. Data are expressed as standard error of mean (SEM). * p<0.05

Data pertaining to the novel object recognition and EEG experiments performed on the transplanted mice can be found in our recent publication (6).

Together, the above data demonstrated that successful correction of a monogenic neurological disease can be accomplished with a stem cell gene therapy approach. This along with the success of a similar approach for ALD, MLD, MPSI, and TS/SD in both clinical and preclinical studies highlight the potential for this strategy to be used for SYNGAP1-realted NSID.

RESEARCH PLAN:

Specific Aim 1: Develop and evaluate a SynGAP expressing lentiviral vector in human hematopoietic stem and progenitor cells and in SYNGAP1-related NSID affected B cells. Vector transduced cells will be evaluated for expression and functionality of SynGAP and for normal differentiation of the CD34+ HSPC into lineage-specific colony units and mature macrophages.

Demonstrate the expression and functionality of the SYNGAP1-expressing lentiviral vectors in SYNGAP1-affected B lymphocytes. During this Aim, we will generate the SYNGAP1-expressing lentiviral vectors and evaluate the expression and functionality in SYNGAP1-affected B lymphocytes. This will include western blots to demonstrate and measure expression in transduced cells and a GAP and/or Ras activity assay to demonstrate the functionality of the expressed SynGAP protein.

1A) Lentiviral vector production: The human SYNGAP1 self-inactivating lentiviral vectors will be generated in HEK-293T cells using a multi-plasmid transfection system and concentrated by ultrafiltration. Vector titers will be calculated based on transduction of HEK-293T cells and integrated vector measurements by quantitative PCR. The vector constructs will be designed to contain only the therapeutic expression cassette so data produced can be directly translated to future clinical work. Vectors will be produced in a GMP-compatible setting with GMP-compatible reagents. Our lab produces all of our own preclinical and clinical vectors with the same protocols so translating vector production to a clinical trial will be an easy transition.

1B) Evaluation of expression and functionality in SYNGAP1-affected B lymphocytes: To initially evaluate the levels of expression of SynGAP and its functionality post-transduction, human B lymphocytes obtained from a patient affected by SYNGAP1-related NSID will be transduced with the SYNGAP1 lentiviral vectors. Cells will be transduced at an MOI of 20 and left to culture for fourty-eight hours. Control cells including nontransduced (NT) B lymphocytes and empty vector (EV) transduced B lymphocytes will also be generated. Cellular protein extracts and culture supernatants will be collected and evaluated for SynGAP expression by western blots and compared to control cells.

Functionality of the SYNGAP1 lentiviral vectors will then be evaluated in the transduced B lymphocytes by a GAP and/or Ras activity assay which are commercially available. Protein extracts from the transduced cells are evaluated for GAP activity and/or Ras inhibition and fluorescence readouts are then compared to control cells. Together, this data will initially evaluate the expression and functionality of the SYNGAP1-expressing lentiviral vectors.

Demonstrate the expression and initial safety profile of the SYNGAP1 expressing lentiviral vectors in human CD34+ HSPC and their derived myeloid cells. To further demonstrate expression of the SYNGAP1 expressing lentiviral vectors, human CD34+ HSPC will be transduced with the vectors. These cells will be subsequently derived in vitro into mature macrophages and evaluated for SynGAP expression by western blots. During the derivation of the macrophages, colony forming units will be counted and compared to control cells. These experiments will not only demonstrate expression of SynGAP via lentiviral vector transduction but will also demonstrate initial safety of the cells as the transduced CD34+ HSPC. The proliferation and differentiation of the vector transduced cells will be determined in the CFU assay and their ability to generate mature myeloid cells.

1C) CFU assay: Due to the over expression of SynGAP in transduced cells, it is possible that adverse effects or dysfunction of the transduced HSPC could occur. Therefore, to evaluate the safety of the SYNGAP1 lentiviral vectors, an in vitro CFU assay will be performed. Human CD34+ HSPC will be transduced with the SYNGAP1 lentiviral vectors (MOI 20) and cultured in methylcellulose media for 14 days. Both CFU colony counts and colony phenotypes (BFU-E, GEMM, GM) will be evaluated and compared to control NT and EV vector transduced cells. Total cell expansion will also be calculated by counting the number of input cells compared to the number of cells obtained after 14 days of culture. ANOVA statistical analyses will be performed to determine any significant differences in results.

1D) Myeloid cell development from CFUs: Upon completion of the CFU assay, colonies will be further differentiated into mature myeloid cells by culturing in media containing granulocyte-macrophage colony stimulating factor (GM-CSF) and macrophage colony stimulating factor (M-CSF) for four days. Once mature cells have attached and developed, the phenotypic profile of these cells will be determined by flow

cytometry. Cells will be stained with antibodies specific for the myeloid lineage including CD14, HLA-DR, and CD4. SYNGAP1 lentiviral vector transduced cells will be compared to NT and EV vector transduced cells.

1E) Expression of SynGAP in vector transduced immune cells: Cellular protein extracts and culture supernatants from SYNGAP1 vector transduced human CD34+ HSPC derived macrophages will be obtained and evaluated for SynGAP expression by western blots. Expression in lentivector transduced cells will be compared to control NT and EV cells.

Specific Aim 2: Develop an immunodeficient mouse model of SYNGAP1-realted NSID (23) and evaluate the in vivo efficacy of our stem cell gene therapy strategy in these mice. Adult heterozygous mice will be transplanted with the SYNGAP1 lentiviral vector and evaluated for behavioral and motor assays including open field and rotarod as previously described (24). Expression of SynGAP will also be evaluated in the brains of transplanted mice by IHC. Homozygous neonates will be transplanted and monitored for an extension of their lifespan.

Generate an immunodeficient and SynGAP deficient mouse model for use in therapeutic development. In this Aim, we will generate an immunodeficient and SynGAP deficient mouse model by crossing the B6-IL2-/- mice with the B6-SYNGAP1-/+ mouse models (23,25). Motor and behavioral assays will be performed on the crossed mice to demonstrate that the new strain retained the clinical phenotypes of the original SYNGAP1-/+ mouse model.

2A) Mouse models and breeding strategy: In order to evaluate the in vivo efficacy of our therapeutic candidate which uses human CD34+ HSPC to express functional SynGAP, we will need to generate an immunodeficient version of the SYNGAP1 mouse model which would be capable of accepting human CD34+ HSPC for transplantation, engraftment, and multi-lineage hematopoiesis in the peripheral blood and lymphoid organs. We have successfully made disease-specific immunodeficient mouse models for both Sandhoff disease (a model for Tay-Sachs and Sandhoff disease) and Angelman syndrome (5-6). These mice were crossed with B6-IL2-/- knockout mice and the double knockout genotypes were determined by PCR according to standard protocols. The IL2-/- knockout genotype is ideal for generating immunodeficiency as displayed in some of the original humanized immunodeficient mouse models, the NSG and NRG mice (21-22). Therefore, to generate the IL2-/-SYNGAP1-/+ mice, we will cross the B6-IL2-/- and B6-SYNGAP1-/+ mice. Both strains are on the B6 background and therefore, fewer crossings will be needed to create the new model. Genotypes will be confirmed by PCR. The SYNGAP1-/+ mice are fertile and will be used for crossing and breeding since SYNGAP1-/- mice die within one week of birth.

2B) Motor and behavioral assays to confirm SYNGAP1-/+ phenotypes: To successfully generate a disease-specific immunodeficient mouse model to be used for in vivo efficacy studies, we need to make sure that after the IL2-/- cross and the eventual immunodeficiency, the disease-specific phenotypes are retained. The original SYNGAP1-/+ strain, displays phenotypes including significantly higher motor activity relative to wild type mice in an open field assay, shorter latency to fall in a rotarod assay due to grasping deficiencies, and decreased anxiety-related behavior by spending more time in open areas (24). Therefore, once genotypes have confirmed a successful cross of the SYNGAP1-/+ and IL2-/- mice, we will perform several experiments to verify that the SYNGAP1-/+ phenotypes were retained. Both males and females will be used and an N=6 (3 females and 3 males) will be included. All behavioral and motor studies will be blinded by genotype. Mice will habituate in the testing room 30 minutes prior to performing the test and testing will be performed as close to the same time of day as possible. All behavioral/motor experiments will be performed on adult mice at 16 weeks of age and again at 48 weeks of age. Data obtained from the IL2-/-SYNGAP1-/+ mice will be compared to the original SYNGAP1-/+ and IL2-/- control mice.

Open field: Exploratory locomotor activity will be measured in low lighting conditions in an open field measuring 45x45 cm, over a 30-min period. A photocell-equipped Accuscan apparatus will automatically record and analyze the behavioral parameters, including total distance traversed, horizontal activity, vertical activity, and center time. The apparatus contains eight photocells in each horizontal direction and eight photocells elevated 4.0 cm to measure vertical rearings. Locomotor parameters are automatically calculated from the software analysis of photocell beam breaks during the 30-min testing session. This open field assay will assess both the increased motor activity and the decreased anxiety-related phenotypes of the IL2-/-SYNGAP1-/+ mice through calculation of total distance traversed both horizontally

and by vertical breaks and also the amount of time spent in the center of the open field compared to the sides.

Rotarod: The ability to maintain motor coordination on a rotating cylinder will be measured with a standard Ugo Basile accelerating mouse rotarod apparatus. The cylinder is 4.0 cm in diameter and is covered with textured rubber. Mice will be confined to a section of the cylinder 8.75 cm long by plastic dividers. Each mouse will be placed on the cylinder as it gradually accelerates to 35 rpm over a 5-minute test session and measured for 4 trials. Mice which fall within 15 seconds and mice which tend to grip the textured rubber and ride the cylinder around will be given a second trial. Latency to fall off the rotating cylinder will be measured. This assay will be performed to assess the deficiency in grasping observed in the original SYNGAP1-/+ mice.

Demonstrate improved clinical phenotypes in IL2-/-SYNGAP1-/+ mice with human CD34+ HSPC transduced with the SYNGAP1 lentiviral vector. To evaluate whether our therapeutic candidate displays in vivo efficacy, we will transplant Il2-/-SYNGAP1-/+ mice with human CD34+ HSPC transduced with the SYNGAP1 lentiviral vectors. We will evaluate the transplanted mice in motor and behavioral skills and identify any recovery in phenotypes. We will also evaluate engraftment of the transplanted cells in the peripheral blood, lymphoid organs, and in the brain. Expression of SynGAP will also be evaluated in the brain by IHC.

2C) Evaluation of the in vivo efficacy of SYNGAP1 vector transduced human CD34+ HSPC in IL2-/-SYNGAP1 deficient mice: Human CD34+ HSPC will be isolated from human cord blood obtained from the UC Davis Umbilical Cord Collection Program or from a commercial source and transduced (MOI 20) with either the SYNGAP1 lentiviral vectors or with the empty control vector (EV). Eight week old adult mice (N=6 per cohort, 3 females and 3 males) will be conditioned with busulfan and transplanted with the specific cell type according to the cohort list below:

Cohort 1. Nontransplanted wild type IL2-/-SYNGAP1+/+ mice
Cohort 2. Nontransplanted mutant IL2-/-SYNGAP1-/+ mice
Cohort 3. IL2-/-SYNGAP1-/+ mice transplanted with empty vector (EV) control transduced human CD34+ HSPC
Cohort 4. IL2-/-SYNGAP1-/+ mice transplanted with SYNGAP1 lentiviral vector transduced human CD34+ HSPC.

Two months post-transplant, mice transplanted with the EV or SYNGAP1 vector transduced cells will be bled via the tail vein and analyzed for engraftment by flow cytometry for human CD45. We routinely obtain between 20-65% human CD45 engraftment in the peripheral blood of transplanted immunodeficient mice (5-6). Engrafted mice will then be used for the open field and rotarod assays. Brains will be collected from euthanized mice after completion of the open field and rotarod assays. Data will be collected and compared to control mice.

2D) Evaluation of human cell engraftment and SynGAP expression in the brains of transplanted mice: To evaluate whether SYNGAP1 vector transduced cells engrafted in the brains of transplanted mice and whether successful expression of SynGAP had occurred, immunohistochemistry will be performed on the brains of transplanted mice. As described above, separate cohorts of mice (N=6) will be transplanted with the SYNGAP1 vector transduced cells, allowed to engraft, and will be perfused once the behavior and motor assays are performed. Brain sections will be taken and probed for human myeloid cell specific markers. We will also probe for SynGAP expression in these brains to demonstrate successful expression in the brains of mice engrafted with the SYNGAP1 vector transduced cells.

2E) Evaluation of an extension of lifespan in transplanted neonates: It has been previously shown that homozygous SYNGAP1-/- mice die within the first week of life. Therefore, to evaluate whether the SYNGAP1 vector transduced cells are capable of extending their life span, 1-2 day old SYNGAP-/- mice, conditioned by irradiation from a Cesium source, will be transplanted with either empty vector control transduced human CD34+ HSPC or SYNGAP1 expressing lentiviral vector transduced human CD34+ HSPC. As we are not able to genotype the mice in time prior to the transplants, entire litters will be transplanted and genotypes will be performed at a later time depending on the survival of the mice. Transplanted neonates will be closely monitored twice a day and any mice that die within one week of birth will be collected, genotyped, and evaluated for engraftment of the human CD34+ HSPC.

TIMELINE:

Step	Goal	QUARTERS							
		1	2	3	4	5	6	7	8
AIM 1	**Demonstrate the expression and functionality of the SYNGAP1 expressing lentiviral vectors in SYNGAP1-affected B lymphocytes**								
AIM 1	**Demonstrate the expression and initial safety profile of the SYNGAP1 expressing lentiviral vectors in human CD34+ HSPC and derived myeloid cells**								
AIM 2	**Generate an immunodeficient and SYNGAP1-deficient mouse model for use in therapeutic development**								
AIM2	**Demonstrate improved clinical phenotypes in immunodeficient SYNGAP1-deficient mice transplanted with human CD34+ HSPC transduced with the SYNGAP1 lentiviral vectors**								

RESOURCES AND ENVIRONMENT:

Laboratory: The PI has his own lab space within a larger translational laboratory in the UC Davis IRC building which is 109,000 square feet. There is room for 6 people to work at the bench and to have desks. There are 7 tissue culture rooms with biosafety cabinets, one of which is designated for the PI and lentivector work. There is also a FACS Core equipped with a 5-laser Cytopeia Influx sorter, a BD FACS ARIA, and Fortessa, and a large amount of shelving for reagents. This Center contains a state of the art GLP facility and a mouse barrier facility, as well as bench space for multiple PIs and their teams.

Animal: The PI also has a dedicated animal room for the disease-specific and immunodeficient mouse colonies in the IRC vivarium equipped with a biosafety cabinet. Two technicians care for the mice, breeding and weaning, monitoring each cage twice daily, preparing antibiotic-treated water and irradiated food, and changing microisolator cages. The health of the animals is checked daily. Sublethal conditioning is done by irradiation, using the animal irradiator purchased for the Stem Cell Program, and located in that facility, across the hall from the animal suite. Health screenings for each strain are done on a regular basis.

Specific Pathogen Free Barrier Facility (SPF) Vivarium: The shower-in barrier facility consists of approximately 10,500 sf of disease-free housing for experimental animals comprised of twelve holding/ procedure rooms, and two that can be configured as holding or procedure rooms. These rooms are separated into four suites of three rooms and one suite of two rooms, supporting 15 senior investigators and multiple junior investigators. The following resources are situated within the animal facility: A tissue culture room for cell isolation, the animal irradiator for transplantation procedures, and four separate imaging/behavioral assessment rooms.

Major Equipment: The main laboratory contains 7 tissue culture hoods, 4 centrifuges, 2 ultracentrifuges, 5 inverted phase microscopes, 2 fluorescence microscopes, 2 water baths, PCR machines, two AB 7300 Real Time PCR machines, cryotome, FC500 5-color flow cytometer, ELISA plate reader, CO2 and low oxygen tissue culture incubators, high-powered dissection microscope, microcentrifuges, 6-refrigerators; 6-freezers (-20); (-80); water purifier, LN2 tanks; large and small Western blot apparatus, power supplies and transfer equipment, 2-sequencing gel apparatus and power supply; 15-gelboxes, power supplies for nucleic acids, gel dryer and hydro-tech vacuum pump, and gel reader. We share the use of 4 preparative centrifuges, 2 scintillation counters, 2 gamma counters, lyophilizers, speed vac, and autoclave. The Stem Cell Program has an Influx high speed sterile cell sorter equipped with five lasers and an automated cell deposition unit.

UC Davis IRC: The UC Davis Stem Cell Program has a strong track record in translational and clinical science including a dedicated translational mouse core and a GMP facility which is cpable of producing clinical grade lentiviral vectors and processing and manufacturing patient cell products for stem cell and gene therapeutics.

Building on the scientific assets and expertise of one of the largest life science faculties in the nation, the UCD Stem Cell Program has provided a visible intellectual home and a potent coordinating force for 150 faculty investigators who are collaborating on regenerative medicine research, organized into more than 15 disease teams. Each team is comprised of leading researchers and clinicians who are exploring the most promising approaches that can lead to the greatest advances in improving patient health. These groups are supported by an NIH-funded Clinical Translational Science Center (CTSC) and a spectrum of institutional resources such as access to a wide range of animal models including outstanding mouse facilities, an immune deficient mouse core and an NCRR-funded non-human primate facility, sophisticated analytical and imaging technologies and expertise, a 6 suite Good Manufacturing Practice facility, and an integrated academic/clinical services health system structure poised to effectively move promising innovations in regenerative medicine into clinical trials.

REFERENCES:

1) Kilinc M, et al. 2018. Species-conserved SYNGAP1 phenotypes associated with neurodevelopmental disorders. Mol Cell Neurosci. 91:140-150.

2) Holder JL Jr, et al. 2019. *SYNGAP1*-Related Intellectual Disability. In: Adam MP, et al. editors. GeneReviews® Seattle (WA): University of Washington, Seattle. 1993–2021.

3) Gamache TR, et al. 2020. Twenty Years of SynGAP Research: From Synapses to Cognition. J Neurosci. 40:1596-1605.

4) Jeyabalan N, et al. 2016. SYNGAP1: Mind the Gap. Front Cell Neurosci. 10:32.

5) Beegle J, et al. 2020. Improvement of motor and behavioral activity in Sandhoff mice transplanted with human CD34+ cells transduced with a HexA/HexB expressing lentiviral vector. J Gene Med. 22:e3205.

6) Adhikari A, et al. 2021. Functional rescue in an Angelman syndrome model following treatment with lentivector transduced hematopoietic stem cells. Hum Mol Genet. 30:1067-1083.

7) Biffi A, et al. Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. Science. 2013. 341:1233158.

8) Tucci F, et al. Update on Clinical Ex Vivo Hematopoietic Stem Cell Gene Therapy for Inherited Monogenic Diseases. Mol Ther. 2021. 29:489-504.

9) Biffi A, et al. Gene therapy for leukodystrophies. Hum Mol Genet. 2011. 20:R42-53.

10) Eichler F, et al. Hematopoietic Stem-Cell Gene Therapy for Cerebral Adrenoleukodystrophy. N Engl J Med. 2017. 377:1630-1638.

11) Cartier N, et al. Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. Science. 2009. 326:818-823.

12) Fraldi A, et al. Gene therapy for mucopolyssacharidoses: in vivo and ex vivo approaches. Ital J Pediatr. 2018. 44:130.

13) Penati R, et al. Gene therapy for lysosomal storage disorders: recent advances for metachromatic leukodystrophy and mucopolysaccaridosis I. J Inherit Metab Dis. 2017. 40: 543-554.

14) Capotondo A, et al. Brain conditioning is instrumental for successful microglia reconstitution following hematopoietic stem cell transplantation. Proc Natl Acad Sci USA. 2012. 109:15018-15023.

15) Sergijenko A, et al. Myeloid/Microglial driven autologous hematopoietic stem cell gene therapy corrects a neuronopathic lysosomal disease. Mol Ther. 2013. 21:1938-1949.

16) Naldini L, et al. Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. Proc Natl Acad Sci USA. 1996. 93:11382-11388.

17) Yam PY, et al. Design of HIV vectors for efficient gene delivery into human hematopoietic cells. Mol Ther. 2002. 5:479-484.

18) De Ravin SS, et al. Lentiviral hematopoietic stem cell gene therapy for X-linked severed combined immunodeficiency. Sci Transl Med. 2016. 8:335ra57.

19) Cavazzana M, et al. Gene therapy targeting haematopoietic stem cells for inherited diseases: progress and challenges. Nat Rev Drug Discov. 2019. 18:447-462.

20) Barclay SL, et al. Safety and efficacy of a tCD25 preselective combination anti-HIV lentiviral vector in human hematopoietic stem and progenitor cells. Stem Cells. 2014. 33:870-879.

21) Shultz LD, et al. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. J Immunol. 2005. 174:6477-6489.

22) Pearson T, et al. Non-obese diabetic-recombination activating gene-1 (NOD-Rag1 null) interleukin (IL)-2 receptor common gamma chain (IL2r gamma null) null mice: a radioresistant model for human lymphohaematopoietic engraftment. Clin Exp Immunol. 2008. 154:270-284.

23) Kim JH, et al. 2003. The Role of Synaptic GTPase-Activating Protein in Neuronal Development and Synaptic Plasticity. J Neurosci. 23:1119-1124.

24) Nakajima R, et al. 2019. Comprehensive behavioral analysis of heterozygous Syngap1 knockout mice. Neuropsychopharmacol Rep. 39:223-237.

25) Schorle H et al. 1991. Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. Nature. 352:621-624.

Biographical Sketch

Institution: University of California Davis

Name: Joseph S. Anderson, PhD, MAS

Position	Institution	Year(s)
Associate Professor	University of California Davis	2016-present
Assistant Professor	University of California Davis	2010-2016
Senior Research Associate	University of California Davis	2008-2010

Education / Training:

Institution or Location	Degree	Year(s)	Field of Study
University of California Davis	MAS	2013-2015	Clinical Research
Colorado State University	Post-doc	2005-2008	Microbiology
Colorado State University	PhD	2002-2005	Microbiology
University of Wisconsin-La Crosse	BS	1997-2000	Microbiology

1. **Major accomplishments/ honors**

2000 *ASM undergraduate research award*
Fellowship for undergraduate research
Dean's undergraduate summer research fellowship
University of Wisconsin-La Crosse Microbiology scholarship
2003 *Infectious Disease Colloquium Graduate Research Award*
2004 *Keystone Symposium on HIV Pathogenesis scholarship*
2008 *ASH 2008 Presentation Travel Award*
2009 *UC Davis Medical Student Research Symposium Presentation Award*
2011 *Academic Federation Travel Grant*
2012 *Academic Federation Innovative Development Award*
2013 *UC Davis Mentored Clinical Training Program*
2014 *Patent awarded for triple combination anti-HIV lentiviral vector*
2014 *Approved IND for Phase I clinical trial in AIDS-lymphoma patients*
2015 *UC Davis CTSC KL2 Clinical Training Award*

2. Expertise and relevant experience

The objective of this research proposal is to develop a stem cell and gene therapy approach for SYNGAP1-related NSID including the generation of an immunodeficient SynGAP1 deficient mouse model to evaluate the efficacy of the therapeutic candidate. Dr. Anderson has been in the field of stem cell gene therapy for the past 20 years and has developed numerous therapeutic lentiviral vectors for HIV, Tay-Sachs/Sandhoff disease, and Angelman syndrome and has evaluated their safety and efficacy both in vitro and in vivo in adult and pluripotent stem cells. He performed the in vivo safety/efficacy data for the current pre-selective anti-HIV lentiviral vector being used in a Phase I clinical trial for HIV-lymphoma patients. He has also developed stem cell gene therapies for both Tay-Sachs/Sandhoff disease and Angelman syndrome that are currently in the IND-enabling experiment stage. Dr. Anderson has successfully written, submitted, and received approval of NIH RAC, pre-IND, and IND applications for his HIV stem cell gene therapy work. He has written pre-IND packages for his Tay-Sachs/Sandhoff disease and Angelman syndrome therapies as well. He has the necessary knowledge and expertise to oversee the proposed experiments and to develop a therapy for SYNGAP1-related NSID. Once discovered, he also has the expertise to move the proposed therapeutic candidate through development and into pre-clinical studies and a Phase I clinical trial. The techniques and experiments being proposed in this application have already been established in Dr. Anderson's lab and his expertise and knowledge in stem cell gene therapy and what experiments are required to receive IND approval will enable a smooth transition from the pre-clinical studies to a Phase I clinical trial. He has direct interaction with the UC Davis GMP facility, our bone marrow transplant team, and with the UCD Stem Cell Program's Regulatory team for future advancements.

3. Selected peer-reviewed publications/patent applications/patents (in chronological order)

Adhikari A, Copping NA, Beegle J, Cameron DL, Deng P, O'Geen H, Segal DJ, Fink KD, Silverman JL, **Anderson JS**. 2021. Functional rescue in an Angelman syndrome model following treatment with lentivector transduced hematopoietic stem cells. Hum Mol Genet. 30:1067-1083.

Dahlenburg H, Cameron D, Yang S, Bachman A, Pollock K, Cary W, Pham M, Hendrix K, White J, Nelson H, Deng P, **Anderson JS**, Fink K, Nolta J. 2021. A novel Huntington's disease mouse model to assess the role of neuroinflammation on disease progression and to develop human cell therapies. Stem Cells Transl Med. 10:1033-1043.

Beegle J, Hendrix K, Maciel H, Nolta JA, **Anderson JS**. 2020. Improvement of motor and behavioral activity in Sandhoff mice transplanted with human CD34+ cells transduced with a HexA/HexB expressing lentiviral vector. J Gene Med. 22:e3205.

Barclay SL, Yang Y, Zhang S, Fong R, Barraza A, Nolta JA, Torbett BE, Abedi M, Bauer G, and **Anderson JS**. 2014. Safety and efficacy of a tCD25 Preselective Combination Anti-HIV Lentiviral Vector in Human Hematopoietic Stem and Progenitor Cells. Stem Cells. 33:870-879.

Kalomoiris S, Lawson J, Chen RX, Bauer G, Nolta JA, **Anderson JS**. 2012. CD25 preselective anti-HIV vectors for improved HIV gene therapy. Hum Gene Ther Methods. 23:366-375.

Walker J, McGee J, Nacey C, Bauer G, Nolta JA, **Anderson JS**. 2012. Generation of an HIV-1-resistant immune system with CD34+ hematopoietic stem cells transduced with a triple-combination anti-HIV lentiviral vector. J Virol. 86:5719-5729.

Anderson J, Walker J, Nolta JA, Bauer G. 2009. Specific transduction of HIV susceptible cells for CCR5 knockdown and resistance to HIV infection: a novel method for pre-exposure prophylaxis by intracellular immunization. J AIDS. 52:152-161.

Anderson J, Javien J, Nolta JA, Bauer G. 2009. Pre-integration HIV-1 inhibition by a combination lentiviral vector containing a chimeric TRIM5α protein, a CCR5 shRNA, and a TAR decoy. Mol Therapy. 17:2103-2114.

EXHIBIT B

BUDGET with Funding Schedule

Budget: $128,000 total for 2 years of funding which includes $38,300 in mouse and vivarium costs, $40,043 for cell culture/lentiviral vector/supply costs, and $49,657 for personnel costs.

Personnel: $49,657. These costs will include a 5% effort for Dr. Anderson (PI), 5% effort for Joehleen Archard (research associate), and 10% effort for Julie Beegle (research associate) for the two years.

Reagent costs: $40,043. These costs will include the purchase of critical reagents and supplies required to perform the experiments. These will include the cord blood units for purification of the human CD34+ HSPC, purchasing the human CD34+ HSPC from a commercial source, generation of the SYNGAP1 clones and the SYNGAP1 lentiviral vectors, western blot reagents, quantitative PCR reagents, mouse genotyping reagents, cell culture cytokines, plasticware, buffers, media, and gases, GAP and Ras activity assays, immunohistochemistry supplies, antibodies to detect engraftment of human cells in the transplanted mice.

Mouse and vivarium costs: $38,300. These costs will include the purchase and cryorecovery of the SYNGAP1-/+ mice from JAX, vivarium recovery costs for tail clippings, breeding, and weaning. Cage charges per day are $1.50/cage/day and will include the B6-IL2-/- mice, the B6-SYNGAP1-/+ mice, the newly generated B6-IL2-/-SYNGAP1-/+ mice, and the experimental mice. These mice are maintained in a strict pathogen-free environment since these strains are immunodeficient. Their import is strictly regulated and are required to arrive from a pathogen-free source.

Sponsor Payment Schedule:

	Sponsor's Contribution	University's Contribution (*)
Upon signing the Agreement, but no later than ten days following the Effective Date	**$68,000**	
On or about the first anniversary of the Effective Date:	**$60,000**	
Total:	**$128,000**	**$75,796** (*)

(*) *University provided cost-sharing in the form of federally negotiated indirect cost (currently 57%, and rising to 60% during the terms of the Agreement, as shown below) not recovered from the Sponsor:*

- 3/1/2022 through 6/30/2022 57.0%
- 7/1/2022 through 6/30/2023 59.5%
- 7/1/2023 through 2/28/2024 60.0%

AHC