

ALZHEIMER'S SYMPTOMATIC RELIEVER

**NEURON LEVEL
MECHANISM OF ACTION
EXPERIMENT**

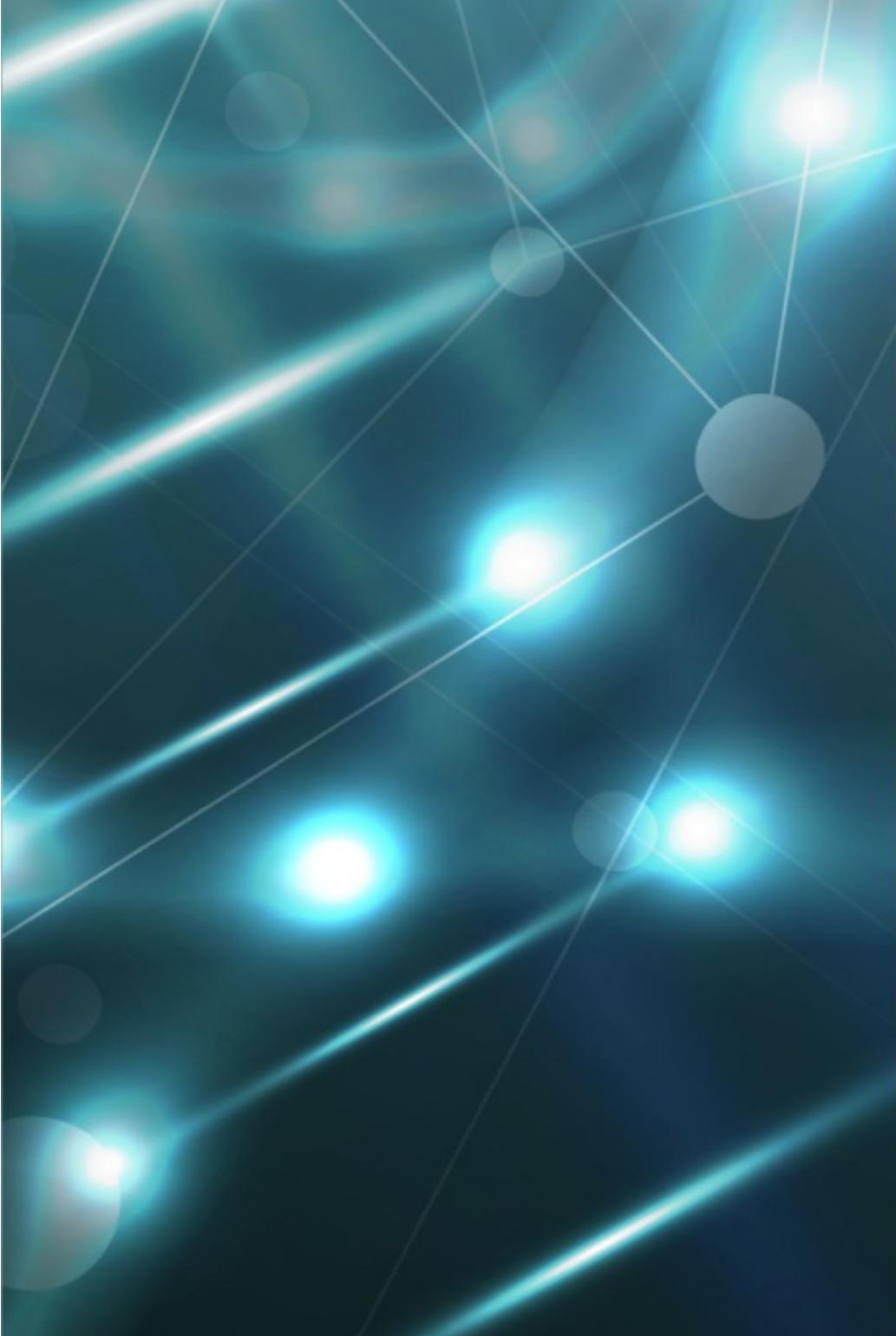
PROJECT PLAN

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ALZHEIMER'S SYMPTOMATIC RELIEVER NEURON LEVEL MECHANISM OF ACTION EXPERIMENT

PROJECT PLAN

1. SCIENCE SUMMARY

A new breakthrough can mitigate Alzheimer's symptoms by *reducing stress* and *increasing mental acuity*. The technology lowers stress by promoting *alpha frequency brainwaves*, which characterize *relaxation*. It sharpens mental acuity by increasing *theta frequency brainwaves*, which signify states of enhanced *cognitive clarity*.

This project demonstrates the neuron-level mechanism of action for a genetic Alzheimer's symptomatic reliever. In its final form with human subjects, the treatment will edit RNA to produce temporary neural changes which enhance cognitive capacity.

Once subjects become well-acclimated to their new mental capacities, and are educated in using their abilities to the greatest advantage, the edits can be committed – if they wish – to DNA and become permanent.

Although not likely to be an issue with Alzheimer's patients, the neuron edits themselves are not hereditary, and hence the treatments cannot be passed on to progeny.

Evidence: Dozens of experiments have demonstrated the efficacy of alpha and theta brainwave states to help specifically with Alzheimer's.

Treatment: Our treatment increases alpha and theta brainwave activity by physically modifying certain neurons. It will generate consistent, reproducible effects across a wide population.

Method: CRISPR is used to enhance cognition in adults by reducing the electrical excitability of a small number of certain types of neurons. This change promotes the production of lower-frequency brainwaves which are experimentally correlated with cognitive ability.

Our design affects neuron structures which have been extensively studied in 600 drug discovery experiments and are safe to modify in limited dosages. These structures are most densely expressed in a brain region experimentally correlated with distraction, inattention, and mind-wandering. Lowering neural activity in this region provides an extra boost to attention, focus and mental clarity.

Advantages: Since gene therapies can precisely target neurons, they are not absorbed by other cells in the body the way drugs are, thereby avoiding side effects. Hence, they will be the treatment of choice for patients who cannot tolerate (or would prefer to avoid) side effects.

2. PROJECT OVERVIEW

Project Name:	Alzheimer's Symptomatic Reliever Neuron Level Mechanism of Action Experiment
Sponsoring Organization:	Cognigenics, LLC
Project Team:	Dean Radin, PhD, Barry Linder, MD, John Mee
Science Advisors:	Jim Fallon, PhD, Randal Koene, PhD Garrett Yount, PhD
Project Consultants:	David Hitt, JD, John Andreadis
Contract Research Lab:	Charles River
CRISPR Fabricators	Millipore Sigma, Aldevron, Synthego, Vigene Biosciences
Start and End Dates:	Start: 1Q 2020 End: 2Q 2020
Stakeholders:	Project team, angel investors, project consultants, science advisors, vendors

Expected deliverables

- Project initiation review
- Project design review
- Project status review
- Project completion review
- Industry partner review

Success criteria

- CRISPR vendor quality verified by DNA sequencing of first edited neurons
- Edited neurons display longer pulse rise time compared to baseline neurons
- Repeatability established
- Project completion review held

Funding timeline

- Payment # 1 (50% of project fee): Upon project approval
- Payment # 2: (40% of project fee): At project midpoint
- Payment # 3: (10% of project fee): Upon project completion

Success implications

- Validates foundational science for company's AD symptomatic reliever.
- Raises company valuation.
- Strengthens negotiating position in large-scale project discussions with Merck and Celgene
- Stimulates VC interest in co-funding project with Merck Ventures

3. PROJECT PLAN

In Vitro Experiment Goal

The experiment's overall purpose is to prove CRISPR can change neuronal activity by modifying neuron excitability. Specifically, the experiment will demonstrate CRISPR can lower neuron excitability.

Strategy

1. Establish neuron pulse rise time baseline.
2. Administer CRISPR plasmids for lowering neuron excitability to neurons *in vitro*.
3. Measure increase in neuron pulse rise time.

Tactics

1. Lower neuron excitability by raising electrical resistance.
2. Raise electrical resistance by reducing receptor population.
3. Reduce receptor population by modifying DNA or RNA to make fewer receptors.
4. Receptor-of-choice has been extensively studied in drug discovery experiments and is well understood.

Step I - Project Initiation Review

1. Product business plan (PBP)
2. Master project plan (MPP)
3. Budget
4. Corporate plan
5. License agreement
6. Management contracts

Outcome: Approval to proceed and funding for Step II.

Review board: Board of directors

Presenters: D. Radin, J. Mee, B. Linder

Step II - Project Design Review

1. Review experiment design options

a) Option 1: Brain tissue sample

Description – Generate mice without the target receptor by editing zygote with CRISPR. Take brain tissue from mature mice. Test excitability of edited tissue vs. control group.

Pros – Avoid risks of individual neuron method.

Cons – Longer timeframe (for mice to mature). Somewhat higher cost.

Risks – *Developmental compensation issue*. Mice may increase expression of other receptors during brain growth stage to compensate for missing target receptor.

Plan for addressing risk: Research scientific literature and bring in neurobiology consultants. Present conclusions and recommendations at Step II review.

b) Option 2: Individual neurons

Description – Administer CRISPR to neurons in petri dish. Test excitability vs. control group.

Pros – Avoids risks of brain tissue sample method. Faster schedule. Somewhat lower cost.

Cons – More risks.

Risks –

1. *Timing of effects*. Neurons may temporarily repair receptors without accessing DNA, affecting accuracy of measurements.

Plan for addressing risk: Research scientific literature and bring in neurobiology consultants. Present results and recommendations at Step 1 review.

2. *Target receptor population* may vary from one neuron to another, complicating comparisons between edited and control group neurons.

Plan for addressing risk: Run assay on cells to measure receptor population. Present plan at Step II review.

c) Option 3: Neural network in micro array

Description – Administer CRISPR to neurons in microelectrode nanowire array. Test excitability vs. control group.

Pros – Avoids risks of brain tissue sample method. Faster schedule without KO mice. Avoids risks that neurons may temporarily repair receptors without accessing DNA, affecting accuracy of measurements, since microelectrode array testing, unlike patch-clamp systems, is non-destructive. Thus, tests can be run for several weeks.

Cons – In-house projects require greater management attention to details otherwise left to CROs.

Risks –

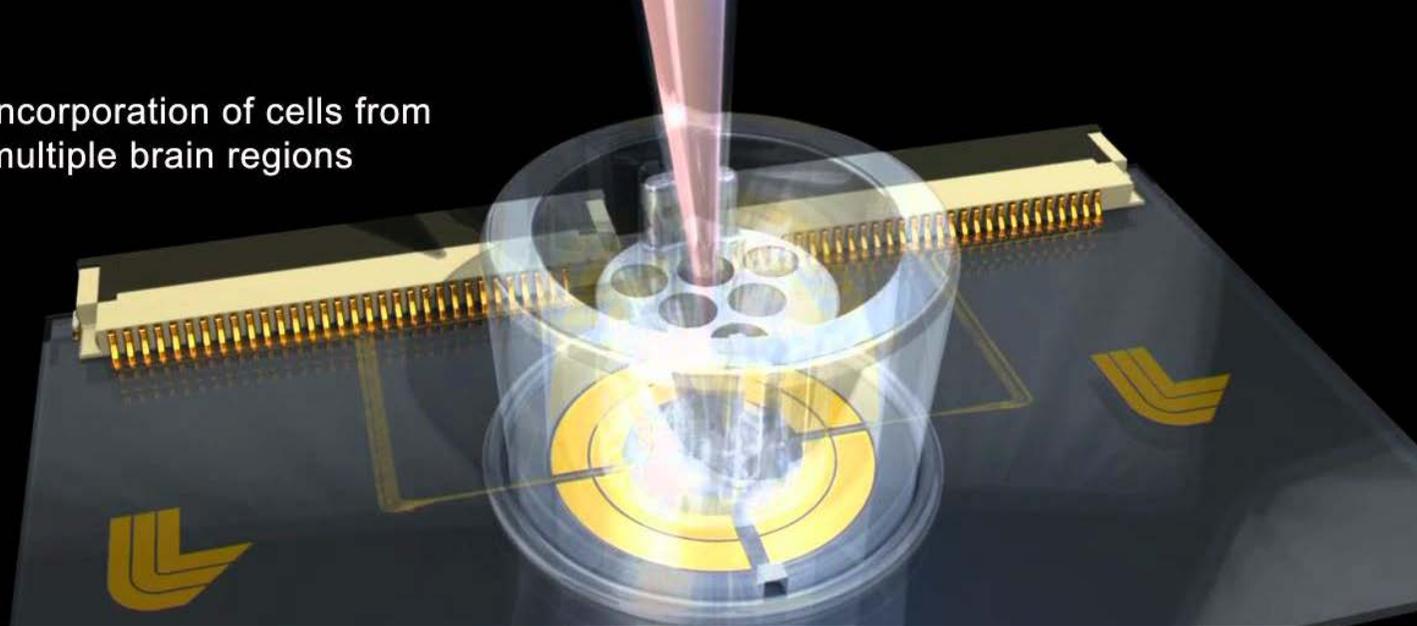
1. *Target receptor population* may vary from one neuron to another, complicating comparisons between edited and control group neurons.

Plan for addressing risk: Run assay on cells to measure receptor population. Present plan at Step II review.

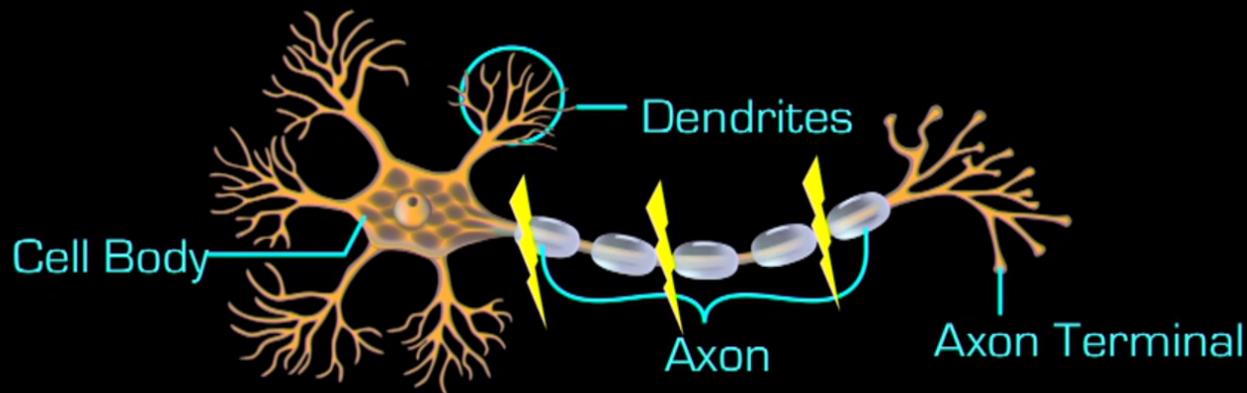
2. Choose experiment design.

Option 1, 2 or 3.

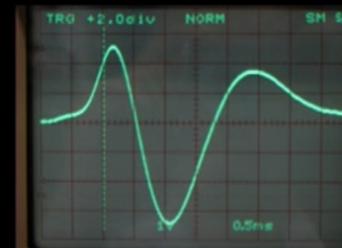
Incorporation of cells from multiple brain regions



Electrodes measure electrophysiology of neurons



Electrode side view



Extracellular recording

3. Review and approve corresponding CRO contract which will include Experiment Project Specification (EPS) for chosen design.
 - How the two groups of mice are obtained & housed
 - Method used for obtaining the acute brain slices
 - Measurement methods (e.g. patch-clamp, local field potentials)
 - Measurement durations (e.g. recording 5 mins, 1 hour, 2 days, 3 weeks)
 - Measurement sampling frequency (e.g. 30kHz)
 - Are multiple cells recorded simultaneously?
 - Neuron types recorded (e.g., pyramidal cells, interneurons)
4. Review CRISPR fabrication options.
 - a) Knockout
 - b) Knockdown
5. Choose CRISPR fabrication design.
6. Review and approve corresponding CMO contract which will include Engineering Product Specification (EPS) for chosen design.
7. Discuss project management CRO monitoring and quality control plans.
8. Review project timetable.

Outcome: Approval to proceed and funding for Step III.

Review board: Scientific advisors and business consultants

Presenters: D. Radin, J. Mee, R. Koene

Notes: This plan recites the issues we know about today. Other unknown problems will surface as the program moves ahead. There are unanswered questions, measurement parameters to determine, experts to consult, research papers to review. The goal is to eliminate all the potential mistakes, variables and tangents, but there is never a way to identify and resolve all the risks and problems in an engineering program before it begins. The only way to do this is to tackle the project with a competent team and work through the unknowns as they arise.

The challenge is to run an experiment which precisely measures the conditions we want to measure rather than some other set of conditions. The accuracy of the conditions we think we are measuring can go sideways in several different ways.

In the neuron case, the edited neurons could temporarily repair the receptors out of recycled proteins, which means the effects of the editing could show up in the cell after we take the measurements. Alternately, the neurons we receive could lack 5-HT_{2A} receptors altogether, in which case the editing makes no difference.

In the KO mouse case, the mouse might build more other receptors to make up for the deficit in serotonin 5-HT_{2A} receptors. Or the lack of 5-HT_{2A} receptors could affect its brain development in some other way that throws off our measurements.

In either case, the Crispr biologics themselves may be defective. Or part of the batch may be bad and we certify the good part and then test with the bad part. If the Crispr has off-target effects, it could change other properties in the cell which affect the measurements. And there could be other potential problems we cannot presently foresee.

The mechanism of action we are testing is firmly grounded in the principles of physics and neuron electrodynamics. There is really no question about what is going to happen to pulse rise time when a neuron's resistance is increased. Ohm's Law dictates increased resistance lowers current. A reduced current takes more time to fill up the neuron's electron reservoir to its threshold value for releasing a pulse. Debate over these facts ceased in the last century.

The only challenge is getting laboratory conditions which reflect what we actually want to measure. We are using CROs to slash cost, but we must bear in mind that CROs usually do not work on the leading edge. Most of their work is repetitive drug discovery experiments which are similar to projects they have done before. And although Charles River has done 3500 mouse knockout experiments for drug discovery projects, this project is different. We can leverage their formidable scientific skills and knowledge, but we are going into unexplored territory. We have to be vigilant and alert for potential curve balls at every step of the way. Only the first explorers can qualify for patents, but new pathways can be full of surprises. Good engineering teams have overcome these kinds of challenges time and again as long as they are astutely managed and properly motivated.

Step III - Project Status Review

Visit Lab.

CRO presents how they are conducting the experiment per the Experiment Project Specifications to minimize the risks identified in Step II and achieve positive results. Review project schedule and deliverables timetable.

Outcome: Approval to continue project.

Review board: Board of directors

Presenters: CRO

Step IV - Project Completion Review

1. Experiment results
2. Evaluation of results
3. Conclusions
4. Recommendations
5. Phase II In Vivo project plan
6. Teambuilding discussion

Outcome: Decision to share results with Merck and select individuals.

Review board: Board of directors

Presenters: D. Radin, J. Mee, B. Linder

Step V - Industry Partner Review

1. Experiment results
2. Evaluation of results
3. Phase II In Vivo project plan and budget
4. Business discussion

Outcome: Decision to collaborate.

Review board: Company chairman, Thomas Ehmer (Merck), Ulrich Betz (Merck)

Presenters: D. Radin, J. Mee, B. Linder, J. Andreadis

4. PROJECT TIMETABLE	DATE	RESULT
Step I – Project Initiation		
Prepare business and project plans and budgets, licenses and contracts		
Hold Step I review	Month 1	Decision to initiate project
Step II – Project Design		
Begin project team meetings		Track action items, resolve issues, coordinate team
Develop experiment design specification options, pros/cons, and risks		
CRO contacts ready		
Develop quality control plans		
Hold Step II review	Month 2	Experiment design selection
Step III – Project Status		
Sign CRISPR and CRO vendor contracts		CRISPR delivered to lab
Prepare lab and begin experiment		
Begin experiment		
Run 5-HT _{2A} assay on neurons		Confirm receptor population.
Edit a test neuron with delivered CRISPR		
Sequence edited neuron's DNA		Verify correct gene clean edit
Hold Step III review	Month 4	Decision to continue
Step IV – Project Completion		
Measure unedited neuron pulse rise time		Establish baseline
Measure edited neuron pulse rise time		Longer rise time
Evaluate initial results		Lowered excitability
Repeat experiment		
Evaluate secondary results		Repeatability
Prepare presentations		
Hold Step IV review	Month 5	Decision to announce privately
Step V – Industry Partner		
Prepare presentations		
Hold Step V review	Month 6	Decision to collaborate

5. PROJECT TEAM

The core team comprises executives who have exclusive knowledge and expertise in the breakthrough science of genetic cognitive engineering.

Dean Radin, Ph.D. is Chief Scientist at the Institute of Noetic Sciences (IONS) and Associated Distinguished Professor at the California Institute of Integral Studies. He earned an MS in electrical engineering and a PhD in psychology from the University of Illinois, Urbana-Champaign. Before joining IONS in 2001, he held appointments at AT&T Bell Labs, Princeton University, University of Edinburgh, and SRI International. He is author or co-author of hundreds of technical and popular articles, four dozen book chapters, and four popular books: *The Conscious Universe* (1997), *Entangled Minds* (2006), *Supernormal* (2013), and *Real Magic* (2018). Dean is also co-inventor of genetic neuropsychology, a new branch of science for improving behavior and cognition by genetically optimizing brain functioning.

Barry J. Linder, MD is a seasoned medical technology executive, physician business leader, and entrepreneur with over 30 years of extensive healthcare experience. Skilled in developing innovative, next generation medical devices and information systems to address unmet needs in healthcare. Barry has been awarded ten US patents. He has extensive management and operational experience in executive leadership positions in privately financed growth and commercial stage medical device companies, as well as senior positions across multiple divisions within a large, integrated, healthcare delivery network

John Mee holds multiple patents pending on genetic engineering methods for improving human cognition. The technology these patents introduce is grounded in Mr. Mee's deep and pragmatic understanding of engineering. One of the architects of the Information Age, John Mee directed R&D programs involving thousands of engineers which produced new computer systems hardware designs. He is the father of a mainframe, having managed the engineering development of an advanced-technology large computer at Honeywell Information Systems (IBM's top competitor in the 20th Century). Combining his electrical engineering acumen with a lifelong interest in meditation, he discovered genetic engineering methods for optimizing brainwaves to enhance human cognition after four decades of research.

Randal Koene, Ph.D. earned his doctorate in Computational Neuroscience at the Department of Psychology at McGill University, and his M.Sc. in Electrical Engineering at Delft University of Technology. He has served as Director of the Department of Neuroengineering at the Fatronik-Tecnalia Institute in Spain, the third largest private research organization in Europe, as well as Professor at the Center for Memory and Brain of Boston University, and lead scientist at Kernel. He is also co-founder of the Neural Engineering Corporation of Massachusetts.

Job Descriptions

Research Director (D. Radin)

Review and approve project plans and budgets

Review and approve vendor selections and contracts

Management decisions and direction

(a) project initiation

(b) project design

(c) project status

(d) project completion

Investor relationship management and communications

Academic liaison

Review and approve project external communications

Hire scientific advisors and review board members

Merck KgAA liaison

Team building

Co-ordinate with project manager to identify any IP opportunities which may arise in the course of designing and conducting the experiment and help prepare patent applications to capitalize on them.

Project Manager (J. Mee)

Planning

Define project, experiments and metrics. Prepare project plans and budgets.

Management

Hold weekly project team meetings to coordinate project. Track and follow up action items.

Conduct management reviews.

project initiation / project design / project status / project completion

Prepare presentations for all team meetings and project reviews.

Manage vendor relationships and contracts.

Monitor experiment closely and make any necessary course corrections.

IP

Monitor project closely to identify any IP opportunities which may arise in the course of designing and conducting the experiment.

Prepare and file patent applications to capitalize on identified opportunities.

Stay up to date on the latest CRISPR industry and technology trends.

Prosecute patents pending.

Post-experiment

Evaluate results.

Prepare and give business presentations reporting project results.

Prepare news release (private).

Phase II planning

Prepare phase II in vivo experiment project plans and budgets during phase I to accelerate phase II project initiation.

Prepare phase II presentations for investors and Merck.

Line up CRISPR vendors, CRO and IRB board for animal experiment

Prepare animal IRB board presentation.

Chief Medical Officer (Barry J. Linder)

Phase I

- Interface with regulatory, quality, and clinical program company experts
- Formation and management of Scientific Advisory Board (SAB)
- Coordination of SAB meetings
- Ensure regulatory strategy is understood in collaboration with regulatory expert
- Support management in corporate strategy development
- Participate in investor meetings (seed, equity, debt or strategic investors)
- Assist with business development activities

Phase II planning

- Line up IRB board for animal experiment
- Prepare animal IRB board presentation

Business Advisor (John Andreadis)

- Assist in business strategy and planning
- Chair review boards
- Assist with investor relationship management and communications
- Merck US liaison
- Team building

Biologics Manufacturer (Sigma Aldrich)

- Provide high-quality research grade CRISPR plasmids for experiment
- Present at weekly project team meetings
- Present at management reviews

Research Laboratory (Charles River)

- Prepare laboratory experiment plan
- Receive biologics
- Conduct experiments
- Present at weekly project team meetings
- Present at management reviews

BUDGET SUMMARY (\$K)		
	Area	Cost
1	Staff	\$112
2	Manufacturing	\$9
3	Experiments	\$70
4	Legal and Professional	\$21
5	Board	\$0
6	Overheads	\$10
7	Capital	\$2
8	Marketing	\$0
9	Office	\$6
	Project Total	\$ 230



Contact

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SUPPLEMENTARY INFORMATION

Alzheimer's symptomatic reliever gene therapy Long range project timetable

Phase	Title	Description	Schedule
Preclinical	In vitro experiments	Demonstrate the treatment's neuron-level mechanism of action by modifying neuron excitability and activity with CRISPR.	2020
	In vivo experiments	Establish the therapy's efficacy in mammals by using behavioral tests for measuring cognitive ability in laboratory animals receiving the treatment.	2021
Phase 1	Safety	Determine safety and dosage in 20-50 healthy adult volunteers for an RNA version of the therapy with temporary effects. Monitor subjects to learn more about how the therapy works in the body and the effects associated with increased dosage. Gain early information about efficacy and how best to administer the treatment to limit risks and maximize benefits.	2022
Phase 2	Efficacy	Measure the RNA therapy's efficacy in relieving Alzheimer's symptoms in a group of several hundred patients at the early and moderate stages of the disease. Closely monitor subjects to identify any side effects.	2023
Phase 3	Efficacy and adverse reactions	Measure the RNA therapy's efficacy in relieving Alzheimer's symptoms in a group of 300 to 3000 patients at the early and moderate stages of the disease. Closely monitor subjects to identify any side effects.	2024

Alzheimer's symptomatic reliever gene therapy Tools Roadmap					
Stage	Edit Type	Target	Current Tools	Effect	Remarks
Preclinical	Gene knockout	DNA	CRISPR Cas9	Permanent	Lowest cost way to demonstrate mechanism of action
Preclinical	RNA interference	RNA	CRISPR Cas13	Temporary	
Preclinical	Gene silencing	DNA	CRISPR dCas9	Permanent / Reversible	
Human	RNA interference	RNA	CRISPR Cas13	Temporary	Safest method for first human trials
Human	Gene silencing	DNA	CRISPR dCas9	Permanent / Reversible	Final product

Note: This roadmap mentions specific CRISPR tools for purposes of illustration. The field is moving so fast that by the time preclinical studies are complete and human trials begin, there will be a different set of tools than the ones we have today.

Reference Documents

No.	Title	Format
1	<i>Genetic Neuropsychology Science Summary</i>	Slide deck
2	Alzheimer's symptomatic reliever neuron level mechanism of action experiment project budget	Spreadsheet
3	<i>Method for sustainable human cognitive enhancement</i>	Patent pending
	<i>Adjustable method for sustainable human cognitive enhancement</i>	Patent pending
	<i>Reversible method for sustainable human cognitive enhancement</i>	Patent pending
3	<i>Method for treating neurological conditions and improving human cognition</i>	Patent pending
4	<i>A New Approach for Treating Alzheimer's Symptoms</i>	Slide deck
5	<i>Relieving Alzheimer's Symptoms: Proof-of-Concept Experiments</i>	PDF