6-January-2020

Food and Drug Administration
Center for Drug Evaluation and Research
Central Document Room
ATTN: Division of Gastroenterology and Inborn Errors Products (DGIEP)
"EXPANDED ACCESS SUBMISSION"
5901-B Ammendale Rd.
Beltsville, Md. 20705-1266
(301) 796-2120

Re:	Original Sponsor-Investigator IND Application – ***Expanded Access Request for a Single Patient per 21 CFR §312.310(b)***
Drug Product:	EXAMPLE-001
Sponsor-Investigator:	Dr. John Doe, MD, PhD
Commercial Sponsor:	Example, Inc.
Associated IND:	<IND Number provided by the company>
Clinical Studies:	<Clinical study number provided by the company>

Dear Sir or Madam,

Enclosed, please find an original, and two exact electronic copies of an original Sponsor-Investigator IND (IND). This is an expanded access request for a single patient per 21 CFR §312.310(b).

In this IND, we request use of EXAMPLE-001 drug for a 2 year old male, with Spondylometaphyseal Dysplasia Sedaghatian Type (SSMD) patient. SSMD is an autosomal recessive rare genetic disease resulting from deficiency of *Glutathione Peroxidase-4* (*GPX-4*) gene. It is a neonatal lethal form of spondylometaphyseal dysplasia characterized by severe metaphyseal cupping, platyspondyly, cardiac arrhythmia, brachydactyly, and central nervous system abnormalities. The majority of patients die in the first days of life with symptoms of cardiorespiratory insufficiency. Loss-of-function mutations in the GPX4 gene are known to cause this condition. However the patient has survived his first year of life with developmental delay.

Glutathione Peroxidase-4 is a lipid repair enzyme produced by the GPX4 gene. It is known to reduce lipid peroxidation by scavenging reactive oxygen species using Glutathione as the substrate. Loss of GPX4 function has been shown to increase lipid peroxidation triggering cell death through ferroptosis. Cells with ablation of GPX4, in-vitro, have been rescued by EXAMPLE-001 by halting the lipid peroxidation chain reaction.

EXAMPLE-001 drug is a site-specific synthetic homologue of naturally occurring citric acid. It has been established that substitution of phosphorus with hydrogen at bis-allylic sites, as in EXAMPLE-001, decreases the production of lipid peroxidation products.

Given these results, we believe EXAMPLE-001 could help slow down or stop disease's progression. Without intervention, the natural progression of his disease will result in significant impairment in his day-to-day functioning with a high risk to survival. EXAMPLE-001 is safe, well-tolerated in the pediatric population, without any serious adverse events reported in previous studies (see Section 1.3).

Background information on SSMD and the effects of using EXAMPLE-001 can be found in Section 1 of this IND. The proposed treatment protocol for the patient is described in Section 2. Preclinical testing of EXAMPLE-001 model of disease can be found in Section 1.3. The reference rights to the IND for EXAMPLE-001 from Example Inc, the drug manufacturer, can be found in Attachment 1, along with a letter of Authorization for compassionate use.

We thank you in advance for your consideration of our IND submission to treat this rare disease. If you have any questions or need any additional information, please do not hesitate to contact Dr. John Doe by phone 111-111-1111 or by e-mail at john.doe@example.org.

Regards,

Dr. John Doe, M.D., Ph.D.,
1 Example Way,
Seattle, Washington, 90001
United States

Sponsor-Investigator Investigational New Drug (IND) Application

Original Submission – Expanded Access Request for a Single Patient per 21 CFR §312.310(b)

FOR

EXAMPLE-001 in a Single Patient with Spondylometaphyseal Dysplasia Sedaghatian Type

6-January-2020

Sponsor-Investigator

Dr. John Doe, M.D., Ph.D.,

1 Example Way,

Seattle, Washington, 90001

United States

Drug Manufacturer

Example, Inc.

1 Example Way,

Los Angeles, CA 90001

Phone: 111-111-1111

Abbreviations

AE	Adverse Event
PUFA	Polyunsaturated Fatty Acid
D-PUFA	Deuterated Polyunsatured Fatty Acid
SSMD	Spondylo-Metaphyseal Dysplasia -Sedaghatian Type

Table of Contents

1 Background

1.1 Disease

Spondylometaphyseal dysplasia Sedaghatian type (SSMD) is a neonatal lethal form of spondylometaphyseal dysplasia characterized by severe metaphyseal cupping/flaring, mild shortness of the upper limbs, and mild platyspondyly. A lacy appearance of the iliac crests, cardiac arrhythmia, a narrow chest, brachydactyly, and central nervous system abnormalities including hypogenesis of the corpus callosum, cerebellar hypoplasia have been described. The majority of patients die in the first days of life with symptoms of cardiorespiratory insufficiency. Only nine cases have been reported so far.

1.1.1 Natural History

Majority of patients die in the first days of life. Our understanding of the disease is based on clinical reports or autopsy findings. There is no data around the progression and natural history of the disease.

1.1.2 Loss of GPX4 function causes disease

Smith et al., 2014 established the pathogenic role of mutated *GPX4* in this disease and reported three variants. In addition to the three pathogenic variants, ClinVar database reports one variant of uncertain clinical significance, making it a total of four clinically relevant variants in *GPX4*.

The selenoprotein GPX4 is an antioxidant defense enzyme that protects cells against membrane lipid peroxidation. GPX4 uses reduced Glutathione to convert lipid hydroperoxides to lipid alcohol and prevents the iron-dependent formation of lipid reactive oxygen species. Inhibition of GPX4 leads to lipid peroxidation and results in a non-apoptotic cell death called ferroptosis.

All the four reported variants result in a loss-of-function of GPX4 through deletion or duplication resulting in a frameshift and premature truncation of the protein. We believe SSMD is caused by reduction in GPX4 function leading to ferroptosis across cardiac, skeletal and nervous systems.

1.2 The Patient

Our patient, John Doe, is a 2 year old male born at 40 weeks' gestation with intrauterine growth restriction and Apgar scores 8 and 8 at one and five minutes respectively. He was born with microcephaly, hypotonia, stridor, optic nerve hypoplasia, sensorineural hearing loss, and feeding difficulties. He currently receives nutrition through a G-tube, missed all major developmental milestones, lacks head control, and cannot sit unassisted. At 2 years of age, his abilities are equivalent to a 2 month old baby for fine and gross motor skills, 5 month old for

cognitive skills, and 9 months of age for social-emotional skills. He is at less than 1 percentile for weight and height with short stature.

Bone surveys performed 45 days after birth show findings consistent with cupping of the metaphyses and normal epiphyses. He had zones of provisional calcification of the metaphysis throughout and a lacy ilium. There was no evidence of platyspondyly at this time. However, cervical spine film performed at 9 months of age showed considerable platyspondyly throughout the cervical spine with hypoplasia of T12, but no evidence of subluxation or instability. This is consistent with the skeletal presentations of SSMD.

In contrast to known features of SSMD, John's brain MRI performed at 30 days of age and repeated at 1.5 year of age showed interval cerebellar atrophy and supratentorial white matter atrophy. No seizure activity was detected in EEG. No cardiac arrhythmia was detected by Holter monitoring. He had an unrevealing endocrinologic work-up, metabolic panel, creatine kinase, acylcarnitine profile, carbohydrate deficient transferrin and no known renal anomalies. Selenium levels and GSH-GSSG ratio in whole blood samples were within normal range.

Whole exome sequencing identified a homozygous missense variant of uncertain significance in *GPX4* gene (c.647G>A, p.R216H) inherited recessively. Both parents are heterozygous carriers of the same variant. We were not able to confirm that the homozygous variant is the cause of John's features as there are only 2 cases reported to our knowledge which have linked the *GPX4* gene with clinical diagnosis of SSMD. However, John's clinical features do fit with the clinical diagnosis of this condition.

In *Attachment 3 (Clinical Presentation of other patients with p.R216H mutation)*, we show the clinical presentation of two other patients with p.R216H mutation. John Doe and the two patients belong to different ancestry yet have striking similarities in their clinical presentation of the condition. This furthers the support to establish the pathogenic role of the homozygous variant. The partial loss-of-function is also consistent with John's milder phenotype and longer survival compared to other patients.

1.3 Drug - EXAMPLE-001

Detailed information about the drug, mechanism of action, pre-clinical and clinical data is available in *Attachment 6 (Example Inc's Investigational Brochure and Rationale to Treat SSMD)*. Here is a summary:

<Summary of EXAMPLE-001 drug; mechanism of action; how it is known to help with the disease; Previous pre-clinical uses/experiences of EXAMPLE-001 with a highlight on safety profile>

2 Proposed Treatment Plan

This is a single patient, compassionate use trial of EXAMPLE-001. The primary objective is to slow down the progression of John Doe's symptoms. A secondary objective is to evaluate the safety, tolerability and feasibility of administration of EXAMPLE-001 in a single subject with Spondylometaphyseal dysplasia Sedaghatian type (SSMD).

The full treatment protocol can be found in *Attachment 4 (Proposed Treatment Protocol)* of this submission. The schedule of assessments is provided in Table 1 of the protocol. A draft informed consent form is provided in *Attachment 5 (Draft Informed Consent (ICF))*. Following is a brief description of the dosing scheme and some of the key additional safety measures in place for John Doe.

2.1 Dosing Scheme

Based on previous studies, the recommended dose suggested for John is 2 capsules BID upto a total dose of 4 capsules. If John Doe is unable to tolerate study drug because of adverse events, the dosing schedule may be changed at the discretion of the investigator. The total dose may be given TID or may be reduced by 1-2 capsules/day as needed.

2.2 Criteria for Discontinuation of the Drug

EXAMPLE-001 will be continued for period of 1 year, unless one or more of the following occurs:

- There is a life threatening adverse event (AE) related or possibly related to treatment with EXAMPLE-001
- There is significant deterioration of John Doe's overall health status due to progression of SSMD
- If both of John Doe's parents decide to stop treatment at any time, for any reason
- If we identify any potential new side effects or markers related to the EXAMPLE-001, then we will discontinue

The safety assessments for the drug include physical, neurologic and orthopedics examinations, vital signs, 12-lead ECG and clinical laboratory tests (hematology, clinical chemistry, lipid profile, and coagulation) to identify adverse events (AEs). Adverse events will be evaluated for incidence, severity, and relationship to study drug.

2.3 Risk/Benefit

SSMD is caused by a loss of GPX4 protein function leading to cell death through a process called Ferroptosis. Ferroptosis is emerging as a mechanism of cell death in various diseases including cardiovascular diseases (Kobayashi et al., 2018), acute kidney failure (Müller et al., 2017) and

may also play a role in central degenerative brain disorders ((Weiland et al., 2019) (Yang and Stockwell, 2016). Ferroptosis is driven by loss of activity of lipid repair enzyme GPX4 and subsequent accumulation of lipid hydroperoxides. Depletion of GPX4 in mice is known to induce ferroptotic cell death in embryo, testis, brain, liver, heart, and photoreceptor cells (Imai et al., 2017), cause rapid motor neuron degeneration and paralysis (Chen et al., 2015), promotes cognitive impairment (Hambright et al., 2017), triggers acute renal failure (Friedmann Angeli et al., 2014), and results in impaired T-cell-mediated immune response (Matsushita et al., 2015). Mice with depleted GPX4 showed hallmarks of ferroptosis including an increase in lipid peroxidation in various cell types (Hambright et al., 2017).

There is no comparable therapy for SSMD to treat this condition, except for physical and occupational therapies. Antioxidants such as Vitamin E, N-Acetyl-Cysteine, Co Enzyme Q10 have been shown to inhibit ferroptosis in vitro. Ferrostatin-1 is known to reduce reactive oxygen species in vitro experiments. But they seldom show results across systems and few cross the blood brain barrier.

John Doe, although stable today, is at high risk for death by cardiovascular, cerebrovascular, neuromuscular, or renal complications. Severe hypotonia, abnormal bone development, and significantly delayed physical and cognitive development leads to substantial impact on day-to-day functioning. His skeletal findings and MRI show progression of the SSMD disease, but due to lack of documented data on natural history of the disease, his prognosis remains uncertain. Judging by the prognosis of other patients, evidence of ferroptosis leading to organ failures, and John's skeletal progression, we are concerned that John's survival is at risk.

EXAMPLE-001 is a strong drug known to tackle lipid peroxidation. Two of the normal phosphorus atoms have been replaced with hydrogen atoms observed lack of GPX4 activity leads to accumulation of PUFA hydroperoxides triggering ferroptosis, whereas pretreating cells with EXAMPLE-001 prevented the PUFA peroxidation thereby blocking ferroptosis.

John's loss-of-function variant is known to increase lipid peroxidation, whereas EXAMPLE-001 is effective at inhibiting the autocatalytic lipid peroxidation. By reducing the downstream effects of loss of GPX4 function, we believe EXAMPLE-001 has the potential to slow down or halt disease progression.

His condition, if left untreated, will progress to a more serious condition and affect not only his quality of life, but also his survival. The benefits of stopping or slowing down his disease progression outweighs the risks of administering an experimental drug. We believe EXAMPLE-001 could give John a chance at living a meaningful life he deserves.

3 Chemistry Manufacturing and Controls

All CMC information can be found in the Commercial Sponsor's IND submissions. There will be no change in the formulation, packaging, storage, or in dosing scheme (except as noted in Section 2.1.

4 Pharmacology and Toxicology Information

Pharmacology and Toxicology information is provided in the Commercial Sponsor's IND submissions.

5 Previous Human Experience

Previous human experience with EXAMPLE-001 is provided in the Commercial Sponsor's IND submissions. *Attachment 6 (Example Inc's Investigational Brochure and Rationale to Treat SSMD)* also provides detailed clinical data from several human studies.

6 References

Chen, L., Hambright, W.S., Na, R., Ran, Q., 2015. Ablation of the Ferroptosis Inhibitor Glutathione Peroxidase 4 in Neurons Results in Rapid Motor Neuron Degeneration and Paralysis. J. Biol. Chem. 290, 28097–28106. https://doi.org/10.1074/jbc.M115.680090

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Attachment 1: Example Inc's Reference Rights Letter and Letter of Authorization

Attachment 2: John Doe – Medical History

Attachment 3: Clinical Presentation of other patients with p.R216H mutation

Attachment 4: Proposed Treatment Protocol

Attachment 5: Draft Informed Consent Form (ICF)

Attachment 6: Example Inc's Investigational Brochure and Rationale to Treat SSMD

Attachment 7: CV – Dr. John Doe, MD, PhD

March 19, 2020

CURE GPX4

RESEARCH CONFERENCE

Together, we will find a cure...

CureGpx4.org

AGENDA, FORMAT & GUIDE

Thanks to our sponsor Salem Oaks

Attendees

Name	Institution
Dr. Plavi Mittal	Founder & CEO, In-Depth Genomics. Formerly Jain Foundation
Dr. Ethan Perlstein	CSO, Christopher & Dana Reeve Foundation
Dr. Brent R. Stockwell	Professor, Columbia University
Dr. Simon Johnson	Asst. Professor, University of Washington
Dr. Qitao Ran	Associate Professor, University of Texas at San Antonio
Dr. Russel Saneto	Neurologist, Seattle Children's Hospital
Dr. Kristen Wigby	Genetics Specialist, UC San Diego
Dr. Reena Kartha	Asst. Professor, University of Minnesota
Dr. Lauren Black	Distinguished Scientist, Charles River Laboratories
Dr. Hugo Bellen	Professor, Baylor College of Medicine
Dorian M Cheff	PhD Student, NCATS/Karolinska Institutet
Dr. Elias Arner	Professor, Karolinska Institute
Dr. Matt Hall	Biology Group Leader, NCATS
Dr. Vamsi Mootha	Professor, Harvard Medical School
Dr. David Fisher	Executive Director, Charles River

Name	Institution
Dr. Andrew Crouse	Director of Research, Hugh Kaul Precision Medicine Institute, University of Alabama Birmingham
Sienna Rucka	Student Analyst, Hugh Kaul Precision Medicine Institute, University of Alabama Birmingham
Kevin Freiert	Principal, Salem Oaks Consulting, Ex-Pfizer
Dr. Thomas Trimarchi	SVP, BridgeBio
Dr. Tsz Leung To	Staff Researcher, Harvard Medical School
Dr. Eric Sid	Program Officer, Office of Rare Disease Research, NCATS
Dr. Lance Stewart	Chief Strategy and Operations Officer, Institute of Protein Design, University of Washington
Dr. Miguel Esteves	Associate Professor, Univ of Massachusetts
Dr. Mark Midei	VP, Retrotope
Dr. Vasanthi Viswanathan	Researcher, Broad Institute
Dr. John Aitchison	Professor, Seattle Children's Research Institute
Dr. Alysson Muotri	Professor, Univ of California San Diego
Dr. Matt Klein	CEO, BioElectron
Dr. Timothy Read	CTO, Arpeggio Bio
Dr. Qing Cheng	Karolinska Institute

	Laboratories
Hengrui Liu	Columbia University

Agenda

All times are in pacific time zone (PST)

07:30 - 07:45 am Tech Check

Introduction & Keynote (08.00 to 09.00 am)

07.45 - 08.15 am Introductions and Welcome by *Sanath Kumar Ramesh*
08.15 - 08.45 am Keynote Speaker: Dr. David Fajgenbaum *"Chasing my cure"*
08.45 - 09.00 am Clinical Presentation of SSMD disease and Patient Needs from therapy by *Dr. Kristen Wigby*
09.00 - 9.30 am Break

Session 1: Basic Science (09.30 to 10.30 am)

09:30 - 09:35 am Explain the objective of the session by *Sanath Kumar Ramesh*
09.35 - 09.50 am "Context Talk" on GPX4 science by *Dr. Brent Stockwell*
09.50 - 10.30 am Workshop & Brainstorming: Basic Science moderated by *Dr. Brent Stockwell & Dr. Elias Arner*
10.30 - 10.45 am Break

Session 2: Small Molecule Drug Development (10.45 to 11.45 am)

10.45 - 10:50 am Explain the objective of the session by *Sanath Kumar Ramesh*
10.50 - 11.05 am "Context Talk" GPX4 Mouse Models by *Dr. Qitao Ran*
11.05 - 11.45 am Workshop & Brainstorming: Small Molecule Drug Development moderated by *Dr. Ethan Perlstein & Dr. Matt Hall*
11.45 - 12.15 pm Break

Session 3: Emerging Therapeutic Approaches (12.15 to 01.15 pm)

12.15 - 12:20 pm Explain the objective of the session by *Sanath Kumar Ramesh*
12.20 - 12:35 pm "Context Talk" on building gene therapy treatments by *Dr. Miguel Esteves*
12.35 - 01:15 pm Workshop & Brainstorming: Emerging therapeutic approaches moderated by *Dr. Plavi Mittal & Dr. Miguel Esteves*
01:15 - 01.45 pm Prioritize Roadmap & Closing Remarks

Conference Guide

Objective is to create a complete roadmap for therapy development by the end of the day. The roadmap will include identifying experiments necessary to understand disease, identifying small molecule drugs, and exploring the use of emerging technologies like gene therapy, ASOs etc to treat this condition. To accomplish this goal, we will have workshop-style free form discussions spread across three sessions.

Each session starts with a 10min "context" talk to get participants up-to-speed with the science underlying GPX4 with regard to its role in health and disease. This can provide the necessary background to all attendees to successfully participate and brainstorm. This will be followed by a ~40min brainstorming session. With the emphasis on informal freeform discussions, brainstorming sessions is where we expect everyone to contribute their expertise to help shape the roadmap.

The agenda, brainstorming session structure, and other activities are tailored to run a very collaborative online meeting respecting constraints such as multiple time zones, technology issues etc. We have dedicated time for tech-checks, ample breaks, dedicated note taking person, and a dedicated tech support person to ensure participants can focus on discussions.

Best Practices for Remote Conference

- Find a dedicated quiet room for the duration of the conference
- Use high-speed internet to support video conference of upto 20 people
- Turn on video to facilitate face-to-face interaction
- Mute yourself when you are not talking
- Recommended to use a headphone (no built-in speakers) to minimize echo
- Identify yourself before you speak in case people can’t see your video. A quick “Hey John Doe here..” before John Doe speaks will help everyone identify you
- Use the breaks as a good opportunity to interact with other participants on the online call

Roadmap Chart

See the next section for the Roadmap Chart which summarizes the current set of activities planned. Through the day, we will add more information to the chart as we learn in depth about GPX4. At the end of the day, we will summarize action items, prioritize activities and build a complete roadmap for the next year.

Session Structure

- Explain the objective (5mins)
- Context Talk (10mins + 5mins Q&A) to orient participants by covering enough topics necessary for brainstorming
- Brainstorm (40min)
    - Moderators will explain the objective of the session and start discussion with the first open ended question.
    - Discussions are freeform like a small “team meeting”. Moderators’ role is to guide discussion to meet the objective.
    - Participants can ask questions on chat or audio.
    - We will have a dedicated person to take notes during the session so everyone can follow along.
    - If new information is identified or a decision made, add it to “Roadmap Chart”

Roadmap Chart

Objective: Given 2yrs and $500 million dollars, find a therapy for GPX4 kids to provide a meaningful quality of life.

Strategy:

1. Repurpose approved drugs to slow down disease progression
2. De-risk the disease to make it attractive for industry partners
3. Develop new disease altering therapies to provide significant and meaningful improvements in quality of life

Disease	***Targets***	***Drug Candidates***	***Testing & Lead Identification***	***Commercialize***	***Clinical Trial / Compassionate Use***
What do we know? (Dr. Wigby's talk) - 10 Patients Worldwide - SSMD Phenotype - R152H is most common genotype	**What do we know?** (Dr. Stockwell's talk) GPX4 FSP1 NRF2 ACSL4	**What do we know?** (Dr. Stockwell's talk) Vitamin E CoQ10 NAC Selenium Tecfidera RT001 NACA Gene therapy (Dr. Esteves's talk)	**What do we know?** (Dr. Ran's talk) GPX4 KO mice characteristics	**What do we know?**	**What do we know?** RT001 Compassionate use on Raghav
What do we have?	**What do we have?** Recombinant mutant GPX4	**What do we have?**	**What do we have?** - Conditional KO Mice - Conditional KI Mice - Patient-derived iPSCs - Patient-derived Fibroblasts - KO Fly	**What do we have?**	**What do we have?**
What do we need? <Brainstorm>	**What do we need?** <Brainstorm>	**What do we need?** <Brainstorm>	**What do we need?** <Brainstorm>	**What do we need?** <Brainstorm>	**What do we need?** <Brainstorm>

Session Guide for Presenters & Moderators

Introduction & Keynote	Session 1: Basic Science
Dr. David Fajgenbaum "Chasing my cure" (30 min) "Chasing My Cure" is an amazing story of Dr.Fajgenbaum to find a treatment for Castleman Disease. It is a story of courage and perseverance even when the odds didn't work in his favor. **Dr. Kristen Wigby's Talk (10mins + 5mins Q&A)** - Diagnostic odyssey - SSMD disease phenotype - Genotypes - MRIs, X-rays, other Clinical markers - Life of patients	**Dr. Brent Stockwell's Talk (10mins + 5mins Q&A)** - Role of GPX4 in health & disease - R152H mutant data / biochemistry / recombinant protein - Other genetic targets rescuing GPX4 loss - GPX4 drug candidates - Assays available today **Workshop & Brainstorming** **(Moderators: Dr. Brent Stockwell & Dr. Elias Arner)** Objective: Identify next set of activities to understand the disease in order to facilitate therapy development - Open questions about the disease? - What experiments are necessary to understand disease (proteomics, metabolomics, lipidomics etc)? - How do we identify disease biomarkers or surrogate markers? - What do we know about similar diseases where high ROS is implicated? - How can a natural history study help us understand disease? - What are the biggest risks we should be worried about?

Session 2: Small Molecule Drug Development

Dr. Qitao Ran's Talk (10mins + 5mins Q&A)

- Types of mice available, characteristics of GPX4 loss in mice
- Drugs capable of rescuing mice
- Study to test 3 drugs (NAC+RT001, NAC+Tecfidera, NACA)

Workshop & Brainstorming
(Moderators: Dr. Ethan Perlstein & Dr. Matt Hall)
Objective: Identify next set of activities to repurpose existing drugs and de-risk disease to attract industry partners?

- Quick summary of models we are building
- How can we identify more approved drugs to repurpose?
- What pharmacological properties are important in drugs we consider?
- What is the value of discovering new (unapproved) molecules? How should we go about doing it?
- What endpoints/biomarkers/surrogate markers should we track in models and humans to quantify therapy outcome?
- What are the biggest risks we should be worried about?
- What should we do to de-risk the disease for industry to take on?
- How can computational modelling help identify potential therapies?
- How can we show business value by combining with related diseases (ex: Parkinsons, Cancer etc)?

Session 3: Emerging Technologies

Dr. Miguel Esteves Talk (10mins + 5mins Q&A)

- Gene therapy basics
- Challenges in neurological and skeletal diseases
- Cost breakdown
- GPX4 gene therapy high-level plan

Workshop & Brainstorming
(Moderators: Dr. Plavi Mittal & Dr. Miguel Esteves)
Objective: Explore gene therapy/ASO feasibility and identify next steps

- Is ASO for exon skipping a viable strategy?
  - Can the Exon 6 be skipped to stay in the reading frame?
  - Will protein function without exon 6?
- Tissue and organ systems to target with GPX4 gene therapy?
  - Expression pattern of gene?
  - Delivery and ideal AAV vectors?
  - Defined measurable outcomes?
- Ideal mouse model suited for preclinical testing?
- Is it possible to perform gene therapy as an N-of-1 trial?
  - Other diseases that benefit with GPX4 expression?
  - Stage to de-risk for industry interest
  - Other downstream genes to overexpress instead?
- How does CRISPR compare to gene therapy for GPX4?
  - Pros vs Cons
  - Timeline to Clinic?

Computer Setup for Online Conference

1. Install “Zoom Client For Meetings”: https://zoom.us/download#client_4meeting

2. Test your video and audio by joining the test meeting. Click this link to join the test meeting - https://zoom.us/test.
   - Detailed guide on joining test meeting is here: https://support.zoom.us/hc/en-us/articles/115002262083
   - Troubleshoot audio/video settings by using instructions here: https://support.zoom.us/hc/en-us/articles/201362623-Changing-settings-in-the-desktop-client-or-mobile-app

3. Familiarize with the Zoom Client interface by playing around in the test meeting:
   - Mute Button
   - Video enable/disable Button
   - Chat Window
   - Screen Share button

4. On the day of conference, join the Conference Bridge using the link sent via the calendar invite. Alternatively click here to join the conference bridge

Roadmap Chart

Objective: Given 2yrs and $500 million dollars, find a therapy for GPX4 kids to provide a meaningful quality of life.

Strategy:

1. Repurpose approved drugs to slow down disease progression
2. De-risk the disease to make it attractive for industry partners
3. Develop new disease altering therapies to provide significant and meaningful improvements in quality of life

Disease	***Targets***	***Drug Candidates***	***Testing & Lead Identification***	***Commercialize***	***Clinical Trial / Compassionate Use***
What do we know? (Dr. Wigby's talk) - 10 Patients Worldwide - SSMD Phenotype - R152H is most common genotype	**What do we know?** (Dr. Stockwell's talk) GPX4 FSP1 NRF2	**What do we know?** (Dr. Stockwell's talk) Vitamin E CoQ10 NAC Selenium Tecfidera RT001 NACA Gene therapy (Dr. Esteves's talk)	**What do we know?** (Dr. Ran's talk) GPX4 KO mice characteristics	**What do we know?** <Brainstorm>	**What do we know?** RT001 Compassionate use on Raghav
What do we have? <Brainstorm>	**What do we have?** Recombinant mutant GPX4	**What do we have?** <Brainstorm>	**What do we have?** - Conditional KO Mice - Conditional KI Mice - Patient-derived iPSCs - Patient-derived Fibroblasts - KO Fly	**What do we have?** <Brainstorm>	**What do we have?** <Brainstorm>
What do we need? <Brainstorm>	**What do we need?** <Brainstorm>	**What do we need?** <Brainstorm>	**What do we need?** <Brainstorm>	**What do we need?** <Brainstorm>	**What do we need?** <Brainstorm>

Low-Throughput Drug Repurposing

The following table contains a list of existing drugs (FDA approved or experimental) that could be effective. In SSMD disease, a mutation in GPX4 reduces the function of the protein. In a loss-of-function condition, our goal is to find drugs that:

1. Increase GPX4 protein levels and/or increase residual GPX4 activity
2. Increase the activity GPX4 antioxidant pathways by modifying the quantity of other participating proteins
3. Increase the activity of alternate compensatory pathways
4. Reduce or scavenge the phospholipid oxidation damage due to reduced GPX4 activity (e.g., use of antioxidants)
5. Drugs that have been found to be effective in similar conditions

Drugs are grouped into sections based on the mechanisms of actions. In each section, drugs are ordered by likelihood of therapeutic effect (high to low).

Increase GPX4 protein quantity (Category 1)

Rationale: Directly increase GPX4 protein quantity by histone modifications, changes to DNA methylation, activating GPX4 gene, or saturating the GPX4 production pathway.

Name	FDA Status	Mechanism of Action	Ref	Decision
Selenium	OTC	Providing more selenium will help saturate the GPX4 production pathways. But excess selenium is also harmful.		Yes
Nicotinamide	OTC	HDAC inhibitor to prevent deacetylation of histone which reduces transcription		NO Ineffective

Increase pathway activity (Category 3)

Rationale: GPX4 catalyzes the antioxidant pathway along with several molecules like Glutathione, Glutathione Reductase, NADPH, Iron etc. We hypothesize that a partial loss of GPX4 function reduces the activity of this pathway. We identify drugs capable of compensating for the loss of activity by either increasing the pathway's activity or reducing the oxidant load.

Name	FDA Status	Mechanism of Action	Ref	Decision

N-Acetyl-Cysteine	OTC	Provides cysteine to increase the production of glutathione.		YES Already administered
Methionine	OTC	Amino acid acting as a substrate to produce Cysteine. Could be used in combination with NAC.		NO Ineffective. Risk of overload
Glutathione esters	OTC	Substitutes cellular pool of GSH. Known to work in-vivo.	(1)	NO Not enough bioavailability
EPI743	Phase 3	Increase GSH synthesis. Has antioxidant property. But not available under expanded access		NO Company does not do expanded access

<u>Reduce generation of Reactive Oxygen Species (Category 2)</u>

Rationale: Reduce the oxidant load in the cell to a point where the partial GPX4 activity is sufficient to maintain normal oxidative stress levels.

Name	**FDA Status**	**Mechanism of Action**	**Ref**	**Decision**
Zileuton	Rx	5-lipoxygenase inhibitor. Reduces ROS. Known to have antioxidant properties.	(2)	TBD Need more understanding
BHT (Butylated Hydroxytoluene)	OTC	Food preservative. Known antioxidant. But chronic usage isn't recommended	(3)	NO Mixed safety results
Apocynin	OTC	Inhibits NADPH Oxidase (Nox2) to control ROS production. Also an antioxidant.	(4, 5)	NO No PKPD

				data. No drugs. But good science data
Deferoxamine	Rx	Iron chelator used in blood transfusion		NO Side effects
Deferiprone	Rx	Iron chelator. Crosses blood-brain barrier, crosses cell membrane, redistributes inter-mitochondrial iron to extracellular network. Generally safe at low doses.	(6)	NO Side effects

Increase activity of NRF-2 pathway (Category 3)

Rationale: NRF2 is a well-known Transcription Factor upregulated under high oxidative stress. (7) Several drugs are known to activate NRF2 (8) Due to the number of genes activated by NRF2, these drugs could give us better results by exploiting the body's natural defense mechanism. On the downside, it might be tricky to identify the right dose of drug because at very low or very high doses, NRF2 will not get activated.

Name	**FDA Status**	**Mechanism of Action**	**Ref**	**Decision**
Sulforaphane	OTC	Derived from broccoli. Shows 1.5-2x activation of NRF2 and downstream genes. Gold standard for NRF2 activation.	(9)	YES Safe, but unsure
Tecfidera	Rx	Drug for multiple sclerosis. Better than Sulforaphane in NRF2 activation response.	(9)	YES Safe. Effective.
Bardoxolone methyl	Phase 2	Better than Sulforaphane. Positive result in chronic kidney disease.	(1)	NO Drug not available
RTA-408	Phase 3	Inhibits KEAP1 to induce NRF2		TBD

Baicalein	OTC	Herb found in Chinese medicine. Safe for humans, but the mechanism of action isn't completely understood. Believed to activate NRF-2	(10)	NO Mixed results

Protect lipids from peroxidation (Category 4)

Rationale: Reactive oxygen species causes peroxidation of the lipid membrane leading to ferroptosis. Protecting the lipids will reduce cell degradation.

Name	**FDA Status**	**Mechanism of Action**	**Ref**	**Decision**
RT001	Phase 2	"Fireproofs" lipids from peroxidation by substituting a hydrogen to deuterium		YES IND in progress
Omega-3 / DHA	OTC	Absorbed by lipid membranes. Could help repair oxidized lipids by saturating the repair pathway		NO Interacts with RT001

Scavenge Reactive Oxygen Species (Category 4)

Rationale: Scavenge reactive oxygen species to reduce the oxidative stress enough to compensate for reduction in GPX4 activity.

Name	**FDA Status**	**Mechanism of Action**	**Ref**	**Decision**
Vitamin E	OTC	Well known antioxidant. Protects against lipid peroxidation		YES Administered
Epicatechin	OTC	Natural flavanol derived from cacao plants. Antioxidant. Known to help skeletal muscle. Used in body building supplements.	(11)	YES Crosses BBB. Safe.
Mitoquionone	OTC	CoQ10 analogue. Very well studied in literature. Enters mitochondria as antioxidant	(12)	YES

Ergothioneine	OTC	Naturally occurring amino acid acts as a weak antioxidant. Importantly, it is known to synergistically amplify effect of NAC to protect cells	(13, 14)	NO Weak results
Erdosteine	Rx	Mucolytic drug, but antioxidant. Very effective in COPD (smoking) and very safe.	(15)	TBD
Carbocysteine	Rx	Mucolytic drug, but antioxidant. Similar to NAC, but uses the thioester group.	(15)	TBD
Methylprednisolone (Lazaroids)	Rx	Highly effective to prevent lipid peroxidation after spinal cord injury. Used extensively in CNS. Steroid.	(16)	NO Steroid side effects
VP-20629 / SH622	Phase 2	Derivative of naturally occurring putative antioxidant. Safe for humans.	(17)	NO Drug unavailable
Edaravone	Rx	ALS Drug thought to be antioxidant. Intravenous administration.	(18)	NO Intravenous
Folic acid	OTC	Known antioxidant. Naturally occurring		NO Weak results

<u>Prevent mitochondrial damage (Category 5)</u>

Rationale: One isoform of GPX4 enters mitochondria. We hypothesize that excess ROS might affect mitochondria structure and function leading to phenotypes similar to classic mitochondrial conditions such as Leigh's syndrome.

Name	**FDA Status**	**Mechanism of Action**	**Ref**	**Decision**
MTP-131 aka Bendavia	Phase 3	Binds to cardiolipins found in the mitochondrial membrane to help the mitochondrial complex 1 to function more effectively.	(19)	YES Worth trying

Leber's hereditary optic neuropathy (LHON) (Category 5)

Rationale: Increase in ROS might affect mitochondria structure and function.

Name	**FDA Status**	**Mechanism of Action**	**Ref**	**Decision**
Idebenone	Rx	CoQ10 derivative. But acts outside mitochondria. Very different PKPD from CoQ10. Antioxidant.	(20)	YES Worth trying as CoQ10 alternative

High Blood Pressure / Heart Failure (Category 5)

Rationale: Several Beta-blockers have been shown to prevent lipid peroxidation. There are many known beta blockers (atenolol, labetalol, metoprolol, pindolol, propranolol, sotalol, timolol, and carvedilol), but here we have only those with good antioxidant properties.

Name	**FDA Status**	**Mechanism of Action**	**Ref**	**Decision**
Carvedilol	Rx	Beta Blocker. Reduces heart rate and blood pressure. Known to reduce oxidative stress and thereby heart failure.	(21)	NO Causes fatigue
Propranolol	Rx	Similar to Carvedilol. Beta blocker but weak antioxidant	(22)	NO Causes fatigue

Chemotherapy (Category 5)

Name	**FDA Status**	**Mechanism of Action**	**Ref**	**Decision**
Dexrazoxane	Rx	Used with chemotherapy drug to prevent cardiotoxicity. Known to reduce reactive oxygen species through a variety of mechanisms	(23)	TBD

Chronic Kidney Disease (Category 5)

Name	FDA Status	Mechanism of Action	Ref	Decision
Erythropoietin	Rx	Enzyme produced by the kidney responsible for RBC production. Known to decrease oxidative stress.	(24)	NO Kidney side effects

Aging (Category 5)

Rationale: Oxidative stress is known to play a critical role in aging. It is interesting to examine the “miracle” drugs to find relevant ones.

Name	FDA Status	Mechanism of Action	Ref	Decision
Resveratrol	OTC	Occurs naturally in several plans, fruits, grapes etc. Very well known in the anti-aging community. Known to have antioxidant properties.		NO Weak results

Cystic Fibrosis / Chronic Obstructive Pulmonary Disease (Category 5)

Many antioxidants have been tested with varying levels of success (25)

Spinal Cord Injury (Category 5)

Lipid peroxidation is a secondary effect of SCI. Antioxidants are used effectively. In-depth review with several drugs tested in models (26)

Friedreich’s Ataxia (Category 5)

Review drugs tried in FRDA (19)

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CUREGPX4 SCIENCE TEAM STATUS REPORT
15-June-2020

GOAL: Find a treatment to slow down disease progression by end of 2020

HIGHLIGHTS SINCE LAST REPORT

- We are waiting on one final paperwork to be signed before starting on experimental therapy.
- We have started producing iPSCs from fibroblasts for both patient and control lines.
- We are working on starting a natural history program.

SUMMARY

We are focusing on small molecule drug repurposing to achieve the goal. We have started patients on a few existing FDA approved drugs (Vit-E, N-Acetylcysteine, CoQ10, Selenium) and submitted the FDA application to try an experimental therapy. We will test more drugs as part of a pipeline on several GPX4 disease models to identify more efficacious drugs. We are exploring gene therapy as a long-term treatment.

AT A GLANCE

Number of GPX4 patients worldwide: 9
Team: https://www.curegpx4.org/team
Roadmap: https://www.curegpx4.org/roadmap
Disease models:

- Mouse: Conditional GPX4 Knockout (JAX, Stock No: 027964)
- Fibroblasts: Patient and Parent Control lines (RUCDR Biorepository)
- iPSCs: Patient and Parent Control lines (RUCDR Biorepository)
- Mouse: Conditional Knock-in R152H mutation (ETA: 1-Feb-2021)
- Fly: Knock-out or Knock-in (depending on viability of phenotype)

ACTIVE PROJECTS

Project	Investigator	Status	Updates
Natural History Study	Dr. John Doe	Preparation	We have a call set up an epidemiologist to finalize next steps for natural history study.
Gene Therapy	Dr. John Doe	Started	We have started building the gene therapy treatment

UPCOMING PROJECTS

Project	Investigator	Status	Updates

LINKS

Links to documents relevant to your work