2nd Quarter Report

JULY 2017

Executive Director:
Jeffrey Rothstein, Johns Hopkins University

Co-Directors
- Clive Svendsen, Cedars-Sinai
- Merit Cudkowicz, Massachusetts General

Advisory Board Director
- Ed Rapp
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ACKNOWLEDGMENTS

Answer ALS – the program, the research and ultimately our results are predicated on the support from our sponsors, scientists, researchers, participants, and generous donors. Without their solid participation, finding the answer to ALS would remain just another “idea” waiting to be realized. Answer ALS was created in full partnership with our stakeholders to implement an unprecedented results-oriented research program developed with the highest spirit of integrity, collaboration, and transparency.
EXECUTIVE SUMMARY

We kicked-off the second quarter of 2017 with a public webinar to highlight the progress and accomplishments made by the Answer ALS team to date. The webinar provided participants, caregivers, and interested members of the public and science communities alike, the opportunity to hear and ask questions about our goals and development. We were delighted to be joined by 85 participants during the live broadcast and received follow-up questions from many of the 124 individuals who watched the recorded version. We will plan more webinars in the future to help facilitate conversations between the community and our program.

On June 28, the Answer ALS clinical program reached a very significant milestone; our enrollment reached 500 participants, halfway towards our goal! We are grateful to our wonderfully talented and dedicated clinical team for pushing so hard and reaching this enrollment target on schedule – well done team! We are also very proud to announce that the Les Turner ALS Research and Patient Center at Northwestern Medicine is set to join Answer ALS in the Fall of 2017. This partnership will increase the geographical reach of our program. We are delighted to welcome the Northwestern clinic to our team.

At our science centers, work continues to identify the optimal protocol for motor neuron development. As you may recall, at the start of the year our omics teams uncovered some novel observations which suggested that “aging” motor neurons in culture, would help enhance and capture disease signatures. To further investigate this possibility, Dr. Svendsen and his group at Cedars-Sinai differentiated 28 lines of motor neurons and prepared material for transcriptomics from both the short (18 day), and extended (32 day) protocol. Dr. Thompson and her lab at University California, Irvine, recently processed these samples through the transcriptomics assay and are currently analyzing the results. The data captured from this first batch of 28 Answer ALS participant samples, will help to shed light on the length of time required to “age” motor neurons in culture to capture a robust and meaningful disease signature.

On the technology front, we also met a significant milestone during the past quarter. June 30 marked the first launch of the bioinformatics workflow system, Galaxy, on Microsoft’s Azure cloud. For the less tech-savvy amongst us, Galaxy is a computational biology tool which helps biologists to organize and analyze their data. Since data from Answer ALS will be stored and analyzed on Azure, it was first necessary to adapt Galaxy to run on the cloud. Our congratulations to the Microsoft and Galaxy software engineers involved. We are one step closer to creating an environment where researchers from all over the world can access and interface with Answer ALS datasets.

Answer ALS came to fruition when a team of stakeholders including The Fishman Family, ALS Finding a Cure, Travelers, Team Gleason, The Barry Lipp Foundation, the National Football League, the PGA tour and the Robert Packard Center for ALS Research, came together to fund the Research Program. Two years on, we are proud to announce additional partnerships with American Airlines, Warlick’s Warriors and Stay Strong vs. ALS, all of whom have committed to fighting the battle against ALS.
On that note, we would like to extend our sincere thanks to our friends at American Airlines. More than $800,000 was raised for the Answer ALS Research Program through the second annual American Airlines Charity Golf Tournament, held in Dallas, Texas last May. These much-needed funds will allow us to continue the important work we are doing to help solve ALS. Thank you, American Airlines!

As always, we extend our gratitude to all our partners, sponsors, study participants, clinicians and scientists for their continued support and commitment.

Yours truly,

Jeffrey D. Rothstein

Merit Cudkowicz, MD, MSc

Clive Svendsen, PhD

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Executive Director, Answer ALS
Johns Hopkins University, School of Medicine

Merit Cudkowicz, MD, MSc
Co-Director, Answer ALS
Massachusetts General Hospital

Clive Svendsen, PhD
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Cedars-Sinai Medical Center
ANSWER ALS RESEARCH PROGRAM GOVERNANCE

GOVERNANCE UPDATE

Program Oversight
The Answer ALS Research Program is an intensive, in-depth study that extends across multiple institutes and disciplines. As such, it requires significant oversight. Now well into its second year, the leadership and governance has begun to mature. No longer a “startup”, we have developed to the point where our processes and procedures must bring consistency and efficiency to all arms of the program. Our budgets, staffing, and extensive relationships with industry partners, require we have an Advisory Board equal to the task of evolving this unprecedented program.

The Answer ALS Advisory Board, conceived under Jay Fishman (who passed away from ALS in August 2016), is now under the leadership of Ed Rapp. Ed Rapp is a former President for the Caterpillar Group. In December 2015, Ed Rapp announced that he would retire from the Caterpillar Group to focus on his personal fight against ALS. Answer ALS is fortunate to have his wisdom and guidance as we move forward in our fight to end ALS.

Our new Advisory Board is still forming. To date, the following positions have been assigned:

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**Advisory Board Executive Leadership**

- **ED RAPP**
  - Chairman

- **JEFF ROTHSTEIN, MD, PHD**
  - Executive Director

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**Advisory Board Committee Leads & Board Members**

- **MERIT CUDKOWICZ, MD, MSc**
  - Co-Director

- **CLIVE SVENSDON, PHD**
  - Co-Director

- **LUCIE BRUIJN, PHD**
  - Board Member

- **CLARE DURRETT**
  - Committee Chair - Marketing & Communications

- **BEVERLY SCULETE**
  - Committee Co-Chair - Finance

- **PETER WARLICK**
  - Committee Co-Chair - Finance

- **RANDY FISHMAN**
  - Board Member

- **PETER FOSS**
  - Board Member
ANSWER ALS RESEARCH PROGRAM UPDATES

CLINICS

On June 28, 2017, Answer ALS enrolled its 500th participant; we have officially crossed the halfway mark! Reaching this milestone is a tremendous accomplishment, and we are very grateful to all our participants and clinicians. Furthermore, adding to the depth and power of our clinical observations, we are pleased to report that a total of 54 participants have now completed all five follow-up visits. The follow-up visits provide us with a window into the clinical characteristics of disease progression over time—one of the hallmarks of this study.

This quarter also saw the advancement of other tools to help evaluate clinical measures, including newly developed mobile technologies. These tools will allow us to assess disease progression day-by-day. These changes will include measures of speech, mobility, activity and dexterity amongst others.

The following activities will be completed during the upcoming months:

- Activation of our new clinical site at Northwestern. Many processes (regulatory board approval, institutional agreements etc.) must to completed before the site will be ready to enroll participants.
- Creation of blood sample workflow within NeuroBANK to help track samples at Cedars-Sinai Medical Center.
- Rollout of mobile application, “Help Us Answer ALS”, initially at JHU and then MGH (data collected from the app will be analyzed by our partners at IBM Watson).
- Laying out more detailed and mature plans for how we share data and samples.
- Ongoing site support from the Coordination Centers via email and telephone
  - Including monthly site calls.

A summary of our second quarter clinical metrics and progress is provided on the next pages.

Site Status and Metrics

<table>
<thead>
<tr>
<th>Site</th>
<th>Months Since Activation</th>
<th>Total Expected Recruitment</th>
<th>Yearly Recruitment Goal</th>
<th>Target Monthly Average</th>
<th>Actual Participants to Date</th>
<th>Actual Monthly Average</th>
<th>Actual PBMCs Collected</th>
<th>Enrolled in Last 30 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGH</td>
<td>18</td>
<td>211</td>
<td>70</td>
<td>6</td>
<td>114</td>
<td>6</td>
<td>104</td>
<td>1</td>
</tr>
<tr>
<td>Emory</td>
<td>17</td>
<td>211</td>
<td>70</td>
<td>6</td>
<td>122</td>
<td>7</td>
<td>119</td>
<td>3</td>
</tr>
<tr>
<td>JHU</td>
<td>18</td>
<td>211</td>
<td>70</td>
<td>6</td>
<td>136</td>
<td>8</td>
<td>125</td>
<td>15</td>
</tr>
<tr>
<td>WUSTL</td>
<td>18</td>
<td>105</td>
<td>35</td>
<td>3</td>
<td>45</td>
<td>3</td>
<td>44</td>
<td>2</td>
</tr>
<tr>
<td>OSU</td>
<td>16</td>
<td>211</td>
<td>70</td>
<td>6</td>
<td>60</td>
<td>4</td>
<td>59</td>
<td>4</td>
</tr>
<tr>
<td>CSMC</td>
<td>13</td>
<td>53</td>
<td>18</td>
<td>1</td>
<td>29</td>
<td>2</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1000</td>
<td>333</td>
<td>28</td>
<td>25</td>
<td>506</td>
<td>26</td>
<td>479</td>
<td>25</td>
</tr>
</tbody>
</table>

* TABLE 1: PROJECTED AND ACTUAL PATIENT ENROLLMENT NUMBERS AND PBMC COLLECTION AS OF JUL 12, 2017
* ALL NUMBERS ARE ROUNDED TO THE NEAREST WHOLE NUMBER
Answer ALS Enrollment – 12 Jul 2017

FIGURE 1: TARGET AND ACTUAL ENROLLMENT AS OF JUL 12, 2017

Answer ALS Enrollment By Month


Answer ALS Site Visits Completed – 30 June 2017

FIGURE 3: COMPLETED VISITS BY SITE (AS OF JUN 30, 2017)

Answer ALS Site Activity – 30 June 2017

FIGURE 4: SITE ACTIVITY (AS OF JUN 30, 2017)

Sample Type | Name  | #Samples (vials)
-------------|-------|------------------
C           | CSF   | 772              
P           | Plasma | 5519            
S           | Serum | 1628             
W           | Whole Blood | 1142          

TABLE 3: BIOREPOSITORY SAMPLES RECEIVED (AS OF JUL 12, 2017)
Disease progression can be monitored in the clinic using the ALS Functional Rating Scale (ALS FRS). The left-most panel shows an example of slowly progressing participant, while the panel on the right shows a participant with a more rapid rate of disease progression.

TABLE 4: SUMMARY OF PARTICIPANT STATUS (AS OF JUL 12, 2017)

<table>
<thead>
<tr>
<th>Site</th>
<th>Screen Fails; Withdrawals; Deaths</th>
<th>Reason for Screen Fail</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGH</td>
<td>2 - SF; 0 - WD; 11 - D</td>
<td>2 Hep B Positive</td>
</tr>
<tr>
<td>Emory</td>
<td>3 - SF; 0 - WD; 5 - D</td>
<td>1 HIV Positive, 1 Hep C Positive, 1 Hep B &amp; C Positive</td>
</tr>
<tr>
<td>JHU</td>
<td>2 - SF; 3 - WD; 11 - D</td>
<td>2 Hep B Positive</td>
</tr>
<tr>
<td>WUSTL</td>
<td>1 - SF; 0 - WD; 8 - D</td>
<td>1 Hep C Positive</td>
</tr>
<tr>
<td>OSU</td>
<td>3 - SF; 4 - WD; 2 - D</td>
<td>2 Hep C Positive</td>
</tr>
<tr>
<td>CSMC</td>
<td>0 - SF; 0 - WD; 3 - D</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>11 - SF; 7 - WD; 40 - D</td>
<td></td>
</tr>
</tbody>
</table>

FIGURE 5: GRAPHICS OF DISEASE PROGRESSION AND SUBJECTS BY GENDER

FIGURE 6: CORRELATIONS BY GENDER AND AGE
Current Challenges, Issues, and Corrective Measures

To ensure we stay on track to meet our goal of 1000 participants in three years, we are taking the following action:

Cedars-Sinai Medical Center is now operating at an elevated status of a half-site. From June 2017 forward, CSMC will be expected to enroll at least three participants per month.

The Les Turner ALS Research and Patient Center at Northwestern Medicine is in the process of becoming a new site for Answer ALS. The geographic area will help us recruit more heavily from the middle of the US. We expect to see a rise in enrollment due to their efforts. We continue to explore the feasibility and benefits of adding clinical sites across the country to increase our geographical reach.

INDUCED PLURIPOTENT STEM CELL

Blood cells known as peripheral blood mononuclear cells (PBMCs), can be isolated from participants and genetically reprogrammed to become induced pluripotent stem cells (iPSC). iPSCs can then be differentiated in culture to yield motor neurons – the exact cell type known to degenerate in ALS. Motor neurons derived in this manner harbor the participant’s complex genetic make-up and ultimately may hold the key to understanding disease initiation and progression. Although not a biopsy, our goal is to reproduce as many ALS disease features as possible in these cells.

At the start of the year, our multi-omics teams uncovered some novel observations which suggested that “aging” motor neurons in culture, would help to augment the disease signature. To investigate this possibility, Dr. Svendsen and his group at Cedars-Sinai differentiated 28 lines of motor neurons and prepared material for transcriptomics from both the short (18-day), and extended (32-day) protocol.

Studies are continuing so our researchers can determine if the current short protocol may be extended to increase the fidelity of iPSC-derived motor neurons to those found in the participant. There is more on this progress in the next section.

Site Status and Metrics

<table>
<thead>
<tr>
<th>Production Phase</th>
<th># Lines</th>
<th>On Track?</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMC Collection and Processing Total</td>
<td>454</td>
<td>✓</td>
</tr>
<tr>
<td>iPSC Line Initiation</td>
<td>243</td>
<td>✓</td>
</tr>
<tr>
<td>iPSC Line Completion</td>
<td>177</td>
<td>✓</td>
</tr>
<tr>
<td>dIMNs Completed</td>
<td>100</td>
<td>✓</td>
</tr>
<tr>
<td>dIMNs Distribution</td>
<td>52</td>
<td>✓</td>
</tr>
</tbody>
</table>

TABLE 5: IPSC METRICS FOR SECOND QUARTER 2017 SHOWING POSITIVE PROGRESS
MULTI-OMICS

During the second quarter, the consortium generated data from motor neuron lines created using the directly induced motor neuron (diMN) 18-day protocol (the “short protocol”), but given an additional 14 days in culture to mature. To determine the optimal maturation time to yield a robust disease signature, data generated from diMNs aged for either 21 or 32 days, will be compared. The data generated from these lines are being compared to each other and to the original lines generated using the two-step long protocol (iMN).

During this past quarter, the group also conducted a blinded study using 26 Answer ALS participant diMN lines to analyze transcription signatures using a rapid RNA-Seq assay. This higher throughput study was conducted to understand if the current short protocol is providing a sufficiently robust signature to distinguish patient versus control samples. Each of these data sets is being compared to guide us on using the optimal protocol for gaining signatures specific to ALS participant lines.

Second quarter accomplishments:

- Carried out assay on 26 Answer ALS participant lines for RNA-Seq. Data analysis in progress.
- Carried out 32-day diMN cell line processing and data generation. Data analysis in progress
- Continued generation of Answer ALS Participant whole genome sequences (300).

**diMN cell line processing and data generation**

The centers continue to refine methods of data generation and integration using the original induced motor neuron (iMN) differentiation protocol and the shorter diMN protocol with two maturation times. The data shows consistent results between centers on lines maturated to 21 days giving us confidence in the approach, however, the signatures are not as robust as those from the previous study using the longer protocol. To understand these differences, the stem cell core group maintained the motor neurons in culture for a longer period. The hypothesis is that to see the disease signature from the ALS lines, the cells need to mature further. During this past quarter, five cell lines aged to 32 days (2 control and 3 ALS patient lines) were completed and sent to the science centers. Data has been generated using RNA-Seq, ATAC-Seq and proteomics on each of these lines. Once each center has completed the assay data analysis and QC, the data will be integrated to understand if the signatures are sufficiently robust.

**Answer ALS Participant Sample Processing**

Twenty-Eight Answer ALS samples were sent to the Transcriptomics group for a blinded analysis of transcriptional differences between patient and control samples. The samples contained 10 controls and 18 ALS lines in duplicate. To determine whether sufficiently robust transcriptional signatures can be observed in a larger number of biological replicates, thus providing greater analytical power, we used a low coverage, more rapid throughput mRNASeq method. Only 26 of the samples were used since two samples did not pass quality control for RNA concentrations. The 26 lines were processed for RNA and sequenced generating data for subsequent analysis.
These data are currently being put through quality control protocols and aligned to the human genome by the bioinformatics group at our Transcriptomic center.

**Whole Genome Sequencing and Analysis**

The New York Genome Center has sequenced ~300 Answer ALS participant samples and are currently transferring the Binary Alignment Map (BAM) files to MIT. All the Variant Call Files (VCFs) have been downloaded and exist on the MIT servers. The Gladstone group is preparing the transfer to their servers to begin the analysis.

In addition, we have completed generation of the exonic variant analysis pipeline for the 4 **C9ORF72**, 4 **SOD1** and 4 sporadic ALS lines, 4 **Spinal Muscular Atrophy (SMA)** and 4 control lines exonic variants and have provided the results to all NeuroLINCS members. Answer ALS requested that we add the American College of Medical Genetics and Genomics (ACMG) recommended genes and variant lists to our pipeline and this work is underway. In addition, the Gladstone center is currently finalizing the Regulatory Variant pipeline and expects to report the results in the next quarter.

**DATA AND TECHNOLOGY**

**Cloud infrastructure for Data Storage and Analysis**

Progress on computing infrastructure continues in two directions: progress on local computing infrastructure and cloud computing infrastructure. In the coming months, work will continue to develop and deploy the plan to put Answer ALS data on a platform to facilitate data analytics. This is discussed further below.

Locally, the challenge has been setting up a parallel computing environment which operates seamlessly with the Galaxy platform. This quarter we set up and configured the SLURM scheduler to manage the cluster that executes jobs for our local Galaxy server (answer.csbi.mit.edu, “Answer”). The main components are now in place, and we are ironing out the remaining smaller details that will enable the system to work smoothly. In the next quarter, we expect to re-run our processing of basic data types using Galaxy on Answer and improve our pipelines for each omics assay for more reliable analysis.

On the cloud side, months of hard work have come to fruition, and we’ve completed the “first phase” of building the infrastructure to set up Galaxy on Azure. By collaborating with the Galaxy team and Microsoft, we have completed an Azure plugin for the Galaxy “CloudBridge” which provides an API interface abstracted from each cloud provider, from which it is now possible to launch Galaxy instances on any of the CloudBridge-supported clouds. At the Galaxy community conference (GCC) last June, the Galaxy team demoed the ability to launch Galaxy on a single machine on Azure with a single click, important progress for ourselves and a contribution to others in the community who perform analyses on Azure.

To move from launch of a single machine to the scalable, flexible cloud system necessary for this project, a third component is needed. This component is CloudMan (Cloud Manager), which the Galaxy team is rebuilding from scratch. Building CloudMan is the “second phase” which could take up to a year to complete. Once complete, CloudMan will enable scalable Galaxy to set up on
any CloudBridge-supported cloud with a few keystrokes. We are also exploring alternatives to CloudBridge to set up scalable cloud Galaxies on Azure with the help of our collaborators at Microsoft to accelerate progress. Such a solution would rely on recent improvements made to Azure, especially “Azure Batch Shipyard”.

We are moving forward to build the infrastructure necessary for use of the Azure cloud system, while simultaneously establishing a local system for use in the interim, as the cloud infrastructure is being completed. The focus in the third quarter of 2017 will be re-running and improving our pipelines for low-level data pre-processing and de-noising on our local machine.

COMMUNICATIONS AND PUBLIC RELATIONS

The Answer ALS Foundation continues the pursuit of expanding and different funding sources and strategic alliances.

The relationship with Microsoft is strong. Microsoft continues to engage with the Research Team’s use of the Azure cloud.

Due to the great success of partnering with the NFL over the last two years, we are continuing the Answer ALS campaign with the NFL and their digital assets. A new campaign is under development. More details to follow soon.

The Answer ALS relationship with Warlick’s Warriors and American Airlines continues to be positive for all parties. The American Airlines golf tournament recently raised nearly $800K.

In addition, United Airlines just committed funding support to the Answer ALS. We are working on a model to propose to other industry leaders, that would encourage the company and employees to participate in Answer ALS and raise much needed funds.

Because of a recent aviation industry meeting, one individual was inspired to set up a page to raise $10K for Answer ALS by running the Great North Run. We decided to facilitate and expand opportunities for other interested individuals to support Answer ALS. We have added a page for individuals to raise funds for Answer ALS on First Giving.

Web and Social Media:

Web. The Answer ALS Foundation site revisions are under way. The Foundation site is in design, while the Research site is nearly complete. A sneak preview is to the right.

Social Media. We continue to grow in Facebook followers and engagements each week. Nearly 100% in positive responses. We will continue to work on collaborative posting/sharing between all Answer ALS groups and interested parties. Twitter is linked to Facebook and continues to engage and grow as well. Snapshots from recent social media posts are highlighted on the next page demonstrating engagement with thousands of people.
Communications.

Answer ALS Foundation was incredibly pleased to learn that a recent Answer ALS spot “Signed by Steve” was a GOLD winner for the prestigious CLIO award. This was a first award for all parties in the category of “Social Good.”

We have engaged (pro bono) KBS Agency to help with ongoing strategic messaging and a communications plan. Through our overall Advisory Board, we are developing a communications plan for the Foundation and our Research partners for consistent messaging with great impact.

WHAT’S NEXT/FUTURE STATUS

As we move on to the third quarter of 2017, our attention will remain focused on bolstering clinical enrollment across sites. This effort will be aided by the addition of a seventh Answer ALS clinic, the Les Turner ALS Research and Patient Center at Northwestern Medicine. We look forward to welcoming the Northwestern team to the program and expect participant enrollment to commence in the early Fall, once all the regulatory requirements have been fulfilled.

The IPSC and Omics teams will continue to work together closely as we determine the optimal protocol for motor neuron production. The teams are set to meet and exchange data in early August.

Over the coming months, it is also crucial that we continue to develop the infrastructure and technology required to assemble, analyze and share Answer ALS data. Towards that goal, we are grateful to have partnered with some of the world’s leading technology companies including Accenture, Microsoft, IBM Watson and Google Analytics. Plans continue to emerge and solidify with each of these partners.

Finally, under the leadership of Ed Rapp, our governance structure will continue to mature and expand. Each committee has been charged with drafting a charter and setting clear goals for the second half of 2017.