ANSWER ALS

AN INDIVIDUALIZED ALS THERAPEUTIC INITIATIVE

THE PROJECT TO END ALS NOW

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JULY 2016 **2ND QUARTERLY REPORT**

Answer ALS

2ND QUARTERLY REPORT- JULY 2016

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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 ALS stakeholders to implement an unprecedented results-oriented research program developed with the highest spirit of integrity, collaboration, and transparency.

ANSWER ALS PROGRAM GOVERNANCE

GOVERNANCE UPDATE

Answer ALS is a large, multi-discipline, cross-institutional effort. Beyond an expansive internal structure, the goals of our study drive us to form additional relationships with external academic and industry partners, as well as the ALS community at large. During this second quarter, we have taken strong strides to forming the governance necessary to initiate, mature and maintain both internal and external relationships.

Data Access Committee

Mission: Answer ALS is committed to making **all data generated through the work of the consortium freely accessible to the ALS research community.** The Data Access Committee will be charged with establishing the guidelines and policies outlining when and how data are made available.

Development: The committee's first meeting was held in New York on the evening of June 28th. Basic operational procedures were discussed and the overall function of the committee was decided. Work is currently underway to develop a document that clearly defines the roles, responsibilities, policies and procedures associated with sharing data and reagents (e.g. iPSCs) generated during the course of the Answer ALS program. Also under development is an internal web page for information sharing and procedural control.

Program Oversight

General program oversight is maintained in multiple ways. The Answer ALS Directors conduct a high-level review of the program once a month. During this call, progress across each arm of the program is discussed and recommendations to enhance performance are made. Frequent communication exchanges are made between sites and Project Managers to address issues of immediate importance, together with regularly scheduled, bi-monthly calls.

During our first quarter we also established an internal website to house common documents, protocols, calendars and other relevant team communications. This site continues to grow as additional arms of the program become increasingly active.

We believe that the continued maturing of these governance mechanisms will ensure we operate efficiently and effectively throughout the life of the program without adding additional burden or costs.

ANSWER ALS PROGRAM UPDATE

CLINICS

At the onset of the program, Answer ALS selected six Northeast ALS Consortium (NEALS) sites to serve as recruitment centers for the clinical trial. These centers were chosen based on their known potential to successfully recruit and retain participants. We are very pleased to announce that as of June 1, 2016, all six sites are actively recruiting and enrolling participants for Answer ALS. As reviewed in detail below, we are on target to meet our recruitment goal of 1000 participants over the next three years.

Site Status and Metrics

Clinical Study Oversight:

Study oversight is accomplished in a number of ways. Frequent e-mail and telephone communication with the sites and Project Managers occurs on a weekly basis to address issues of immediate importance. During the start-up period, the NCRI Coordination Center had bi-monthly conference calls with all sites and monthly internal team meetings. Now that we have entered the maintenance period of the study, the site conference calls and the internal team meetings occur on a monthly basis with the ability to call a meeting as needed.

Answer ALS clinics are also subject to external review by our monitoring team at Barrow Neurological Institute. To date two of our six centers have been reviewed, while the remaining centers are scheduled for review in the near future.

Recruitment Strategies

Recruitment

Our sixth clinical site, Cedars Sinai Medical Center (CSMC), became fully operational at the start of June. CSMC significantly added to our recruitment with the result that we enrolled 190 participants by the quarter's close; 114% of our target (Figure 1).

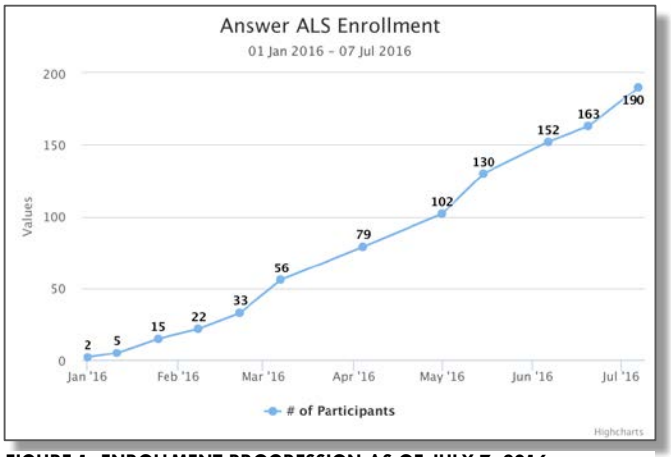

FIGURE 1: ENROLLMENT PROGRESSION AS OF JULY 7, 2016

With the help of Emory's site staff, easy to understand educational materials were created and then refined by a medical illustrator at Johns Hopkins and circulated to all sites (Figure 2). All sites obtained approval from the required regulatory board prior to using these documents. Sites have also been encouraged to create their own study specific Answer ALS recruitment materials.

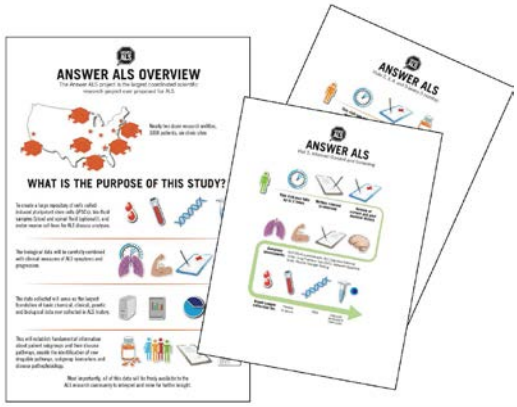

FIGURE 2: PARTICIPANT EDUCATIONAL MATERIALS

Current Challenges, Issues, and Corrective Measures

Current issues that are starting to trend in the first year include:

- Visits may be time consuming for participants
  - To ease the burden on participants, certain assessments can be completed via telephone.
- We'd like to recruit more participants for lumbar puncture (LP; a procedure to collect fluid that surrounds the brain and spinal cord).

- Barriers to this goal include:
  - Scheduling issues and time constraints – The first visit is long and participants are typically tired. Additionally, and especially if participants live far from the clinic, they prefer to limit return visits for additional procedures.
  - It's an elective procedure and more time consuming than a simple blood collection.

- A physician is required to consent the participant for a LP.

- The peripheral blood mononuclear cells (PBMCs) collection rate is up from last quarter, and can be further improved. Issues and opportunities include:
  - Some participants cannot provide enough blood. Much of the blood is saved for future research; PBMCs however are needed right away for generation of the iPSCs. We have asked sites to collect PBMCs first to help eliminate this issue.
  - Some participants sign the consent, but do not have time to complete the procedures on the same day.

    Once a participant returns to complete the screening visit, PBMCs will be drawn.

    We are now collecting metrics on the average delay between consenting a participant and drawing PBMCs.
  - A few participants have been found to have infectious diseases and iPSCs cannot be generated from these participants. A new protocol amendment now excludes these participants.

Decision to return results of whole genome sequencing to participants: With the advances in determining an individual's entire genome, the ways in which this information when, generated in the setting of a research trial, is returned to participants is under active discussion among most medical centers. We need to determine a responsible, efficient and economically feasible mechanism to return genetic data to participants. In developing such a process, there are several considerations:

- Samples require re-testing in a CLIA (Clinical Laboratory Improvement Amendments) certified lab before results can be shared with participants. This is mandatory and absolutely required for licensed physicians.
- Participants should have access to genetic counseling before/after testing. No typical Neurologist or research scientist has the proper training to truly educate participants about all of the genetics results and these discussions require a trained genetics counselor
- Physicians are required to consent participants if there is the option of returning data

Since there is currently no standard practice in the field, we have sought the guidance of a bioethicist at the Berman Institute of Bioethics, Johns Hopkins to help develop a potential plan. We hope that with authoritative expertise, we can formulate an ethical and responsible mechanism that allows participants the choice of receiving their genetic test results.

INDUCED PLURIPOTENT STEM CELLS

Under the supervision of Dr. Clive Svendsen at Cedars-Sinai, the IPSC team has continued to make excellent progress towards their milestones. To briefly review, the lab receives participant blood samples from all our contributing clinics. From this blood, the lab isolates a cell type known as peripheral blood mononuclear cells (PBMCs). These cells can then be transformed into induced pluripotent stem cells (IPSCs) and, following extensive quality control and assurance (a process that can take up to 12 weeks to complete), ultimately motor neurons (MNs) – the cell type which degenerates in ALS (Figure 3). This procedure has been **highly refined and optimized by the team to produce large numbers of high quality cells.**

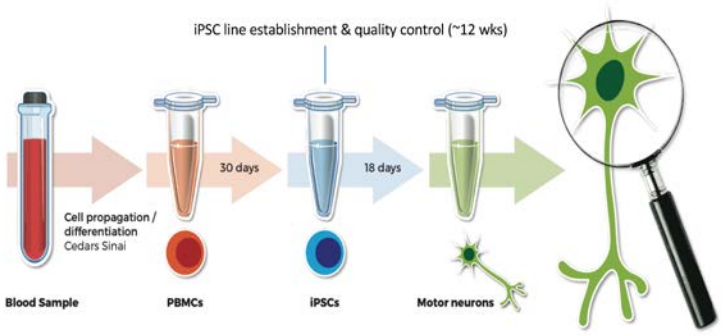

FIGURE 3: GENERATION OF IPSC-DERIVED MOTOR NEURONS

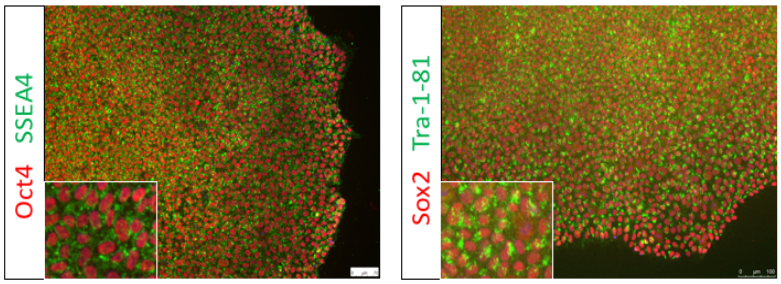

FIGURE 4: IPSCS DERIVED FROM ANSWER ALS PARTICIPANT STAINED WITH PLURIPOTENCY MARKERS OCT4, SSEA4, SOX2 AND TRA-1-81

Status and Metrics

During the last quarter, PBMCs were processed from an additional 65 participant samples. On target with our milestones, IPSC reprogramming was initiated on a further 15 lines, and completed on 12 more (bringing our total to 21 established IPSC lines). Finally, three lines have progressed to MN differentiation. A summary of production totals to date can be found in Table 4.

Production Phase	# Lines	On Track?
PBMC Collection and Processing Total	133	✓
iPSCs Total	54	✓
iPSC Line Initiation	33	✓
iPSC Line Completion	21	✓
iMNs Total	3	✓
iMNs Initiated	3	✓
iMNs Banked and Ready for Distribution	0	✓

TABLE 1: SUMMARY OF IPSC AND MN PRODUCTION DURING THE SECOND QUARTER OF 2016

MULTI-OMICS

Based on our timeline for MN production, sample distribution to our 'omics sites will begin in October 2016. Until that time, the centers continue to prepare the infrastructure and tools required to process and analyze Answer ALS participant samples. As previously reported, our Answer ALS pilot program NeuroLINCS, continues to educate and guide our thinking as we develop each 'omics program. Although sample processing for NeuroLINCS occurs at a much smaller scale, it has allowed our 'omics centers to optimize sample processing protocols, assess workflow timelines and begin to define the needs and requirements for data management surrounding sample analysis. Emerging from that experience was the clear need for a Laboratory Information Management (LIM) system to track samples and monitor workflows at each of our centers. We have now identified such a system and explain in further detail below how it will be implemented across all our work centers.

As mentioned in our first quarter report, it was essential to optimize and scale-up IPSC production to facilitate the needs of our program. The Svendsen lab successfully developed a novel protocol to produce large numbers of high quality MNs. However, the crucial next step was to have our 'omics centers validate this protocol with in-depth biological interrogation of the newly developed MNs. We are very happy to announce that this was achieved during our second quarter.

Second Quarter Accomplishments:

- Omic centers have validated the new MN protocol for replicate consistency
- A LIM system vendor has been selected and system planning is underway

Status

'Omic Center Data Generation

As indicated in the previous report, the 'omic centers have been successful in optimizing protocols for data generation. During the second quarter, the centers focused on validating the optimized MN protocol developed by Dr. Svendsen's group in the previous reporting quarter. The new protocol produces what we refer to as "direct from IPSC motor neurons" (diMNs). The diMNs protocol produces a purer neuronal population that reduces cellular aggregation and facilitates imaging. Approximately 70% of all cells at day 18 express neuronal markers, indicating a higher yield of motor neurons. Finally, it is important to note that this protocol can produce motor neurons from iPSCs derived from both fibroblasts and blood derived cells.

During this past quarter, the 'omic centers validated the consistency and robustness of the diMNs produced by this optimized protocol. A pilot run consisted of samples from two lines (one derived from a Spinal Muscular Atrophy patient (a disease like ALS that affects infants and children) and one from a control patient) in triplicate. diMN pellets were shipped to the 'omic centers for analysis of batch and replicate consistency. **The data we have received for each 'omic test shows high correlation between replicates indicating that the new protocol is robust and capable of producing high quality samples for 'omics analyses**.

Management: Sample Tracking and Management

As indicated in the previous quarterly report, having a sample tracking and workflow system is critical to the Answer ALS program. During this past quarter, we have produced a list of features and functionalities that are required to track our samples from IPSC generation to 'omic data production. We identified two suitable vendors. To assess their capabilities, we produced and distributed a list of initial requirements needed to appropriately track samples through the 'omic workflow. From this

assessment, we identified one vendor currently working with the Cedars Sinai Stem Cell Core facility that also met the Answer ALS criteria for sample tracking and workflow management.

Current Challenges, Issues, and Corrective Measures:

One challenge has been producing a sufficient volume of MN sample for the vast array of 'omics studies required by Answer ALS. Pilot experiments conducted through NeuroLINCs have given us insight into the quantity of cells we will need to manufacture in order to generate sufficient sample material for all of our analyses.

DATA AND TECHNOLOGY

Central to the success of Answer ALS will be the ability to build, curate and integrate large datasets from all components of the study: the clinic, wearables/handheld devices and multi-omics analyses. We have made tremendous progress during this last quarter. Some highlights of this progress include:

- Development of task modules for disease progression assessment via handheld device/wearables
- Establishment of a computational workflow from 'omics data published on the Galaxy platform
- Development of a requirements table for a searchable database of all data generated during the Answer ALS program
- Exploration/maturation of collaborations with industry partners. Major industry partners are keenly interested

Wearables and Hand-Held Android/iOS Devices

The software team at the Johns Hopkins Brain, Learning and Movement (BLAM) lab are well into the development phase of the project. The BLAM Lab has completed development of a user interface for the portable device together with construction of three of the four currently proposed task modules (finger trace, finger tap and gyro trace; Figure 5.) Each task module has been carefully designed to assess specific aspects of upper limb function. The final planned module for the portable device will assess speech to monitor **both physical and cognitive disease progression in collaboration with an industry leader in this area.**

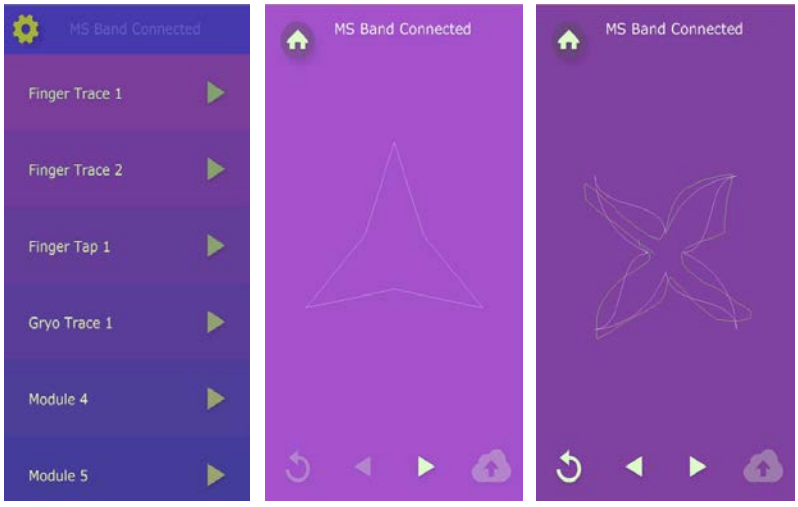

FIGURE 5: SCREENASHOT EXAMPLES OF TASK MODULES DEVELOPED FOR IPHONE/ANDROID HANDHELD DEVICE

Computational Platform for 'Omics Data

In an effort to make our data analysis transparent, reproducible and accessible to the scientific community, we will be releasing our computational workflows. To accomplish this goal, we have adopted the Galaxy platform, which allows both skilled bioinformaticians as well as experimental biologists to view and run computational workflows using a graphical interface. During this past quarter, we implemented a Galaxy instance that runs our analysis pipelines for ATAC-Seq (epigenomics) and RNA-Seq (transcriptomics). This instance will allow users to reproduce and share complete analyses at no cost to them. **Eventually, all 'omic workflows will be available in Galaxy for use by the scientific community**. We have also released several of our analysis modules on the Galaxy Tool Shed. Tool Shed serves as an app store for all Galaxies worldwide allowing other Galaxy users (administrators) to install our workflows and tools into their own Galaxy instance. This will make it easier for scientists to compare the data from their studies to that of Answer ALS. Moreover, to boost our capabilities for large-scale analysis, our Galaxy instance operates on a secure cloud-computing platform, allowing us to process, analyze and integrate new data as they are generated by the Answer ALS research community.

Answer ALS Research Data Portal

In order to make the data generated by the Answer ALS team easily accessible, we are drafting user requirements and specifications for a data portal. The Answer ALS team has established a Data Management team that will guide the implementation of the data portal. Gathering user input will be valuable during this phase of the portal development, and the Data Management team will begin generating a user requirement/specification table. Some of these inputs have already been collected through the NeuroLINCS program. Through this program, we have gathered input from users downloading and using the NeuroLINCS data and this input will be used to draft the initial framework and list of user requirements for the Answer ALS data portal. Once these inputs are documented, we will engage with several developers in order to generate proposals for this body of work. Their responses will help guide our decision on which vendor to use for the development and implementation of the Data Portal.

COMMUNICATIONS

Overview

The participants and the ALS Community are also our biggest drivers for recruitment and retention. Periodic news articles and social media posts continue to create positive energy and encouragement in the ALS community. Online/Internet activity continues to be a leading communications method for the Community.

FIGURE 6: CONCORD MONITOR- APRIL 2016

FIGURE 7: DR. KOLB'S OUTREACH WITH PARTICIPANTS IS ANNOUNCED ON TWITTER

Additionally, and in concert with, other efforts are moving forward through strategic alliances in an effort to accomplish community goals while not spending a great deal in capital. We are continuing aggressive effort for funding Answer ALS

- Initiated year one public strategy campaign with a major sponsor. **Year two is agreed upon and will kick off in September.**
- In talks with a different major sponsor for campaign sponsorship
- Through Team Gleason's relationships with a leading web services company and software development company, Answer ALS is in a unique position for data, application support and secure cloud services.

FINANCIAL STATUS

Overall Status

This second quarter has been very productive in formalizing budgets and milestones. The lead investigators are maturing their efforts and have achieved timely accomplishment of milestones. No payments have had to be delayed or withheld due to lack of performance/achievement.

Our donor commitments have been arriving on time and we are well positioned to fund our milestone efforts through 2016 and 2017.

WHAT'S NEXT

The second half of 2016 involves our continuing maturation and operational expansion. We will be moving from "pilot" efforts in 'omics to ramping up to full production of Answer ALS lines. We will also keep the press on in our ambitious recruitment/retention of participants and collection of samples.

We will continue to refine our data and technology backbone for data collection and analysis working with our Industry partners. This will involve refining milestones, budgets and beginning the build of the architecture.

There is also considerable excitement about our Wearables program and seeing the technology come to life is inspiring. We look forward to bringing our existing sponsors more closely into the program and getting participants enrolled in this facet of the program.

We continue to pursue new resource opportunities and potentials with new and existing partners. Outlook is strong. We will continue to develop those opportunities and others to keep Answer ALS in continued good financial health.

Overall – the last six months have been exceptional. There is even more to anticipate as we enter into our next operational phase with 'omics. We look forward to presenting our continued progress in our next report.
