STUDY DESCRIPTION – SECTION A

TITLE OF PROTOCOL	**ALS Translational Research Program**		
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Sponsor/Funding Source	**ALS Therapy Development Institute (ALS TDI)**		

A1. PURPOSE OF PROTOCOL

Specific Aims

The aim of this proposal is to characterize the relationships between clinical phenotype, genetic background, representation of phenotype in induced pluripotent stem cells and correlation to drug response in ALS patients.

These goals will be accomplished by obtaining a patient's consent to collect tissue samples (blood) for DNA sequencing and molecular profiling (RNA and protein) and a skin biopsy to develop an induced pluripotent stem cell line (iPS) for banking and high content drug screening at ALS TDI.

Patients will be asked to wear accelerometers on their wrists and ankles for one week per month to collect unbiased movement data and compare to the ALS FRS questionnaire, a self reporting web based questionnaire to monitor disease progression.

A database will be developed to link a patient's genetic data, molecular data, and high content screening data with disease progression assessment methodologies.

Specific Aim 1: Reprogram skin fibroblasts from patient biopsies into pluripotent stem cells for banking.

Specific Aim II: Differentiate iPS lines from the skin biopsies of newly diagnosed ALS patients into neurons and astrocytes for phenotypic screens and drug screening assays. ALS TDI has developed several high content screening assays from primary neurons and immortalized cell lines that are related to ALS clinical phenotype including cell stress, oxidative stress, and protein mis folding.

Specific Aim III: Sequence the genome of each patient entering the study using whole exome and full genome (30X coverage) sequencing technologies.

Specific Aim 4: Collect accelerometer data for one week a month to compare the sensitivity of unbiased movement to data to web based ALS FRS self reporting questionnaire.

A2. SIGNIFICANCE AND BACKGROUND FOR THE STUDY

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disorder resulting in progressive muscle weakness, atrophy, and paralysis. ALS is an orphan disease with an incidence rate of approximately 5,000 annual cases diagnosed in the United States on an annual basis. The median survival times after symptom onset is five years resulting a prevalence of the disease in the United States being approximately 30,000 individuals. There is only a single FDA approved treatment for ALS, Rilutek, which offers patients no improvement in quality of life and a minor improvement in survival benefit of 90 days. The development of effective treatments for ALS is a significant unmet need that is hampered by patient heterogeneity. The vast majority of ALS cases are sporadic in nature (90%) with the remaining 10% of genetic cases being associated with over twenty different genes with varying biological functions.

This proposal has several innovative objectives that are critical to developing new treatment strategies for ALS and other chronic neurodegenerative disease conditions. There have currently been limited studies that carefully track patient history and disease progression in ALS and correlate the clinical phenotype to tools that are now used for drug development such as patient derived induced pluripotent stem cells. Greater than 90% of ALS is sporadic in nature highlighting the need to correlate clinical phenotype and phenotype from iPS cells to background genetics. Therefor this study will also sequence the entire genome from each patient enrolled in the study in order to elucidate genetic background to clinical phenotype.

Rationale

In the last decade more than 20 different phase III clinical trials have failed to reach clinical endpoints in ALS clinical trials. Recent advances in genomics and proteomics technologies have opened the door for personalized medicine strategies in clinical oncology. Mutations in v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 (Erbb2, Her-2) and epidermal growth factor receptor (EGFR) have led to genetic screening for these mutations and the development of the FDA approved drugs trastuzumab, Lapatinib, Erlotinib, and Gefitinib.

A critical issue that has hampered the successful advancement of treatments through the clinical trial process in neurodegenerative diseases is patient heterogeneity.

A major advancement that this proposal will create is the software and database infrastructure to link clinical phenotype, genetic background to phenotypic outcomes from cell based assays using iPS cell lines from patients. Patterns from a database of sporadic ALS patients may lead to new targets for drug development in ALS.

A3. DESCRIPTION OF RESEARCH PROTOCOL

A. Study Design – Overview, Methods, Procedures

The proposed study is a research proposal to obtain the consent of ALS patients to donate blood samples for DNA sequencing, RNA profiling, protein profiling, and a skin biopsy for the generation of an induced pluripotent stem cell line. Subjects will asked to wear accelerrometers in their wrists and ankles for one week per month for at elast eight hours per day. Acceleromoters will be sent back to ALS TDI and redeployed to subjects by ALS TDI via Fedex pre paid shipping envelopes.

The proposal will recruit Three hundred ALS patients and fifty controls in the next twelve-eighteen months. Patients will be asked to waive their HIPAA rights so that an online database can be developed to track disease progression rate and correlate genetic information from DNA sequencing to phenotypic outcomes.

Recruitment
An email blast will be sent to internal supporters of ALS TDI describing the program after IRB approval. The email blast will be confidential and sent to individual patients registered in ALS TDI's database. The email will provide a description of the procedures, the aims of the study, a description of the required consent and waivers, and contact information if you are interested in participating in the study.

Prescreen:
Patients who respond via appropriate contact that was provided in the email will be contacted via phone by the project coordinator at ASLTDI who will describe the program and review the goals and, consents, and waivers and answer any additional questions.

Scheduling
Patients who are interested after the prescreen and have read the waivers and consent will coordinate the visit to ALS TDI through the project coordinator. The project coordinator will contact Mass General Hospital Dermatology Clinic (MGH) and arrange the scheduling for the patient's visit to MGH for sample collections. MGH is acting as a contract research organization for tissue collection for the project. Subjects will be sent required patient consent and waivers two weeks prior to scheduling their clinical visit. Signed documentation will be verified before scheduling their appointments for skin biopsy, blood collection, and cheek swab at MGH.

Appointment
Patients will be driven to MGH by an ALS TDI staff person. ~~Copies of all required consents and waivers will be brought to MGH at the time of visit~~. ALS TDI will provide barcoded sample collection materials for each sample which will contain an anonymous barcode and no patient information. The barcode identifier will link to relevant patient history in ALS TDI's database. Patient and samples will be delivered back to ALS TDI by an ALS TDI program manager. The skin biopsy will be sent to Charles River by ALS TDI for viral screening and safety then shipped to ALS TDI.

Tissue Collections:

Blood Draws

- ALS TDI will provide the phlebotomist at MGH with barcoded PAX gene and heparin collection tubes containing a unique identifier.

- The phlebotomist will collect three tubes for 5 mls of blood into a each gene tube for molecular profiling and DNA sequencing

Skin Biopsy Collection

ALS TDI will provide the dermatologist at MGH with a collection tube containing a unique barcode identifier. The dermatologist will rub numbing cream on the site location for the biopsy, usually from your forearm, thigh or lower back. After the skin is numb, the site will be cleansed with an antiseptic solution and an additional, injectable numbing medication will be given. The injection may sting a little, but the numbing cream should lessen the burning sensation. Once the skin is completely numb, a 3 millimeter piece of skin (about the size of a pencil-end eraser), will be removed. The biopsy site may be closed with a stitch. Participants will be provided with instructions on the care of the skin biopsy site.

B. Statistical Considerations

a. *Sample Size Justification*: This is a research proposal with no prior knowledge of samples size estimates to determine the number of samples required to correlate genetic background to phenotypic outcome. Given that 90% of ALS cases are sporadic in nature with no known genetic mutation we are assuming it will take greater than 100 established cell lines and genomic sequences to allow un supervised clustering of phenotypic outcomes to genomic sequence. The goal of this phase is to build the tools and data integration systems necessary to perform a first pass analysis of genomics data to clinical phenotype and phenotypic analysis of a patients iPS lines when challenged with exogenous stimuli.

b. *Data Analysis:* ALS TDI has a robust data management system that assigns a unique identified and barcode to each tissue sample that is generated from any source. Each unique identifier can then have multiple clinical attributes associated with it including age, gender, age of diagnosis, site of onset, medication history, tissue type ect. ALS TDI has robust genomics capabilities including Affymetrix RNA profiling and whole genome sequencing data infrastructures built in SciTegic Pipeline pilot and a backend using Bioconductor and R code. Data from the project will be entered into these systems for downstream data quality control and processing. The Bioconductor packages have a suite of clustering tools to analyze multidimensional complex data structure to identify patterns in genomics, RNA profiling and clinical data obtained from this project,

C. Subject Selection

Participants will be screened by phone prior to visiting ALS TDI. Patients will sign and return all consent forms and waivers prior to visiting ALS TDI.

Inclusion Criteria

Participants eligible for inclusion in this study have to fulfill all of the following criteria:

1. Male or female healthy volunteers or patients
2. Any background ethnicity
3. Age 18 years or older.
4. Sporadic or familial ALS diagnosed as possible, laboratory-supported probable, probable, or definite as defined by revised El Escorial criteria.
5. Capable of providing informed consent.
6. Geographically accessible to the site.

Exclusion criteria

Participants meeting any of the following criteria during screening evaluations will be excluded from entry into the study:

1. History or excessive bleeding of on anti coagulants of any kind including but not limited to daily aspirin, Plavix
2. Treatment with an immunosuppressant medication within 30 days of the Baseline visit.
3. Active infection (acute or chronic).
4. Presence of tracheostomy.
5. The presence of unstable psychiatric disease, cognitive impairment, or dementia that would impair ability of the subject to provide informed consent , according to PI judgment, or a history of active substance abuse within the prior year.
6. Clinically significant history of unstable or severe cardiac, oncologic, hepatic, or renal disease, or other medically significant illness.

A4. POSSIBLE BENEFITS

There are several beneficial aspects of enrolling in the program. One important aspect is having ones whole genome sequenced as part of the program. The vast majority of ALS cases are sporadic in nature (~90%) with no known genetic association. For patients with the familial form of the disease more than 70% of these cases now have an ability to identify the underlying genetic cause that is associated with about 27 genes. Recent data suggests that it is very common for sporadic cases of ALS to have mutations associated with common ALS genes including SOD1 and C9ORF72. This will allow these patients to know if they have mutations in genes commonly associated with ALS. For sporadic cases with no known mutations the long term goal of clustering the data into clinical phenotypes based on iPS cell phenotypes may allow us to identify new genetic risk factors associated with the disease that maybe relevant to specific ALS cases and their family members.

Another benefit is that the program will bank pluripotent stem cell lines for each patient after reprogramming. From a clinical perspective this technology is still very new and future application to the treatments of patients is quickly evolving. A banked stem cell may prove valuable for a patient as new applications are developed and maybe useful for other family members in the future.

Finally the development of research tools for high content screening from a patients owns cells could lead to early opportunities fro drug repurposing for a patient in the program.

There is a significant benefit for the ALS community as a whole. The construction of a comprehensive database that couples clinical phenotype to genomic sequence data and phenotypic screens on patient cell lines will lead to a more understanding of disease mechanisms, drug development opportunities, and effective treatments for patients. The coupling of genomics to these endpoints will also lead to rare genetic associations with ALS that have not been characterized.

A5. POSSIBLE RISKS AND ANALYSIS OF RISK/BENEFIT RATIO

The actual clinical procedures have little risk for patients outside of bleeding from the skin biopsy which in some cases may require a suture to stop the bleeding. The potential for some scarring around the skin biopsy. There may be localized discomfort from the blood draws.

The most important factor to consider is the need for genetic counseling should a patient choose to wan to know any mutations that are identified in the genome sequencing component of the proposal. All ALS clinics have genetic counseling available and for those patients that have a desire to discuss their genetic information ALS TDI will facilitate genetic counseling at their ALS clinical prior to discussions.
With human subjects research, there is always the risk of the loss of confidentiality. ALS will take all reasonable precautions to protect the participant and their data.

A6. RECRUITMENT AND CONSENT PROCEDURES

There will be limited and confidential recruitment of patients via an email blast and website information to patients that currently support ALS TDI. The website will contain a brief description of the project goals to link patient medical information to genetic sequence and research on an induced pluripotent stem cell line derived form the patient. It will ask patients who are interested to call ALS TDI for further information and prescreening. ALS TDI has more than 10,000 ALS patients in their database and a confidential solicitation will go out describing the program. There will also be information on ALS TDI's website but all members of the ALS TDI community are required to register to utilize these functions on our secure website.

Both of these advertisements will have a website link to a contact information page. The patient will be asked to enter their name, email address, and phone number. Once submitted the patient will be notified that they will be contacted shortly. The data will be entered into a database at ALS TDI and an internal email will be sent to the project manager. The project manager will contact the patient by email/phone to set up the prescreening interview.

Patients who are interested will be directed to a call in number to speak with our project coordinator about the program and help decide if a patient fits the inclusion criteria and understand the patient consent requirements and HIPAA requirements for the project.

Consent –
During the prescreen the patient will be asked questions as outlined in the prescreen document attached. All data will be entered in the database. The program manager will also take the time to discuss the tissue samples being collected and the purpose for these collections. The program manager will also describe the consent form and HIPPA waiver with the patient.

If a patient fits the criteria and can travel to ALS TDI they will be asked if they would like to participate in the program. If they would like to participate the program manager will ask if their care taker or spouse would like to participate as a healthy control in the study

a) If yes then program manager asks patient to have spouse initiate a new contact via the web portal to start a new record for the spouse

2) Program manager emails them web link to electronic consent questionnaire and HIPPA waiver to be completed by patient and healthy volunteers

Schedule Visit
Once the electronic documents for consent and waiver are received a program manager will reach out

to the patient and/or control to schedule a visit to ALS TDI and schedule the appointments at MGH

HIPPA
Patients will sign a complete HIPPA waiver allowing ALS TDI to decode their medical history and information and assign these data to all data associated with the project including but not limited to genomic data, molecular profiling data, and data generated from cell lines and tissues.

A7. STUDY LOCATION

All prescreening interviews will be conducted over the phone by the project coordinator at ALS TDI. Patient consent forms and waivers will be distributed to patients electronically. The verification of written consent and verbal consent will be conducted at ASL TDI on the day of the visit prior to transport to MGH for tissue collection.

Blood and skin will be collected at MGH Hospital according to the study protocols.

No further patient follow up will be required