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NORTHWESTERN UNIVERSITY

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Mr. and Mrs. Robert Blais
Blazeman Foundation for ALS
18 Maple Avenue, PMB 121
Barrington, RI 02806-3560

Dear Bob and Mary Ann,

I certainly regret that we have been so involved with our ALS projects and applications for direly needed funding that this update has been long in coming. For the first time since 1985 there will be a gap in Federal funding for my work, so we have written six applications this year. It is ironic that this difficult period in funding comes at a time when we have made very important discoveries and are on the threshold of new and exciting developments in ALS. These developments are not yet known to the wider community.

Following is a brief summary of where we are with the iPSC project. This is a time consuming, highly technical project that requires great care at each step. It is also groundbreaking work from which we are learning as we go.

From our application to you, I know you are aware of the many steps required. I think it's easiest to summarize what we've done thus far as it relates to each objective.

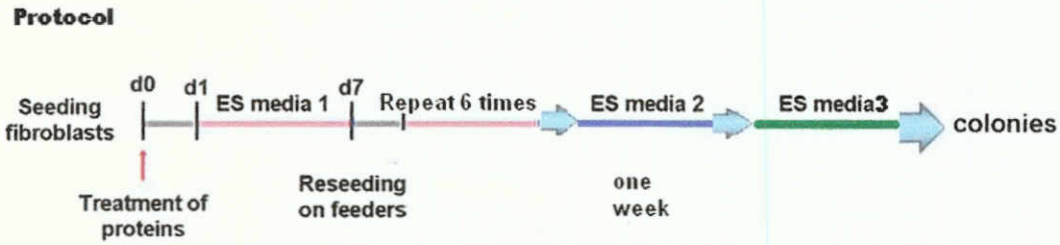
Plans

1. We have continued to collect skin samples through the Registry and culture fibroblasts from them. We are looking for samples from specific people: 50 patients with SALS, 50 patients with known mutations in SOD1, TDP-43 and FUS, as well as age and gender matched controls (such as unaffected siblings without mutations for FALS). While we are aiming to do this within 2 years we may be limited by the scarcity of families with ALS from known mutations.

   We are well on our way with our collection. We have sampled 64 people thus far (61 with ALS), including 8 SOD-ALS patients, 1 FUS-ALS patient, 1 TDP-43 patient and 1 patient with X-ALS, 6 patients from families with unknown genetic mutations, and others from families with related disorders or sporadic ALS. We have started collecting control samples as well, with 3 thus far. Fifty two cell lines have been obtained, with a failure of two. Six are under culture and one was resampled so, thus far we have seen only one failure.

   Thus we have been able to establish fibroblast lines from 52 of these samples. The remaining samples are in various stages of that process, something that generally takes from 6-8 weeks.

2. Establishment of iPSC lines. Once skin fibroblasts are optimized to 20% of cell density, they are ready for transduction of four recombinant proteins (C-myc, Klf4, Oct4 & Sox2). Just the induction process takes 8-10 weeks, and then the iPSC clones can be dissected under the microscope and transferred to feeder cells of mouse embryonic fibroblasts for growing and expanding. This is a brief diagram of that process.



Yi Yang is evaluating different conditions that can influence outcome so that we will be able to do this as successfully as possible. Our original plan was to use the above method only, but a newer, quicker strategy using lentivirus is now available. Each method has strengths and challenges, so our plan now is to use both, the strength of one obviating the shortcomings of the other. The lentivirus strategy requires special training, which Dr. Yi Yang has taken here at the University Cancer Center in the virology laboratory of Dr. Hope. Additionally, the University Office for Research Safety requires we obtain special certification before we can use this strategy. We have applied for lentivirus usage and expect to receive it next month. It has taken an inordinately long time because Northwestern does not have an active lentivirus program which has meant extra scrutiny. In the meantime, we have ordered our supplies and are ready to go once we have the authorization.

As you will remember from our proposal, there are additional steps from here to generate iPSC, and even more steps in testing potential therapies. However, we have learned over the years that careful, solid underpinnings serve us well, so we are solidifying these stages so we can then jump into the next phases, generation of stem cells and disease specific motor neurons. As you know, we take the long view, that this kind of work, which will enable us to understand how ALS develops and provide us with molecular targets for therapeutic intervention, is the only sure method for devising effective treatments for ALS.

In the mean time the Blazeman Fellow has also significantly contributed to a very important paper describing a new gene that causes ALS and ALS/dementia. The protein coding this gene is abnormal in all of ALS and ALS/dementia as well as dementia without ALS. This protein may be key in the regulation of the degenerative process. The paper is under review in one of the top scientific journals in the world.

I am extremely grateful for your generous funding and your foresight in understanding that this is a step-by-careful step process that can yield groundbreaking results. At this stage of research and the funding climate your help and supports provides oxygen for survival to continue this important work to which I have dedicated my best years.

Sincerely,

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