

REPORT ON THE ICSCN WORKSHOP ON PROGRESS TOWARDS CLINICAL TRIALS USING STEM CELLS FOR ALS/MND

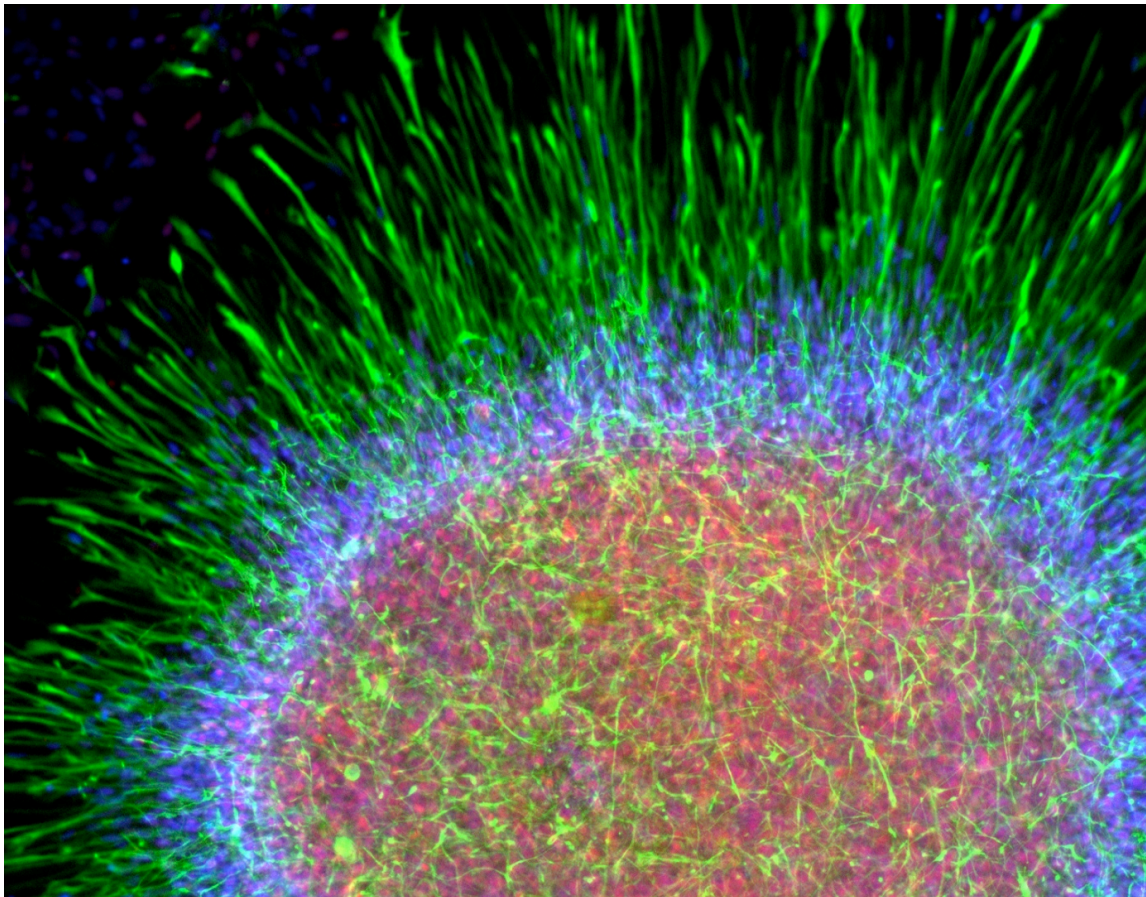


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This report was written by Maya Chaddah (freelance science writer), and edited by Dr. Lucie Bruijn (The ALS Association), and Dr. Brian Dickie, (Motor Neurone Disease Association).

INTRODUCTION TO THE WORKSHOP

On January 24, 2011, 63 participants gathered in New York City for the *International Consortium of Stem Cell Network (ICSCN) Workshop Towards Clinical Trials Using Stem Cells for Amyotrophic Lateral Sclerosis (ALS)/Motor Neuron Disease (MND)*. The workshop was co-hosted and sponsored by the ICSCN, The New York Stem Cell Foundation, The ALS Association, the Motor Neurone Disease Association, UK National Stem Cell Network, and the Stem Cell Network (Canada).

Kevin Eggan, on behalf of the New York Stem Cell Foundation, and Ben Sykes, on behalf of the ICSCN, welcomed the international contingent that hailed from fourteen countries and included representation from sixteen universities, ten associations and foundations, nine institutes, four hospitals/centers, three biopharmaceutical companies and the regulatory field. The workshop piloted a series of international meetings, conceived by the ICSCN in 2009 with the intention to bring together stem cell researchers from disparate global communities in order to catalyze broad discussions in disease areas where there is limited expertise in any one country. The successful collaboration among the ICSCN, The ALS Association, MND Association and other leading charities and associations in the USA and Canada provided the means for coalescing the unique composition of multidisciplinary and experience-rich speakers and participants, who together explored six broad themes during the workshop.

1. *Overview of ALS/MND*
2. *Cell Derivation and Characterization*
3. *Pre-Clinical Research Using Stem Cells*
4. *Phased Clinical Trials Involving Stem Cells*
5. *Latest Developments in Induced Pluripotent Stem (iPS) Cell Technology*
6. *Industry Involvement and Investment*

Punctuating the second day of research-oriented talks were three panel sessions: *Unregulated ALS/MND Treatments and Public Education; Review of the State of the Art in Stem Cells for ALS/MND* and *Barriers to Progress and Future Prospects for Stem Cell Based therapy for ALS/MND*. By the close of the workshop, the moderators had ample food for thought to distill the key messages emanating from participants around the opportunities for using stem cells in clinical trials for ALS/MND. [Throughout the report, ALS/MND is referred to as ALS.]

1. OVERVIEW OF ALS

Dr. Peter Andersen introduced the three session speakers who together set the stage for the downstream presentations by providing an in-depth review of ALS. Dr. Leonard van den Berg made the case for describing ALS as a complex disease where the interaction of genetic and environmental susceptibility factors contributes to a clinical and pathological spectrum of disease phenotypes. To unravel this complexity, the field needs to initiate large exposome-wide studies, including incident populations, occupations, nutrition, exercise (especially marathon) and electromagnetic fields; to discover more genes, better biomarkers and predictive algorithms for identifying endophenotypes; and to perform genome wide association studies that identify single nucleotide polymorphisms common to different neurodegenerative diseases. The results from these types of studies may identify variants with intermediate penetrance, and thus contribute to a changing concept of the etiology of familial versus sporadic ALS. Some of the foremost technologies that hold promise for studying ALS include exome sequencing, MRI scans, novel neurophysiological techniques, proteomics and metabolomics of tissue and fluids, and iPS cells to make motor neurons from ALS patients.

Since 2001, a number of genes implicated in familial ALS have been identified, and massive parallel sequencing is providing opportunities for rapid progress toward finding and characterizing cells that have other endogenous mutations. Five genes have been shown to segregate with classical ALS +/- FTD (frontotemporal dementia): SOD1, FUS, TDP-43, Optineurin, and VCP. In asking which could be used to model disease and develop therapies, Dr. Chris Shaw described the TDP-43, FUS, and VCP mutations and suggested that multiple pathways may converge to give rise to the proteinopathies observed in most forms of ALS and some forms of FTD. The challenge will be to identify which pathways are mechanistic and which contribute to cell death. Creating better models that replicate disease, characterizing samples from normal individuals and generating iPS cell lines from patients may help to validate the idea that precise morphology and subcellular localization of mutant aggregates map to different disease phenotypes.

Dr. Lucie Bruijn's talk delved into the opportunities for stem cell research for ALS. In the context of disease mechanisms, drug screening/therapeutics and transplants, the important questions to address will be the cell types (motor neurons, spinal, cortical) to use in creating assays, the maturity of cells when developing assays for screening, mimicking the disease phenotype within the assays, and making them relevant for high throughput screening of therapeutic compounds. One of the main opportunities for stem cells lies in their ability to mimic disease in a dish. This is an important requirement moving forward given the inherent challenges with creating new mouse models. The generation of motor neurons from embryonic stem (ES) and iPS cell lines from SOD1 wild-type and mutant mice as well as iPS lines from patients with FUS or TDP43 mutations will provide powerful avenues for comparing mouse models and tissues and exploring changes in phenotypic expression via RNA sequencing. Techniques such as automated microscopy can provide a window onto disease phenotype and an opportunity to look at individual cells. In addition, trophic withdrawal assays offer the ability to test whether small molecules added to iPS or ES-derived lines could improve survival and identify hit compounds.

"These are early days with regards to iPS cell methodologies."

2. CELL DERIVATION AND CHARACTERIZATION

Session chair Dr. Don Cleveland began by challenging the participants to work towards identifying the best motor neuron lines for future use. This is no simple task, given the ever-increasing number of studies on making iPS lines, and understanding the fundamental question of what makes a neuron a motor neuron will be critical to success.

Dr. Kevin Egan reviewed two strategies for developing motor neurons for studying ALS. The first involves benchmarking iPS cell lines, from healthy individuals or ALS patients, against ES-derived lines for their ability to generate functional motor neurons. Starting with a brute force approach followed by whole genome bisulfite sequencing and genome-wide transcriptional profiling of large panels of lines, he learned that there is no obvious signature that sets iPS lines apart from ES lines and that both make motor neurons with similar efficiencies. Of great interest to the participants was his development of a simple scorecard for predicting stem cell behavior. When used to characterize iPS cell lines, a picture emerges where their unique propensities originate from the donor cells and from HOX gene pattern expression. The second strategy, motor neuron transdifferentiation, uses different transcription factors to directly program fibroblasts from adult mouse or human ES cells into functional motor neurons. Transdifferentiation was achieved for both cell types, albeit with less efficiency for human ES cells. His next step will be to optimize the ideal set of factors, to identify those that increase motor neuron survival and to study the effects of glial cells on motor neurons.

Dr. Hynek Wichterle's work on differentiating spinal motor neurons from ES cells teaches us that there is more than one class of motor neuron. In fact, motor neuron subtypes are diverse and can be distinguished by the region they innervate and the expression of different transcription factors, such as *Hoxa5*, *Wnt* and *FGF*. His preliminary in vitro studies also show that changing culture conditions can shift one motor neuron subtype to another. In the pursuit of developing a cell based screen, his next experiment will be to vary in vitro survival conditions to test iPS cell lines from ALS patients and controls. An ongoing challenge for the field will be to identify the best controls for downstream experiments; for example, either using iPS cell lines from healthy people or from subjects with known, and then corrected, mutations.

Central to Dr. Siddharthan Chandran's work on motor neuron diversity and modeling is the premise that every motor neuron is spatially defined in situ by extrinsic signals, and that such diversity will be important for establishing in vitro platforms of inherited ALS. One arm of his in vitro studies at University of Edinburgh described a method for generating retinoid independent motor neurons from human ES cells. The motor neurons show functionality, exhibit distinct subtype diversity and a bias towards caudal identity. Dr. Chandran has also developed an in vitro human iPS cell platform, modeling TDP-43-related neurodegeneration, that will be useful for quantifying the expression of TDP-43 and applying stress paradigms and parallel biochemical studies. The generation of new in vitro assays should take stock of questions about ALS, such as whether the disease is purely a disorder of motor neurons and also what happens to motor neurons post-mitotically, given that ALS is an age-related disease.

Session II: Barriers and Challenges to Progress

Cells and lines

- *Identifying good controls*
- *Finding better in vivo markers for motor neurons and astrocytes*
- *Identifying phenotypes for human iPS-derived motor neurons*
- *Developing culture conditions for long term differentiation (e.g., for co-cultures of motor neurons and myotubules)*
- *Understanding epigenetic memory of iPS cells*

Assays

- *Developing fluorescent iPS cell lines for screening assays*
- *Preventing ongoing neurogenesis in survival assays*
- *Ensuring synchronous cell line production from lab to lab*
- *Developing robust screening assays from human cells*
- *Optimizing the purification of human cells (e.g. to maximize numbers for FACS sorting)*

“Curing ALS will be difficult but slowing progression may be possible.”

3. PRE-CLINICAL RESEARCH USING STEM CELLS

Dr. Pamela Shaw introduced the four speakers who presented data on pre-clinical research using ES- and iPS-derived cells to develop drug screens and cell-based therapies. Dr. Christopher Henderson presented insights from his work on designing screening assays and looking at co-cultures of mouse embryonic motor neurons. A trophic survival assay developed in his lab and established at a French company Trophos to test over 50,000 compounds on pure rat embryonic motor neurons lead to the identification of a compound that may target mitochondrial function and prove promising to patients. The compound is currently being tested in clinical trials in Europe. One of the major challenges in the survival assay is trying to control the noise due to the tendency of stem cells to generate low levels of motor neurons. From the co-culture assays using ES-derived motor neurons seeded on Chinese hamster ovary (CHO) cells he has learned that cervistatins are a 500 fold more potent promoter of axon growth than the Rho-associated protein Kinase (ROCK) inhibitor benchmark. Next he will do similar experiments using ALS mice. In response to queries from participants, Dr. Henderson stressed that the current models are simple and measure readouts of survival and axonal growth rather than synaptic stability which, although more biologically relevant, is much more difficult to measure.

Dr. Stefan Liebau began his talk with the provocative statement that all mouse models had failed in terms of curing and finding new drugs. Because the iPS cell model can be patient specific, it offers the best hope for drug development and cell based therapy. The focus of his lab is to produce model systems of pure motor neuron cultures and/or co-cultures with astrocytes and myotubes to look for characteristics of ALS patient-specific cells, and even possibly to study the pathogenesis of the neuromuscular junction. He intends to collaborate with the pharmaceutical industry in order to identify targets for drug screens. The resources for his project are extensive, and include a huge number of ALS families from whom cells are collected. From preliminary

work, he has learned that one must consider the purity of motor neurons and the variation in motor neurons derived from different patients, and also that iPS cells appear to have an epigenetic memory about their location of origin.

In partnership with investigators in San Diego, including principal investigator Dr. Larry Goldstein, and funded by the California Institute for Regenerative Medicine (CIRM), Dr. Don Cleveland is developing a cell-based replacement therapy for ALS using human ES-derived astrocytic precursors. Chimeric studies have shown that expression of mutant SOD1 in motor neurons influences the onset of disease; however, it is the astrocytes expressing the mutant protein that drive the rapid progression. Eliminating SOD1 mutant protein from the astrocytes in transgenic mice significantly extends their survival. He hypothesized that ALS is not cell autonomous, and that mutant motor neurons can be saved by providing a better neighborhood of astrocytes that might mitigate rapid disease progression. The Californian team intends to convert human ES cells to astrocyte precursors and inject them into the cervical or lumbar spinal cord. The astrocyte precursors will be grown under good manufacturing procedures (GMP) standards and the surgical techniques will be done in partnership with Life Technologies. They have already shown broad engraftment of human neural precursors into mice as well as migration of the grafted cells and astrocyte differentiation. Their next step is to test whether astrocyte grafts will engraft, differentiate, migrate and help to slow neuronal death in rodent models. If successful, they will move to a larger animal model, the mini-pig, and test for engraftment of human ES-derived astrocytic precursors while building in the necessary safety measures, such as testing for aneuploidy, sterility, mycoplasma, and depletion of the remaining undifferentiated cells. These experiments should indicate the best cell line to take forward to the clinical trial, planned for 2014.

Dr. Clive Svendsen reviewed the progress to date in generating clinical grade human neural progenitor cells to improve respiratory output in the SOD1 G93A rat model of ALS. His preclinical work has involved transplanting human neural progenitor cells into the transgenic SOD1 G93A rat model of ALS. Although he has had mixed success with engraftment, he did show that GDNF-secreting human progenitor cells protect dying motor neurons in the rats at 105 days. The lack of functional effect despite excellent cell body survival has been attributed to axonal pullback (inability to repair the neuromuscular junction). Human neural progenitor cell grafts can also significantly improve phrenic motor output, but the survival of such motor neurons in this system has yet to be determined. They are in the process of testing whether neural stem cells alone or those releasing GDNF, IGF-1, VEGF or BDNF could help muscle cells to survive. Currently, the Neuralstem clinical trial is underway and 8 ALS patients have been transplanted with human neural fetal progenitor stem cells isolated from spinal cord.

Session III: Barriers and Challenges to Progress

Animal models

- *Developing larger disease animal models to facilitate longer growing times of transplanted cells (e.g., SOD1 G93A mice live only 3 months)*
- *Creating regimens for preventing graft rejection*
- *Measuring synaptic stability in vitro*
- *Preventing colony drift in SOD1 G93A rat model*
- *Addressing short lifespan of SOD1 G93A rats versus long growing maturation time of human cells*

Pre-clinical research

- *Dealing with the lengthy duration for generating clinical grade human neural progenitor cells free of chromosomal abnormalities*
- *Preventing 'mission-creep' (i.e., by using good administration teams and Gantt charts)*
- *Dealing with overwhelming legal documentation and the unpredictability of the FDA*
- *Partnering early with the FDA during experimental phases to avoid delays when planning the clinical trials.*

"I will remember that there is art to medicine as well as science, and that warmth, sympathy, and understanding may outweigh the surgeon's knife or the chemist's drug."

4. PHASED CLINICAL TRIALS INVOLVING STEM CELLS

Dr. Merit Cudkowicz chaired the session that began with Dr. Nick Boulis presenting pioneering work on the surgical nuances around safety and accuracy of spinal cord transplantation. His team used a pig animal model to optimize the stability, accuracy and consistency of their cell delivery platform, and also to assess drug types, doses and duration required for immunosuppression to prevent graft rejection. Dr. Boulis has taken the procedure into a small phase I trial - the first FDA-approved human stem cell trial in ALS patients in which 18 ALS patients are to be treated with neural stem cells derived from human fetal spinal cord. The data safety and monitoring board (DSMB) oversees the various stages of the trial and if there are no serious adverse events the trial is able to proceed. Advances in their surgical techniques have shortened the duration of the surgery to 3 hours. Although they have overcome a number of challenges, such as loss of anatomical landmarks in the event of bleeding at the injection site, they still have a considerable issues to address prior to taking the procedure into phase 2 trials where the effect of the transplant can be assessed. The team is creating a failsafe switch by transducing the cells with herpes cyclodine kinase, which would kill them in the event that complications arise from the graft. Because neurosurgery is extremely risky, he voiced the need to define what is considered meaningful for patients in order to assess the acceptable level of morbidity.

Dr. Jonathan Glass recapped the perspectives gained from the ongoing Neuralstem phase I trial. The advantages of using neural stem cells derived from human fetal spinal cord – no tumour

formation, minimal HLA (human leukocyte antigen) expression, no ABO blood type activity – result in a low overall antigenicity of the cells. The first surgery of the trial took place a year ago, and the 9th surgery was performed earlier this year, without the need for patients to be on ventilators or to be taken to intensive care post-operatively. The trial was staged, first enrolling non-ambulatory patients, and the first ambulatory patient was enrolled early this year. The main adverse events have been GI upset, transient neuropathic pain, bladder problems and immunosuppression. They have yet to test the mechanism of rejection, but in similar work Geron has not seen a response in either T cells or NK cells. Some looming questions include the number of patients needed for phase II trials, how to measure a response over a short time frame (currently the most reliable measure is changes in survival making trials very long), how to design trials given the ethical and scientific considerations of placebo-controlled trials, and who will pay for them.

Dr. Daniel Offen summarized his work on the impact of neurotrophic factor delivery via mesenchymal stem cells (MSCs). Although it is known that neurotrophic factor (NTF) treatments can protect neurons, many ALS trials have failed, perhaps from the incorrect choice of factor, mode of delivery or experimental model, or due to the enzymatic breakdown of the factors. His approach is to make MSC-NTF secreting cells by modifying adult MSCs to become astrocyte-like cells that can supply endogenous neurotrophic factors. For the purpose of transplantation, the resulting MSC-NTFs have the advantage of being autologous and therefore do not require immunosuppression. From his studies thus far he has gleaned that MSC-NTF conditioned media protects cultured motor neurons and that MSC-NTF derived xeno-free growth media, a technology licensed to Brainstorm, markedly increases the growth of MSCs. In addition, MSC-NTFs derived from ALS patients secrete the neurotrophic factors BDNF and GDNF. Transplantation studies in mice and rats thus far have also proved successful. For example, mouse MSC-NTFs transplanted into hmSOD1 (G93A) mice improved motor functions and survival. The intramuscular transplantation of rat MSC-NTFs into a rat model for sciatic motor nerve injury preserves motor function, protects neuromuscular junctions and accelerates regeneration. Brainstorm recently gained approval for a phase I clinical trial for adult stem cell therapy for ALS patients. The strategy would be to perform intrathecal injections of MSC-NTF cells into early stage ALS patient and to follow them for 3-6 months to evaluate safety and tolerability as well as clinical changes and overall survival.

Dr. Letizia Mazzini described the lessons learned from the first long term follow-up of the second of two phase I clinical trials on autologous mesenchymal stem cell transplantation in ALS in Italy. Nine years after the surgery, 10/11 patients are still alive and, according to her studies, have coped well psychologically, despite the huge expectations and media pressure. In decreasing order, the most common adverse effects were trunk pain, sensory light touch impairment, tingling sensation, and sensory light touch impairment in the sacral region. The lack of tumour formation/abnormal cell growth was the most important outcome of the study. Three of the patients, albeit very young, showed a long period of stabilization after the surgery but for all patients, the quality of life as measured by SEIQoL was similar to that of the point of recruitment. At the last interview, 9/11 patients expressed satisfaction at having participated, 6/11 associated the trial with hope for the future, and 4/11 highlighted the invasiveness and pain. All patients said that family and friendship were most important to their quality of life.

Session IV: Barriers and Challenges to Progress

- *Confining trophic effects to the central horn rather than to the white matter*
- *Defining the duration of immunosuppression*
- *Controlling rejection and tracking when it starts given the inability to image in live patients*
- *Ensuring that cell injection is the same from the beginning to the end of the procedure, and defining the optimal number and spacing of injections*
- *Mitigating the unpredictable nature of the blood vessels on the vascular surface of the spinal cord, and loss of anatomical landmarks in the event of bleeding*
- *Movement of the spinal cord during patient breathing*
- *Knowing what level of graft survival is sufficient upon removal of immunosuppression*
- *Tracking and/or killing the cells in the event of complications resulting from the graft*
- *Recruitment and selection of patients, and multicenter collaboration with multidisciplinary groups*
- *Acquiring consent to do post-mortem studies*
- *High cost of clinical trials*
- *Paperwork required to meet regulatory standards*
- *Impact of publicity on patient selection*

Commercialization of Unproven Stem Cell Treatments

Doug Sipp's talk changed the pace somewhat to address the emergence of opportunism around stem cell therapies. Because the etiology of diseases such as ALS remains poorly understood, there are opportunities for hucksterism. Because the prognosis is poor, patients are more inclined to take chances and their expectations are lower. Because patients are frustrated with the lack of options offered by conventional medicine and progression is rapid, they are more willing to undergo speculative procedures. And, the media's tendency towards hyperbole has exaggerated the promise of stem cell therapies.

Money drives this industry, with companies estimated to bring in revenues of tens of millions of dollars. The tactics used by clinics to convince people to purchase stem cell therapies are numerous and devious: advertising cheap transplants and free information; claiming to have credible scientist, doctors, medical advisory boards, associations or collaborations with societies and scientists or to have registered clinical trials with FDA or EMA in Europe; prominently placing false certifications or claims of regulatory approval or legal status.

One particular dialogue among meeting participants focused on why governments in other countries, as compared to North America, allow stem cell clinics to persist, and whether there is a risk of being Western-centric. From the discussion, it became clear that the laws in every country are different. In some, there is inadequate rule of law or lack of enforcement of the laws, while in others the regulators are unwilling to take ownership of new medical approaches. Contrary to popular belief, similar clinics do exist in the West but the regulatory agencies have yet to address the situation, perhaps because there are relatively few negative reports on stem cell therapies considering the tens of thousands of people claiming to have been treated.

Consensus on Commercialization of Unproven Stem Cell Therapies

⇒ Despite the many challenges that lie ahead, there is hope on the horizon in the form of organizations such as the ALS Association, ALSUntangled and the ISSCR (Patient Handbook) that report on clinics around the world offering stem cells treatments.

Panel A : Unregulated ALS Treatments and Public Education

Dr. Rick Bedlack, a neurologist and founder of ALSUntangled, moderated the panel session comprised of Stephen Byer and Dr. Brian Dickie. ALSUntangled uses social networking via twitter and email, and discussions occur via a website called a NING. Once a critical mass of knowledge about a therapy or clinic comes to light, the organization will begin an investigation, the results of which are published (to date nine) in an open access journal called *Amyotrophic Lateral Sclerosis*. ALSUntangled has created a minimal dataset of questions, vetted among scientists, to help identify the suspect treatments. The organization would welcome input from the stem cell community. Dr. Bedlack posed the question of how to measure success. For example, the community currently relies on clinical trials, and although these are subject to rigorous scrutiny, the fact remains that that published work is not always correct. Another way to measure success would be to find as much anecdotal evidence as possible from those who have undergone a procedure. In the final analysis, nothing replaces an onsite visit and patient follow-up for understanding the context and discerning whether patients are being treated in a way that is likely to create a benefit.

Stephen Byer profiled ALS Worldwide, an organization that serves as a communication link between patients and physicians. On behalf of the scientific community, ALS Worldwide endeavors to express the goals, needs, requirements and willingness to treat patients, and to that end is open to feedback from the scientific community. The organization has helped 6000 families in over 70 countries, and has visited more than 60 labs and hospitals in five continents. There are plans to make additional site visits to 30 facilities. ALS Worldwide has published its recommendations to help the patient community judge the most important criteria for the validity of ALS therapies.

Criteria for Judging the Validity of ALS Therapy

1. Is the therapy part of a trial or procedure in a hospital?
2. Is the trial or procedure subject to rigid IRB?
3. Is trial or procedure closely regulated by countries FDA or other regulatory agencies?
4. Are there carefully designed and mediated clinical blind trials in place?
5. Does the facility in question enjoy international recognition?
6. Does the staff have proven credentials or have they been falsified?
7. Is there a proven collection of data and specifically scheduled collection of data pre and post therapy?
8. Are the claims relevant and supported?
9. Is there detailed information available to families and observers?
10. Does the protocol purport to treat a single disease or is does one process treat everything?
11. Is the informed consent a complete, thoughtful and intelligent document?

Brian Dickie from the MND Association professed that ‘snake oil’ salesmen have been around forever but the difference today is that stem cell clinics and the internet have taken the issues to new level. The MND Association is in touch with 75% of people with ALS in England, Wales and Northern Ireland, and has dealt with significant activity around clinics offering umbilical cord and oligodendrocyte cell therapy in the Ukraine and Beijing. Whether treatments are conventional or unconventional, he stressed that MND Association’s perspective is to provide patients with information that will allow them to make informed choices. In the past, the organization has not asked its membership about the use of alternative therapies, but he suggested that patient associations could integrate appropriate questions within their membership surveys.

Consensus on Unregulated ALS Treatments and Public Education

- ⇒ Despite the obvious issues with emerging stem cell clinics, the scientific community needs to recognize that there is lack of consensus, even in this form, about what constitutes a good scientific approach.
- ⇒ Education is important and measures of success might take the form of information on websites, articles in journals, and patient booklets.
- ⇒ Collaborating with ISSCR, The ALS Association, MND Association and other ALS organizations worldwide is a good tactic.

“The belief that it will be possible to implant and rebuild neural circuitry is shared, but in the case of motor neurons, that process will not be possible any time soon.”

5. LATEST DEVELOPMENTS IN iPSC TECHNOLOGY

Dr. Clive Svendsen chaired the session profiling four speakers whose talks described the latest developments in iPSC technology. Many groups are working with these cells, and although iPSC cells are conceptually straightforward, in reality they are practically very difficult to work with. Regardless of the disease model, everyone encounters the same problems and is looking for similar solutions. During their talks, the three speakers identified the main challenges of working with iPSC technology as timing, disease phenotypes, limitations of in vitro modeling, heterogeneity, gene targeting efficiency and expense.

In his presentation, Dr. Frank Soldner reiterated that the promise of iPSC technology is with respect to in vitro modeling and potential therapies using autologous cells. The ultimate goal would be to introduce or correct disease specific mutations in ES or iPSC cells. That approach could also result in perfect controls through the production of isogenic pairs of lines. In generating different iPSC cells from sporadic Parkinson’s patients, he and others realized that all the cell lines behaved differently, and that because the system is very noisy, it becomes difficult to find meaningful differences. Cluster analyses demonstrated that variations are related to residual transgene expression and also that iPSC cells without transgenes behave more like human ES cells. They posited that removing the transgene might reduce the noise, and began looking for more efficient gene targeting systems for use in iPSC/ES cells. Their search led them to test zinc

finger nucleases which showed much promise: 95-100% targeting efficiency, reproducible expression of a transgene, the ability to target a vector to a locus and target a mutation to both alleles, the ability to target silent genes (e.g., Pitx3) and to build inducible doxycycline dose-dependent transgene expression. The downside of zinc finger nuclease technology is that it is laborious and expensive. Collaborations with commercial companies can offset the former and flexible design the latter. They are in the process of differentiating A53T alpha-synuclein positive dopamine neurons, to test the effect of the mutation on dopaminergic cell function and survival, and in the future will screen lines via exome sequencing to test for off-target mutations.

It has been shown that astrocytes are involved in the progression of ALS pathogenesis and thus could be potential targets for ALS therapies. In his talk, Dr. Su-Chun Zhang discussed the success of growing human astrocytes in vitro and transplanting them into mouse brain. He has shown that human ES and iPS cells grown in a defined medium can be efficiently differentiated into astrocyte progenitors that can be expanded, show basic functionality, produce astrocytes and form connections with blood vessels. Using a variety of differentiation factors, (fibroblast growth factor 8, retinoic acid, and Sonic hedgehog homologue), he also showed that the differentiated astrocytes maintain regional markers during expansion and regional specificity (dorsal or ventral) following transplantation into the brain. To attack the question of whether human iPS-derived astrocytes could be modeled for pathology, they were generated from many patients with different diseases (although not yet from ALS patients). He confirmed that human astrocytes expressing mutant SOD1 specifically impair motor neuron survival, and is working to identify the factors that are responsible. He will also look at whether such factors are secreted by ALS iPS-derived astrocytes. Dr. Zhang's results indicate that astrocytes grown from human ES and iPS cells can be readily modified to express disease genes and thus represent a powerful opportunity for drug screening. A key message from his studies is that astrocytes take considerable time to reach maturity and therefore it will be important to find ways to assess that maturity and determine the levels that are required for efficacy in transplants.

Dr. Jeffrey Rothstein reviewed the ALS Consortium's approach to generating iPS cells from ALS patients. The ALS consortium is part of a larger NINDS initiative that was started a year and a half ago through stimulus funds from the US government. Three disease consortia are represented, ALS, Parkinson's and Huntington's Disease. The ALS consortium is collaborating with iPierian, a biotechnology company based in California that has standardized protocols to generate iPS cell lines, which will then be characterized and made available for investigators worldwide. Part of the project is to genetically profile iPS-derived astrocytes and motor neurons for RNA profiling, RNASeq and microRNAs and to compare this data set with that of in vivo astrocytes and motor neurons. One challenge will be to find extracellular markers to sort pure populations of astrocytes. The plan is to perform drug screens and genetic comparisons in the hopes that they may define a disease phenotype for ALS.

Panel B: Review of the State of the Art in Stem Cells for ALS

Dr. Lee Rubin moderated the panel discussion that gained momentum from his comments and those of panelists Drs. Monica Carrasco and Nicholas Maragakis. Participants stressed the need to keep neurogeneration, not just neuroprotection, as the goal for patients. Although everyone shared the belief that it would someday be possible to implant and rebuild neural circuitry, in the case of motor neurons, that process is not a low hanging fruit. During the course of the workshop, most of the discussion focused on other kinds of cell therapy, ranging from transplanting neural progenitors to engineered neuroprogenitors to astrocytes to MSCs. The common theme centered on providing a local source of trophic factors or astrocytes to detoxify the motor neuron environment. Neurotrophic factors alone have not been successful, perhaps owing to incorrect combinations, delivery, or dosing issues. Although cell therapy offers much promise, cells themselves are more complicated and thus will present more challenges.

Heterogeneity

The appeal in using iPS-derived cells for cell based therapies or modeling ALS is to test drugs on patient-specific lines in order to identify the best therapeutics for different patient subgroups. A stumbling block to the progress is heterogeneity, observed both at the level of the disease itself and also among individual ES and iPS cells. Such marked heterogeneity leads to great difficulty in choosing the most appropriate assays for study and demands the collection of a great many number of patient lines for screening.

Long-Term Cultures

In terms of developing in vitro models for drug screening, it will be important for the field to improve cell survival towards developing long-term cultures. Improved culture technologies such as plating a monolayer of glia over neurons plated on a glass cover slip could provide long-term survival of neurons in vitro and facilitate RNA and sequencing analyses. Another promising technology is microdot cultures in which neurons are plated at low density in order to study synaptic activity and survival. Regardless of the technology employed, the appropriate population of motor neurons to work with must be selected. Moving forward, it is likely that a few models, based on the most efficacious motor neuron lines, will be selected for screening.

Standardization

The high costs and long timelines for developing in vitro models of ES and iPS cells are ongoing concerns. So too is the lack of standardization of iPS and ES cell lines and controls given the importance of being able to better, and more quickly, compare data across laboratories. The field would also benefit from having a few widely used internal controls, similar to rat pheochromocytoma-derived PC12 cells, and standardized protocols that could facilitate access to ready networks for the scientific community. The development of new tools that predict stem cell behavior, such as Dr. Kevin Eggan's scorecard, would also be of tremendous benefit to the field.

“Lack of efficacy and good examples of success are stalling industry.”

6. INDUSTRY INVOLVEMENT AND INVESTMENT

In an introduction to the talks, session chair Dr. Lucie Bruijn voiced the need to be better organized to provide the most current information about stem cell trials, and to keep sight of the fact that stem cells have a utility other than as transplants.

Dr. Ashkan Javaherian is a senior scientist for the iPierian ALS program. It is an integrated iPS drug discovery platform that involves generating iPS cells from ALS patients and collecting clinical information, and that is currently attempting to find lead compounds for spinal muscular atrophy (SMA) and ALS. The company is able to convert patient fibroblasts with 100% efficiency to iPS cell lines, and can differentiate the characterized cells to motor neurons and glia. After having identified 880 compounds and screening them through secondary ELISA, they are going forward with three compounds for SMA, evaluating chemical analogues and doing animal toxicity. For the ALS program, they have derived more than 30 iPS lines, many of which are shared with the iPS consortium for ALS, and have put in place a 200,000+ chemical library to execute high throughput screening. The goal is to develop a drug that slows or halts disease progression and improves survival and quality of life for a range of ALS patients, especially those with sporadic disease. Currently, the company is differentiating sporadic ALS and TDP-43 lines and preliminary results show that TDP-43 may have additional roles in axons and dendrites that are not yet characterized. The company is in the early stages of defining a phenotype for ALS in vitro. Screening of compounds has not yet begun for ALS.

Dr. Timothy Allsopp presented the big ‘pharma’ perspective on developing therapies for regenerative medicine. Pfizer is interested in building a clinical portfolio of tissue replacement and cell integration (autologous or allogeneic) and non-integrating trophic cell therapy. The company has platforms on soluble regulators, autologous stem cells therapies, allogeneic stem cell therapies, and differentiated embryonic iPS stem cells. Their chemogenomic library, covering more than 750 compounds, could be a useful tool for regenerative medicine given that screens could help to identify small molecules that differentiate IPS cells into motor neurons or glia.

Panel C: Barriers to Progress and Future Prospects for Stem Cell Based Therapy for ALS

Dr. Alan Colman moderated the panel discussion. He attended the workshop with the preconception that ALS was ill understood but was most encouraged by the significant progress in the field, recognizing that many challenges still lay ahead for stem cells. Together with the panel members and participants, he explored the range of hurdles around animal studies, pharmaceutical participation, regulation and funding.

Animal Studies

Proof of principle is critical, and having a large animal model with human like physiology, size and genetic lesions that gives an ALS type phenotype would be beneficial. The transgenic pig is potentially a good model, but rejection and immunosuppression are big problems, and for efficacy studies, animals would need to be monitored for a long duration. One solution might be to use a transgenic pig model on a SCID background, but SCID pigs and monkeys have yet to be produced. More recently, investigators have characterized the pathology in a canine model with degenerative myelopathy, an adult onset neurodegenerative disease, caused by a mutation in SOD1, showing many characteristics in common with human ALS. This model could be extremely valuable for testing therapeutic strategies.

Pharma Participation in Stem Cell Therapies

Big pharma's interest in regenerative medicine is attributed to three main points: the aging population and increase in significant co-morbidity factors; the increase in healthcare costs associated with treating chronic conditions and co-morbidities; and the increase in the percentage of the population with multiple chronic conditions. If a business model could be resolved, pharma might take more of an active interest in reprogramming and the concept of making a reparative cell. For developing regenerative medicines, as with small molecule drugs, pharma will need to address significant relative efficacy, safety, and tolerability of transplanted cells; to measure that cells or surrogate markers are present in the right tissue; to measure the persistence, function and expected effect on disease markers; and to demonstrate a measureable relevant effect and safe and well tolerated dose. Although indication selection becomes critical for large pharma in terms of matching technical clinical aspects with commercial realities, there are efforts being made within the industry to focus on rare diseases with unmet market needs.

Key Questions that Drive the Strategic Process for Pharma

1. Is there high unmet need, and it is large enough to justify intervention with cell based therapy?
2. Does the cell base therapy promise to deliver significantly improved outcomes compared with current standard of care?
3. Will payers and/or clinicians appreciate an incremental benefit to patients and pay for and use the therapies?
4. Can the product be manufactured at an accessible cost to drive profitability?

Regulation

As the only regulatory consultant at the workshop, Dr. Chris Bravery brought a singular perspective to the main barriers to developing cell based therapies: money; scientific complexity; uncertain efficacy; biological variation in cell isolation; and time frames. The main problem returns to money given that it costs 1-2 billion dollars to lead a product to market (100 million dollars per medicine with a 90% failure rate). In addition, the timelines are onerous: at least 8 years from non-clinical proof of concept, and an 18-24 month review process. Scientific complexity, where one is effectively searching for mechanisms of action from among 1000s of cellular proteins, and the uncertain efficacy of cell-based products are also major hurdles. And, if the mechanism of action is unknown, then consistent manufacturing also becomes difficult. The biological variation in cell isolation among patients, particularly for autologous samples, will need to be factored into

development as will perhaps the development of more batches of samples or patients to understand the variation. There will also need to be rigorous product analysis to ensure reproducibility. Dr. Bravery noted that the first day of talks hinted that a symptoms-based definition of disease disguises the heterogeneity of disease. But if one cannot define a population, then one cannot show efficacy applicable to large populations. Finally, there is also lack of realism about time frames, which perhaps speaks to scientific complexity, regulatory burden and the need to secure funding. He reminded participants of the long road to success for monoclonal antibodies and juxtaposed their complexity with that of cell therapy to predict a galvanizing task ahead.

Funding

As food for thought, Karen Berry and Margaret Sutherland highlighted the funding opportunities from their respective organizations, The California Institute for Regenerative Medicine (CIRM) and the National Institute of Neurological Disorder and Stroke (NINDS). CIRM has built state-of-the-art research facilities across the state of California and has 28 million in funding for ALS. Two of their funded clinical trials, have originated from CIRM-funded disease research teams, and eight Californian companies, one of them being Ipeirian, have been awarded grants. CIRM is working to improve industry participation and brainstorming about how to get assistance from funders; for example, showcasing grantees work to industry and venture capitalists, or co-funding requests for applications. NINDS sponsors translational research and is using stimulatory funds to support iPS cell development, particularly as a resource for well characterized lines for industry and academic use. NINDS has had competitions on Parkinson's disease and familial ALS, currently has 87 lines with the Coriell repository, and is developing the stem cell scorecard with Dr. Kevin Eggan. Both panel speakers posed the important question of who would fund long-term follow-up studies in stem cells, and both reinforced the value in spearheading collaborations among industry, academia and consortia.

'Working as individual groups is a strength, not a weakness, and inherent in that strength is that each group has its own methods.'

MODERATORS' SUMMARY

After a long and extensive workshop, the moderators reviewed some of the core messages that emerged around the themes of cell therapy and modeling disease, clinical trials, and unregulated stem cell therapies.

Cell Therapy and Modeling Disease

The discussions throughout the workshop focused on the modest goal of providing a source of neurotrophic factors or other combinations of supportive environments, rather than on the more ambitious goal of reconstructing neural circuitry. Participants also discussed the problems of administration, manufacturing and unknown endpoints associated with using cells for therapeutics. There was much excitement about the best way to make neurons and modeling disease. In response to the fundamental question of whether one can model late onset disease,

some participants reasoned that an accelerated disease phenomenon may be observed under the right circumstances.

It is difficult to predict how to move faster towards disease modeling. iPS cells offer tremendous potential to test compounds that come from screens across a broad range of human motor neurons and to assess which compounds have the highest chance of success. Creating iPS cell lines from subsets of patients might facilitate the process and help to establish a personalized medicine approach to therapy. Small animal models are being refined but having larger animal models would be of tremendous value for modeling disease.

Clinical Trials

It is clear that new surgical techniques are valuable and will help to refine extrapolations from pre-clinical studies, such as injection points, the rate of diffusion, and differentiation of stem cells. Participants agreed that the sensitivity for detecting foreign cells is crucial. To that end, there are efforts in industry to develop a platform to detect foreign cells using a collection of preclinical and clinical biomarker approaches. It will also be necessary to devise ways to track autologous cells.

It is important to remember that there are tacit conclusions being made about the issue of cell longevity given that there is little evidence regarding the lifespan of transplanted cells. In the case of transplanting mesenchymal stem cells, that issue is not as critical because the delivery of trophic factors is the main outcome. Ongoing macular degeneration trials should help to define the duration of in vitro generated transplants. There was less discussion at the workshop as to which ALS patients to treat with different types of stem cells.

It will continue to be very expensive and time-consuming to complete clinical trials, and the issue of follow-up after academic money runs out is a consideration. It is crucial to be able to perform post-mortem studies on participants in clinical trials, but obtaining consent will be an ongoing challenge, especially given that many jurisdictions leave the final decision to the next of kin. There is a tremendous need to encourage patients to explore the opportunities to participate in clinical trials. In recognition of that, organizations such as ALS Worldwide, MND Association, and The ALS Association, through the TREAT/ALS NEALS Clinical Trials Network, have made it a primary goal for the upcoming year. Clinicians, scientists, patients, and associations working more closely together will be key to facilitating success.

Unregulated ALS Treatments and Public Education

The key point that emerged from the discussion around unregulated ALS treatments and public education was the tremendous need for more collaboration and better communication between the scientific and patient communities. Communication is a strategic focus for many associations where the key strategy is to empower patients affected by the disease through access to knowledge and the opportunity to participate in dialogues. There is also an opportunity for scientists to become involved through global science media centers that function to ensure accurate reporting of information by linking scientists with the media.

Participants agreed that it is more important to focus their energies on patient and public education than on policing or shutting down 'bogus' facilities. This decision brings up the larger issue of the role of science in society, particularly in relation to the burden placed on society to wade through huge volumes of information. The scientific and medical communities are well

positioned to alleviate this burden, not only by general education but also by helping patients appreciate the difference between scientifically validated evidence and anecdotal or testimonial reporting. By contributing to a more aware patient population, it will be patients themselves who spearhead the closure of such facilities. In the interest of providing patients with unbiased opinions about emerging therapies, participants agreed that there is value in maintaining an arm's length relationship with organizations, such as ALSUntangled, that provide unbiased perspectives on treatments for ALS.

"A cure is not at hand. We don't have perfect answers, but sometimes one goes forward with whatever systems are in place. In the field of ALS, we will forge ahead on all fronts."

CLOSING REMARKS

Drew Lyall, Executive Director of the Canadian Stem Cell Network closed the meeting on behalf of the ISSCR. The onset of iPS technology, which as excited many people for good reason, provided the context for putting together the workshop. At the outset, it was hoped that one tangible outcome might be a consensus statement on the progress towards stem cell therapies for ALS. As the meeting progressed it became clear that the field is not yet ready for such consolidation, but that in itself is an important message to communicate to the broader scientific and patient communities. One consensus, however, was reached: the need to communicate more effectively with patients.

Drew Lyall thanked the meeting sponsors, and especially the New York Stem Cell Foundation for hosting the workshop. Drs. Lucie Bruijn from The ALS Association, and Brian Dickie from the Motor Neurone Disease Association, along with their associates were instrumental in bringing together the speakers and organizing the workshop logistics. The moderators, speakers and participants are to be applauded for their thoughtful and animated discussions, which contributed to a thoroughly enjoyable and productive meeting.

APPENDIX 1: DELEGATE LIST

First Name	Surname	Institute	Country
Timothy	Allsopp	Pfizer Regenerative Medicine	UK
Peter	Andersen	University of Umea	Sweden
Richard	Bedlack	Duke Institute for Brain Sciences	USA
Karen	Berry	California Institute for Regenerative Medicine	USA
Nicholas	Boulis	Emory University	USA
Chris	Bravery	Consulting on Advanced Biologicals Ltd	UK
Lucie	Bruijn	The ALS Association	USA
Stephen	Byer	ALS Worldwide	USA
Monica	Carrasco	Harvard University	USA
Maya	Chaddah	Science Writer	Canada
Siddharthan	Chandran	University of Edinburgh	UK
Don	Cleveland	The Ludwig Institute, UCSD	USA
Gavin	Clowry	University of Newcastle	UK
Alan	Colman	A*Star Institute	Singapore
Merit	Cudkowicz	Massachusetts General Hospital	USA
Brian	Dickie	Motor Neurone Disease Association	UK
John	Dimos	Iperian Inc	USA
Kevin	Eggan	Harvard University	USA
Valerie	Estess	Project ALS	USA
Denise	Figlewicz	ALS Society of Canada	Canada
Lidia	de Filippis	University of Biocca	Italy
Jonathan	Gilthorpe	University of Umea	Sweden
Jonathan	Glass	Emory University	USA
Dorine	Gordon	The ALS Association, Greater New York Chapter	USA
Arthur	Grayzel	The ALS Association, Greater New York Chapter	USA
Amelie	GubitZ	NINDS	USA
Orla	Hardiman	National Neuroscience Centre, Beaumont Hospital	Ireland
Derek	Hei	Waisman Centre, UW-Madison	USA
Christopher	Henderson	Columbia University	USA
Ira	Herman	Tufts University	USA
Ashkan	Javaherian	Iperian Inc	USA
Stefan	Liebau	University of Ulm	Germany
Drew	Lyall	Stem Cell Network Canada	Canada
Nicholas	Maragakis	Johns Hopkins University	USA
Caroline	Marshall	New York Stem Cell Foundation	USA
Cecile	Martinat	I-Stem Institute	France
Letizia	Mazzini	Eastern Piedmont University of Novara	Italy
Amy	McDermott	Columbia University	USA
Sheila	Menzies	Prize4Life	USA
Dani	Offen	Brainstorm Cell Therapeutics	USA
Deanna	Packham	Motor Neurone Disease Association	UK

Hemali	Phatnani	Columbia University	USA
Victor	Rafuse	Dalhousie University	USA
Jeffrey	Rothstein	Johns Hopkins University	USA
Lee	Rubin	Harvard Stem Cell Institute	USA
Chris	Shaw	King's College London	UK
Pamela	Shaw	Sheffield Institute for Translational Neuroscience	UK
Douglas	Sipp	RIKEN Center, Kobe Institute	Japan
Kristin	Smith	New York Stem Cell Foundation	USA
Frank	Soldner	The Whitehead Institute	USA
Susan	Solomon	New York Stem Cell Foundation	USA
Vasanta	Subramanian	University of Bath	UK
Margaret	Sutherland	NINDS	USA
Ben	Sykes	UK National Stem Cell Network	UK
Kevin	Talbot	University of Oxford	UK
Leonard	van den Berg	University Hospital Utrecht	Netherlands
Angelo	Vescovi	University of Biocca	Italy
Robyn	Wallace	University of Queensland	Australia
Hynek	Wichterle	Columbia University	USA
Damian	Williams	Columbia University	USA
Su-Chun	Zhang	Waisman Centre, UW-Madison	USA