CellPress
Cell Stem Cell
Forum

From Dish to Bedside: Lessons Learned While Translating Findings from a Stem Cell Model of Disease to a Clinical Trial

John McNeish,[1,*] Jason P. Gardner,[1] Brian J. Wainger,[2,3] Clifford J. Woolf,[3,4] and Kevin Eggan[3,5,6,*]
[1]Regenerative Medicine Discovery Performance Unit, GlaxoSmithKline, Cambridge, MA 02139, USA
[2]Departments of Neurology and Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, Boston, MA 02114, USA
[3]Harvard Stem Cell Institute, Cambridge, MA 02138, USA
[4]FM Kirby Neurobiology Center, Boston Children's Hospital and Harvard Medical School, Boston, MA 02115, USA
[5]Howard Hughes Medical Institute and Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA 02138, USA
[6]Stanley Center for Psychiatric Research, Broad Institute of Harvard and MIT, Cambridge, MA 02139, USA
*Correspondence: john.d.mcneish@gsk.com (J.M.), eggan@mcb.harvard.edu (K.E.)
http://dx.doi.org/10.1016/j.stem.2015.06.013

While iPSCs have created unprecedented opportunities for drug discovery, there remains uncertainty concerning the path to the clinic for candidate therapeutics discovered with their use. Here we share lessons that we learned, and believe are generalizable to similar efforts, while taking a discovery made using iPSCs into a clinical trial.

Phenotypic assays using mature human cells derived from embryonic stem cells (ESCs) (McNeish, 2004) and induced pluripotent stem cells (iPSCs) (Grskovic et al., 2011) enable researchers to study disease-relevant phenotypes and provide a rich discovery tool for candidate therapeutics (Bellin et al., 2012). Furthermore, iPSCs have the potential for improving the identification of drug targets and candidate compounds as well as contributing to the optimized selection and stratification of trial participants. These applications could lead to more efficient clinical trials and reduced drug attrition during the development process.

While employing iPSCs is clearly attractive, the novelty of the approach has left its place in the drug discovery pipeline uncertain. On one hand, experiments with iPSCs can provide increased confidence in the relevance of a medicine to a patient's genetic makeup and human cellular physiology, suggesting that they may be more relevant to decision-making than existing animal models. On the other, the in vitro assays in which iPSCs are generally deployed leave reasonable questions concerning whether the mechanism of disease and candidate therapeutic identified have relevance in vivo. This natural and understandable tension leaves many engaged in iPSC research uncertain concerning the optimal path to the successful initiation of a clinical trial based on their discoveries. We reasoned it would therefore be useful to share our experiences and generalizable learning from translating a recent discovery made with iPSCs to an approved clinical trial for ALS patients (Wainger et al., 2014; Kiniskinis et al., 2014) (https://clinicaltrials.gov/, NCT# 02450552).

Recently, we showed that iPSC-derived motor neurons produced from ALS patients harboring *SOD1* mutations display reproducible, disease-relevant phenotypes (Kiskinis et al., 2014; Wainger et al., 2014.) These phenotypes included hyperexcitability with increased spontaneous action potentials and reduced survival (Wainger et al., 2014). More specifically, motor neurons from ALS patients displayed a reduced delayed-rectifier potassium channel activity. Further studies showed that this phenotype could be corrected by modulating the Kv7.2/3 class of potassium channels. Evidence that correcting motor neuron physiology was protective came through treatment with the approved antiepileptic and Kv7.2/3 potassium channel agonist ezogabine, which reduced neuronal excitability and improved cell survival (Wainger et al., 2014; Kiskinis et al., 2014). The first important lesson we learned from our desire to translate these studies was that the use of gene editing to correct the *SOD1* mutation, and with it the physiological changes we observed, was critical in building confidence in our findings.

To date, the only approved medicine available to ALS patients is Riluzole (Rilutek, Sanofi). The exact mechanisms by which Riluzole acts remain controversial, but have been proposed to include inhibition of Na^+ channels and glutamate activity (Bellingham, 2011). To date many additional mechanisms of drug action have been clinically evaluated in ALS, with seven compounds demonstrating positive phase 2 results. However, none of these seven have yet delivered positive findings in a pivotal phase 3 study. Thus, the identification of novel targets for ALS, like Kv7.2/3, worthy of being tested in the clinic remains sorely needed.

From the perspective of GlaxoSmith Kline (GSK), and likely other potential industry partners, a key concern with moving findings in ALS forward to the clinic has been the historically unreliable human translation of compounds evaluated in the *SOD1* mouse model (Perrin, 2014; Bellingham, 2011). In point of fact, Riluzole was brought to market prior to the development of this mouse model. Several hypotheses have been advanced concerning why discoveries from this mouse perform poorly in the clinic. One of the most reasonable is that given the genetic heterogeneity of ALS, it could be that features of disease in *SOD1* patients may not be central to disease progression in individuals harboring mutations in other genes. To determine how general

the relevance of our findings related to Kv7.2/3 were, we examined motor neuron physiology and response to ezogabine in a larger cohort of controls and patients harboring mutations in three distinct genes linked to ALS: *SOD1*, *C9orf72*, and *FUS* (Wainger et al., 2014). Our findings from these studies demonstrated similar physiological changes in the distinct patient classes, which were each rescued by ezogabine. Showing that the target phenotype and drug response were generalizable across patient forms was compelling enough to many key decision-makers to allow us to move forward without drug testing in the *SOD1* mouse model. Thus, while testing in animal models may be indispensable in some cases, we found that a higher premium was often placed on our data that supported the notion that the therapeutic approach proposed was valid to a broader portion of the patient population.

Another key factor enabling our clinical efforts was the relatively close alignment between the assays we carried out in vitro using iPSCs with emerging electrophysiological measures being made in the clinic. It has been shown using transcranial magnetic stimulation and threshold-tracking nerve conduction studies that ALS patients have a more excitable motor circuit and that the larger this change in physiology the worse a patient's prognosis (Bae et al., 2013). As a result, a clinical trial could be readily designed that employed clinical physiological measures to test the hypothesis that ezogabine might reduce motor circuit excitability in patients, much as it did in iPSC-derived motor neurons. In addition, we could propose to use physiological measures as pharmacodynamic biomarkers of ezogabine's impact on hyperexcitability, measuring the effect of two doses of ezogabine relative to placebo. Our experience suggests that carefully taking known in-patient biomarkers of disease into consideration when designing in vitro phenotypic assays with iPSCs can pay substantial dividends in later stages of translation. If we had not aimed our studies at understanding the mechanistic underpinning of a known patient phenotype, which could be readily measured clinically, we would have needed to pursue the time-consuming and costly process of developing a biomarker ourselves.

It is also worthy of note that our path to the clinic was aided in part by good fortune. A key driver of enthusiasm for trialing ezogabine in ALS patients, which was only partially in our control, was its well-known chemical and pharmacological properties as an approved drug. As an efficacious drug for epilepsy that acts through the opening of Kv7.2/3 channels, we could rely on preexisting clinical evidence that ezogabine engaged its target in the brain with therapeutic effect. In the absence of such data, expensive studies of compound toxicity, bioavailability, and in vivo half-life, likely coupled with additional cycles of chemical optimization and further testing, would have been needed before we could have considered a clinical trial. Our own experience suggests that many academic labs may find expertise in these pharmacological tests and medicinal chemistry either unavailable or unaffordable. Thus if it is the motivation of an academic investigator to rapidly test their iPSC-derived hypotheses clinically, it may be advisable for them to focus attention on libraries of already approved drugs or compounds that have made substantial progress in the clinic. Furthermore, if several molecules have been discovered with promise, it clearly would be most pragmatic to move forward using a compound with stronger pharmacological data even if another showed marginally better performance in vitro.

In short, we found that the strong scientific foundation we had produced using iPSCs, a clear clinical question that could be tested using an established biomarker, and a compound with strong pharmacological properties were each essential pieces in the puzzle of organizing partnerships between academics, clinicians, patient advocacy groups, and industry that were needed to mount a clinical trial. The basic research supported by Boston Children's Hospital, Harvard, HHMI, Target ALS, the ALS Association (ALSA), and GSK enabled the assembly of a consortium to fund the clinical study, which included the Harvard Stem Cell Institute (HSCI), Massachusetts General Hospital (MGH), GSK, and ALSA. It was our experience that, as has recently been suggested (Saha and Hogle, 2014), when a large federation of scientists, physicians, and drug development experts can be assembled, many of the clinical and regulatory challenges to mounting a clinical trial are more rapidly overcome.

Another critical accelerator of our study was our ability to work with a preexisting clinical research team. The Northeast ALS (NEALS) Consortium together with the MGH Neurological Clinical Research Institute provided robust yet adaptable infrastructure for the rapid translation to a trial of the scale we proposed. Working with a preexisting clinical network allowed us to streamline the processes of developing the clinical trial protocol, trial contracts, and essential measures for subject safety monitoring and obtaining the needed FDA IND exemption for testing Retigabine in ALS patients. Investigators interested in advancing toward the clinic would be well advised to familiarize themselves with similar clinical consortia operating in their indication of interest and then to build strong enabling relationships with such groups. If such a group does not exist, our experience suggests that energy expended to help organize one would be well allocated.

Another important and likely generalizable lesson we learned while preparing our trial design was that enthusiasm from our funding partners for making the needed investment was increased by incorporating provisions for collecting additional samples that would fuel future basic research on ALS. A key component of the ezogabine study is to derive hiPSC lines from participants. This type of parallel study represents a unique opportunity afforded by hiPSC research. It seems highly probable that similar approaches would be viewed as valuable by funders if implemented in other clinical investigations for which highly reproducible hiPSC-based disease models have been developed. We found that adopting this strategy was motivating to our entire consortium, which was eager to see whether the patients enrolling in the clinical trial reflected the biology of those patients that originally drove initiation of the study. The future availability of this iPSC resource to the community will mean that our clinical trial will become "evergreen." It will allow investigators to study correlations between patient outcomes and in vitro phenotypes in motor neurons or other cell types. In addition, with the iPSC resource in hand, variation in drug response could be investigated mechanistically. If the trial is a success,

the iPSCs could become a resource for evaluating compounds emerging from future high-throughput screens for novel Kv7.2/3 agonists. If our trial fails, these iPSC could nonetheless be useful for attempting to further stratify the patient population, for testing additional therapeutics, or for trying therapeutic combinations. Due to the substantial interest in the iPSC resources, a key feature of the partnership agreement that serves as a foundation of our trial is that it makes these stem cells available for both basic and commercial research following completion of the trial.

The combination of careful execution and good fortune outlined above placed us in position to file an Investigator-Sponsored IND-exemption request, which has now been approved by the FDA to evaluate ezogabine in a phase 2 multicenter, randomized, double-blind, placebo-controlled study of neuronal excitability in ALS subjects. Secondary outcomes of the study will include tolerability and safety of ezogabine in ALS patients. This trial will be conducted at 12 U.S. NEALS Consortia sites. Of note, this program progressed from initial discovery to phase 2a study initiation in less than 2 years. We hope that outlining what we feel were the key factors playing important roles in the successful translation of ezogabine, both fortuitous and carefully calculated, will be valuable to those interested in taking their own discoveries made with iPSCs to the clinic.

We recognize that the type of trial we are undertaking and propose could be useful in many other disease contexts, though it is not without its complications and potential limitations. For example, there are substantial challenges to reducing technical variability among more than 100 iPSC lines made from patient samples collected at a dozen sites. Even with iPSCs in hand, improved processes will be needed to efficiently and reproducibly differentiate, culture, and analyze motor neurons from this large number of patients. Still, we are optimistic that such challenges can be overcome and that additional clinical trials will emerge from the many studies of disease-relevant cell types being made from hiPSCs (Grskovic et al., 2011). The ever-expanding reporting of clinically relevant phenotypes in hiPSC disease models, as well as the pharmacological correction of pathologic disease features in these cells, is creating an exciting environment for the development of new medicines. As the reproducibility and robustness of stem cell technologies continues to improve so too will their utility in nominating hypothesis for clinical testing. We believe that interest in hiPSC technologies for applications in drug research and development will continue to grow and that these cells will eventually serve as surrogates for many clinical phenotypes and perhaps even provide a new form of companion diagnostic.

ACKNOWLEDGMENTS

We are grateful to our many colleagues at GlaxoSmithKline (GSK) and The Harvard Stem Cell Institute for helpful discussions that contributed to the experiences and lessons we share here. J.M. and J.G. are employees of GSK. K.E., C.W., and B.W. have received sponsored research support from GSK.

REFERENCES

Bae, J.S., Simon, N.G., Menon, P., Vucic, S., and Kiernan, M.C. (2013). J. Clin. Neurol. *9*, 65–74.

Bellin, M., Marchetto, M.C., Gage, F.H., and Mummery, C.L. (2012). Nature Rev. *13*, 713–726.

Bellingham, M.C. (2011). CNS Neurosci. Ther. *17*, 4–31.

Grskovic, M., Javaherian, A., Strulovici, B., and Daley, G.Q. (2011). Nat. Rev. Drug Discov. *10*, 915–929.

Kiskinis, E., Sandoe, J., Williams, L.A., Boulting, G.L., Moccia, R., Wainger, B.J., Han, S., Peng, T., Thams, S., Mikkilineni, S., et al. (2014). Cell Stem Cell *14*, 781–795.

McNeish, J. (2004). Nat. Rev. Drug Discov. *3*, 70–80.

Perrin, S. (2014). Nature *507*, 423–425.

Saha, K., and Hogle, L.F. (2014). Cell Stem Cell *14*, 559–560.

Wainger, B.J., Kiskinis, E., Mellin, C., Wiskow, O., Han, S.S., Sandoe, J., Perez, N.P., Williams, L.A., Lee, S., Boulting, G., et al. (2014). Cell Rep. *7*, 1–11.