The California Institute for Regenerative Medicine's Human iPSC Initiative:

Large-scale generation of patient-derived induced pluripotent stem cells to accelerate the understanding of disease biology and drug discovery

In 2012, the California Institute for Regenerative Medicine (CIRM) announced its Human iPSC Initiative, an ambitious effort to generate induced pluripotent stem cell (iPSC) lines from 3,000 donors. Most of these donors had been diagnosed with one of several multigenic disorders, including diseases affecting the brain, heart, lung, liver and eyes. Over the next year grants were awarded and contracts signed, and on December 1, 2013 the program officially launched. On September 1, 2015 the first 300 iPSC lines manufactured by Cellular Dynamics International were made available from the CIRM Repository maintained by the Coriell Institute for Medical Research. These lines have been generated under broad consent intended to enable the wide range of activities critical to the development of treatments for these serious and prevalent diseases. The challenges overcome in successfully launching a project of this scope provide a guide for other organisations contemplating their own large-scale reprogramming and banking projects.

By Dr Thomas J. Novak

One of the major challenges facing the biopharmaceutical industry today is the decrease in return on R&D investment as measured by the number of approved drugs per dollar of R&D expenditure[1]. Numerous factors have been proposed for this decrease in efficiency, including increased regulatory scrutiny; focus on short-term growth, especially through mergers and acquisitions, at the expense of long-term productivity; payer resistance that prioritises cost containment over reward for innovation; and poor target selection choices[2-4]. Related to this last point, one intriguing possible cause for this drop in R&D productivity was identified in a highly-cited 2011 paper by Swinney and Anthony[5]. These authors examined new drug approvals in the decade between 1999 and 2008 and determined that more first-in-class drugs were discovered using phenotypic screens than target-based screens. The fact that the absolute numbers favoured phenotypic

screening was surprising given that the pharmaceutical industry had largely abandoned them in favour of target-based screens. Indeed, the more interesting comparison would have been of the percentage of each type of screen that ultimately yielded a new drug. This is difficult to assess accurately since most companies do not divulge the total number of compound screens run in any specific year, but it is likely that during the period of interest the number of phenotypic screens represented only a small fraction of the total. Looked at in this light, the disproportionate success of phenotypic screening in developing first-in-class products appears even more impressive. What then prevented biopharmaceutical companies from making greater use of phenotypic screens, and is this likely to change in the future?

There are several reasons why phenotypic screening fell out of favour in the 1990s. First, expertise in molecular biology and recombinant gene expression technology became widespread in industry. Combined with an explosion in the availability of kits and reagents, especially high-quality human cDNA libraries, this expertise made it possible to develop high-throughput screens in highly controllable systems, generally involving purified proteins or recombinant cell lines expressing a target of interest. Given that many phenotypic screens had used non-human cells or tissues, the ability to utilise human targets was seen as a big advantage. Even when human material was available for screening it suffered numerous deficiencies. Most human tissues of interest to drug hunters cannot be acquired from live donors. The limited availability and variable characteristics of cadaveric tissue (in genetic background, comorbidities, post-mortem interval, dissection/enrichment in target material, etc.) practically guaranteed low assay reproducibility. With chemical libraries exploding in size and high-throughput screening systems coming online, reagent availability became a key factor in the choice of assay format. Immortalised human cell lines solved the problem of availability, despite the associated disadvantages of karyotypic drift and phenotypic instability. Thus, for a number of legitimate reasons human target-based screening became the industry standard. And to be fair, well-executed target-based screens, followed by a dedicated lead optimisation effort almost always resulted in compounds that were highly potent and highly selective. Unfortunately, these candidate drugs too often failed in the clinic. Why was that? Multiple factors undoubtedly contributed, including incorrect choice of target, but a major reason for the failure of target-based screens to yield drugs that worked in vivo was likely the fact that the targets were not screened in their normal cellular milieu. By screening a target outside its normal cellular environment it is possible to miss the effects of key modulators of target function and thereby overestimate a compound's ability to affect cellular function to a therapeutically useful degree. Fortunately, as the limitations of target-based screening were becoming more fully appreciated a possible solution appeared.

The contemporaneous discoveries in 2007 by Yamanaka and Thomson that adult human somatic cells could be converted ("reprogrammed") to a stem cell phenotype pointed a way out of this screening conundrum[6,7]. These reprogrammed somatic cells, called induced pluripotent stem cells or iPSCs, offered several opportunities to improve the process of drug discovery and development. First, stem cells derived from somatic tissues were free of the controversies surrounding embryo-derived stem cells, and because they were made from adult donors it was possible to generate lines from patients with diseases of interest to biomedical researchers. In a relatively short period of time it was shown that somatic cells differentiated from these patient-derived iPSCs often possessed abnormal phenotypes when compared to control cells derived from donors without a disease diagnosis[8]. However, the process of recruiting patients, collecting samples, reprogramming and validating the resulting iPSCs, and differentiating them to cells of interest is both time-consuming and expensive. It was for this reason that the California Institute for Regenerative Medicine stepped up and made a large investment in this exciting new field of patient-derived stem cells.

The California Institute for Regenerative Medicine and the Human iPSC Initiative

In 2004, following President George W. Bush's Executive Order banning the use of federal research funds for work on all but a few embryonic stem cell lines, voters in the state of California approved Proposition 71. This initiative created a state agency charged with disbursing $3 billion to build and equip stem cell facilities free from federal funding restrictions and to support research, training and education in all aspects of regenerative medicine. In addition, efforts would be made to recruit both academic researchers and commercial entities to the state, thereby bolstering California's economy.

In July 2012, CIRM published three Requests for Applications (RFAs) that together comprised

Table 1: CIRM Human iPSC initiative awardees

RFA 12-02 AWARDEES

PI	Institution	Disease indication	Duration	Award amount
Joe Gleeson	UCSD	Infantile epilepsy, autism and cerebral palsy	2 years	$4 million total
Joachim Hallmayer	Stanford	Autism spectrum disorders (idiopathic)	2 years	
Jackie Maher	UCSF	Viral hepatitis (HCV) and non-alcoholic steatohepatitis (NASH)	2 years	
Joe Wu	Stanford	Idiopathic familial dilated cardiomyopathy	2 years	
Doug Galasko	UCSD	Alzheimer's disease (late onset)	2 years	
Kang Zhang	UCSD	Age-related macular degeneration (wet and dry), primary open-angle glaucoma, and proliferative diabetic retinopathy	2 years	
Brigitte Gomperts	UCLA	Idiopathic pulmonary fibrosis	2 years	

RFA 12-03 AWARDEE

PI	Institution	Duration	Award amount
Thomas Novak	Cellular Dynamics International	3 years	$16 million

RFA 12-04 AWARDEE

PI	Institution	Duration	Award amount
Dorit Berlin	Coriell Institute for Medical Research	4 years	$10 million

the Human iPSC Initiative. The stated goal was to "generate and ensure the availability of high quality disease-specific hiPSC resources for disease modelling, target discovery and drug discovery and development for prevalent, genetically complex diseases"[9]. The agency committed $30 million to fund the generation of iPSC lines from 3,000 donors (a mix of cases and controls), distributed into the following categories:

RFA 12-02, Tissue Collection: CIRM committed $4 million to fund the activities associated with donor screening and recruitment, consent and sample collection. Applicants were required to work at California-based organisations and the proposed diseases needed to demonstrate multigenic inheritance and be prevalent (ie, >200,000 affected individuals in the US)[9]. Parkinson's disease, Huntington's disease and Amyotrophic Lateral Sclerosis (ALS) were excluded due to the fact that CIRM and the National Institute of Neurological Disorders and Stroke had already collaborated to generate iPSC lines from patients with those conditions. Following a rigorous review process, seven investigators studying 11 diseases were selected as Tissue Collectors[10] and received two-year grants to collect donor samples along with associated relevant demographic and medical data (see **Table 1**).

RFA 12-03, Derivation: In order to reduce potential variability in the performance of the iPSC lines due to differences in reprogramming methodology and validation assays, CIRM announced that it would select a single entity to derive all of the lines for this initiative[11]. The Derivation lab would receive $16 million over three years and be required to deposit into the Repository three clones per donor, resulting in a total of 9,000 iPSC lines

deposited at the end of the project period. The Deriver also had to perform the work in California, even if they were not currently located there. Ultimately Cellular Dynamics International (Madison, WI) was chosen as the deriving entity[12].

RFA 12-04, Repository: One of CIRM's goals was to make these iPSC lines available globally. Therefore, applicants for the Repository[13] award ($10 million) were required to have managed a cell banking business for at least one year prior to applying and to agree to establish the CIRM Repository in California. After review, the Coriell Institute for Medical Research (Camden, NJ) received a four-year grant to establish and manage the Repository[14].

Challenges

Unlike most CIRM grants, which are awarded to individuals and are usually independent of other awards, the Human iPSC Initiative required close co-ordination between the Tissue Collectors, the Deriver and the Repository. Despite the fact that CIRM's board approved the awards outlined in **Table 1** in March of 2013, finalisation of the grant contracts for these awards required nearly nine months. Multiple challenges needed to be resolved before the project could commence.

As applications for all three RFAs were submitted simultaneously, applicants were unable to account for dependencies between the separate elements of the initiative. A potential Tissue Collector was unaware of what the preferred starting material required by a potential Deriver (skin fibroblasts, blood cells or other tissue types) would be. Similarly, an applicant for the Deriver award was unaware of the diseases being proposed for RFA 12-02, which could (and did) affect the amount of sample allowed to be collected by a potential Tissue Collector's Institutional Review Board (IRB). In the end it was agreed that the starting material would be 80% peripheral blood and 20% skin punches.

Another challenge resulted from the fact that CIRM funding comes from the taxpayers of California. As a result there is a requirement that work funded by CIRM will be performed by people paying California state income tax. This necessitated CDI and Coriell renting and equipping lab space in the Golden State and hiring and training staff. Fortunately, the Buck Institute for Research on Aging (Novato, CA) had just opened a new stem cell building, co-funded by CIRM, and was willing to lease CDI and Coriell the space required for the grant activities.

In addition, CIRM was adamant that the iPSC

Table 2: CIRM's Human iPSC initiative-recruitment goals

DISEASE AREA	PI	# CASES	# CONTROLS
Infantile epilepsy, autism and cerebral palsy	Joe Gleeson	450	200
Autism spectrum disorders (idiopathic)	Joachim Hallmayer	200	
Viral hepatitis (HCV) and non-alcoholic steatohepatitis (NASH)	Jackie Maher	132 (HCV) 34 (NASH)	20
Idiopathic familial dilated cardiomyopathy	Joe Wu	650	30
Alzheimer's disease (late onset)	Doug Galasko	235	300
Age-related macular degeneration (wet and dry), primary open-angle glaucoma, and proliferative diabetic retinopathy	Kang Zhang	500	
Idiopathic pulmonary fibrosis	Brigitte Gomperts	250	
	Subtotals	2451	550
	Grand total	3001	

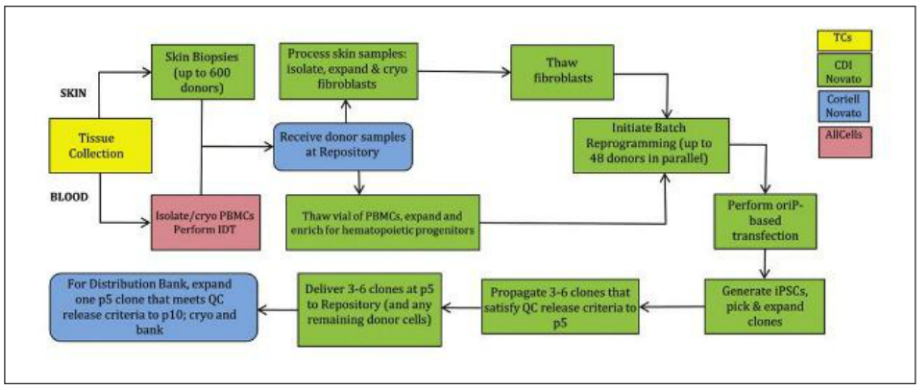

Figure 1
Project workflow indicating key steps and responsible parties

lines generated by this Initiative could be used globally for both non-commercial and commercial research activities. Thus, it was critical that the Informed Consent forms contained consistent language governing future use of the cell lines. Because the Tissue Collectors were spread among four universities, CIRM had to negotiate the consent language with four different IRBs. This process required several months to conclude, but was essential for the Repository's long-term survival as a key resource for both basic research and drug discovery. An important point is that the iPSC lines are the property of CIRM, not CDI or Coriell.

CIRM initially budgeted for 3,000 donors but after awarding the Tissue Collection grants the total number of donors was substantially higher. In order to contain costs, CIRM convened a panel of geneticists who worked with the Tissue Collectors to devise a plan to use shared controls (**Table 2**) in order to reduce the total number of donors to 3,000. However, the plan requires that the shared controls for diseases of the elderly, for example, be screened to make sure they are free of dementia and vision and respiratory problems, thus generating additional unanticipated work for the Tissue Collectors.

The budget for Derivation was tight ($16 million) and the scope unprecedented (9,000 lines in three years). Therefore it was imperative that faster and cheaper methods be developed wherever possible. In addition to the increased use of automation and batch processing, CDI proposed to use a PCR-based gene expression assay to assess pluripotency (replacing teratoma assays) and to use Illumina Infinium® HumanCore BeadChips to verify chromosomal integrity (in place of G-banded karyotyping). CIRM's reviewers endorsed both of these proposals, although they did request that CDI validate its pluripotency assay by running teratoma assays on the first 25 iPSC lines generated.

Myriad other issues had to be worked out as well. Some, such as the creation of barcoded labels, sample submission forms and protocols for sample processing and packaging, were relatively straightforward. Others, such as the collection and transfer of patient demographic information from the Tissue Collectors to the Repository required strict observance of applicable State and Federal laws governing patient confidentiality. Of particular note is the fact that the Deriver only ever sees a barcode and has no knowledge of patient identity or disease status.

Finally, the Repository is required to remain open for 10 years following the end of Coriell's CIRM funding[13] (that is, until the end of 2027). This requires a careful determination of the sale price of the CIRM iPSC lines as this revenue will need to cover Coriell's cost of maintaining a satellite facility in California plus the expenses required to replenish depleted lines.

Project workflow

Despite these and other challenges, the CIRM Human iPSC Initiative project launched on December 1, 2013, with the first donor sample arriving in March 2014. An overview of the project workflow is shown in **Figure 1**. In short, Tissue Collectors send Sample Submission Forms to Coriell prior to overnighting their samples so that Coriell can enter donors into the project database. Blood (80% of the samples) is sent to AllCells, Inc (Alameda, CA) for isolation of peripheral blood mononuclear cells (PBMC). It was felt that having a single lab isolate PBMCs would reduce variability. Associated serum and plasma samples are screened for HBV, HCV and HIV. Positive samples are destroyed and donors informed in accordance

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with state and county laws. Skin punches are sent directly to Coriell in Novato for hand-off to CDI for processing. Because they contain so little blood, skin punches are not subjected to infectious disease testing (IDT) (note that all of the HCV positive donor samples from the lab of Jackie Maher are sent as skin punches). Coriell banks the resulting fibroblasts from skin punches along with PBMCs isolated by AllCells until CDI requests them for reprogramming. Cohorts of 24 or 48 samples are reprogrammed in parallel using CDI's patented non-integrating episomal vector system. iPSC clones are chosen manually and processed robotically until passage 5, when three to six clones are subjected to a panel of QC assays, cryogenically preserved and transferred to the Repository. When requested, Coriell provides one passage 5 clone to CDI for expansion to passage 10. Passage 10 cells are then frozen down as Distribution Lines. It is these Distribution Lines (one clone per donor) that the Repository will make available for sale.

Since launch, the project has been making substantial progress and the first 300 iPSC clones were made available on September 1, 2015. Another 450 lines will be deposited by March 1, 2016. Lines can be ordered from the Coriell website[15], which in addition to listing demographic and disease information for the lines, will also provide pricing information and links to the relevant Material Transfer Agreement (required by all users) and patent licences (for commercial users only).

Prospects for use

Only time will tell how useful these patient-derived iPSC lines will be for understanding disease biology and for accelerating drug discovery. However, it is noteworthy that several pharmaceutical companies have indicated their intention to use lines like these for both new screens and for repurposing existing drugs.

For example, Pfizer has developed its 'Rule of 3' for phenotypic screening (an obvious tip of the hat to their colleague Chris Lipinski's well-known 'Rule of 5' for lead optimisation in medicinal chemistry)[16]. Under this new paradigm assays are to be evaluated in three areas: System, Stimulus and Readout. For System, highest priority is given to patient-derived cell lines expressing a clear disease phenotype, while lowest priority is given to target-based screens using transfected cell lines. For Stimulus, highest priority is given to systems where the disease trigger is inherent in the cell line, as opposed to some exogenous pharmacological 'sledgehammer'. Finally, the most relevant Readout is a 'functional manifestation of disease' as opposed to an assay with little relevance to actual clinical measurements. Together, these guidelines provide a straightforward method for prioritising screening campaigns and will likely lead to an increase in phenotypic screening so long as well-characterised systems, such as the CIRM iPSC Repository, are available.

An outstanding example of the value of patient-derived iPSC lines in drug reproposing was recently provided by scientists at GSK working in collaboration with colleagues at the Harvard Stem Cell Institute[17]. Using motor neurons differentiated from iPSCs from patients with a familial form of ALS (SOD1), they were able to identify a disease-relevant phenotype and elucidate its likely cause as dysfunction of the potassium channel Kv7.2/3. Ezogabine, an approved antiepileptic and Kv7.2/3 agonist, reversed the disease phenotype, as did correction of the SOD1 mutation, thereby definitively linking the known genetic lesion with the observed cellular phenotype. Analysis of ALS iPSC-derived motor neurons from patients with mutations other than SOD1 confirmed the generality of the observations and encouraged the team to initiate a clinical trial of ezogabine in ALS. Having access to multiple ALS iPSC lines with different genetic lesions was an important confidence builder and highlights the value in assembling large repositories of well-annotated patient-derived iPSC lines.

Patient-derived iPSCs can also be used as an entry point into understanding the molecular basis of disease. If successful, initial investigations using iPSCs can identify genes that can be the subject of traditional target-based screening campaigns. For example, Joe Wu, a CIRM Tissue Collector (**Table 1**), and his colleagues have established an iPSC-derived model of dilated cardiomyopathy (DCM) and found reduced β-adrenergic signalling in DCM cardiomyocytes (DCM-CM) compared to controls. Transcriptional profiling showed upregulation in DCM-CM of phosphodiesterases 2A and 3A, enzymes involved in dampening β-adrenergic signalling by hydrolysing cAMP[18]. Thus, even when not used for high throughput screening, patient-derived iPSC models have enormous utility as platforms for target discovery.

While early results are encouraging, patient-derived iPSC lines are not a panacea. Target deconvolution remains an issue at some companies[19] despite advances that promise to reduce the effort required for this activity[20,21]. In addition not all diseases demonstrate cell-autonomous phenotypes, requiring models that incorporate multiple cell types, three-dimensional scaffolds or both[22].

Despite these challenges, patient-derived iPSC

lines offer insights into human disease biology that are only likely to increase as more lines are made available for study. The CIRM Human iPSC Initiative represents a farsighted investment in this exciting new technology that should pay dividends for many years to come. DDW

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