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iPS cells: five years later

By Kai-Jye Lou, Staff Writer

Despite the proliferation of technologies to derive induced pluripotent stem cells since they were first described five years ago, this has been a technology in search of a home. The tide may have finally turned. In March, **Cellular Dynamics International Inc.** said its induced pluripotent stem cell–derived product—iCell Cardiomyocytes—will be added to the drug development toolbox at **Roche**.

Other pharmas are also exploring applications of induced pluripotent stem (iPS) cell–derived systems for R&D, but broad adoption has not occurred due to a dearth of data validating their utility in most drug discovery, drug screening and disease modeling settings. Showing their utility as a basis for therapeutics is even further away.

Researchers and iPS cell companies alike need to show they can produce iPS cell–derived somatic cells in the quantities and at the consistency and purity needed to meet pharma's standards. They also must prove that the derived cells faithfully capture relevant disease phenotypes and biological processes.

iPS cells genetically match the organism or individual from which they are derived and are relatively easy to generate compared with embryonic stem cells (ESCs). Nor does the generation and use of iPS cells raise the ethical concerns of ESCs.

"iPS cells offer the promise of more relevant cell-based assays and better understanding of mechanisms of disease through the derivation of iPS cell lines from disease- or patient-specific samples," said Matthew Singer, manager of scientific development at iPS cell and reagent company **Stemgent Inc.** "Furthermore, the ability to generate patient-specific lines allows pharmaceutical and biotech companies to develop banks of cells with characterized genetic backgrounds upon which to screen for new drugs or test the toxic effects of drug candidates. And similar to ESCs, iPS cells offer the promise of a potentially unlimited supply of cells to replace the high demand and short supply of cadaveric cell sources."

In 2008, **GlaxoSmithKline plc** and the **Harvard Stem Cell Institute** signed a 5-year, $25 million collaborative agreement to explore the use of stem cells, including ESCs and iPS cells, for drug discovery. Later that year, **Pfizer Inc.** launched its Pfizer Regenerative Medicine unit to explore the use of ESC- and iPS cell–derived cell lines for drug discovery.

Other pharmas and big biotechs are at least taking a financial stake in stem cell companies. In 2009, the venture arms of **Astellas Pharma Inc.** and **Genzyme Corp.** (now part of **Sanofi**) participated in a $30 million series B round for adult stem cell and iPS cell company **Fate Therapeutics Inc.** Earlier this month, the Takeda Ventures Inc. arm of **Takeda Pharmaceutical Co. Ltd.** made an undisclosed equity investment in Fate, noting that the move is consistent with the pharma's intention to "develop a stronger foundation in regenerative medicines."

Fate Therapeutics is using its stem cell technology platforms to discover therapeutic small molecules and biologics.

Singer said pharma is primarily looking to generate human cardiomyocytes, hepatocytes and neurons via iPS cell technologies, as those cells are associated with major disease areas and are the key sites of drug toxicity.

"The promise of these cells is to offer more relevant material for drug screening and toxicity assays than currently used immortalized cell lines and animal models," he told *SciBX*.

"From a practical application point of view, the iPS cells are not the end goal," noted Emile Nuwaysir, VP and COO at Cellular Dynamics International (CDI). "The end goal is obtaining a relevant terminal cell type. Until those terminal cells are readily available in unlimited quantity, quality and purity, the practical applications of iPS cells will be limited."

Reprogramming improvements

Sheng Ding, a senior investigator at the **Gladstone Institute of Cardiovascular Disease** and professor in the Department of Pharmaceutical Chemistry at the **University of California, San Francisco**, said there are lots of proof-of-concept data on protocols to reprogram and differentiate iPS cells, but the data on the practicality of such methods are limited.

Ding is a scientific cofounder of Fate Therapeutics.

"The key needs that should be addressed with iPS cells are a steady supply of cells, establishment of simplified methods for culture and differentiation, and cost reduction for their preparation and differentiation," said Atsushi Nakanishi, research manager in the Biology Research Laboratories at Takeda. "In addition, it should be proved that differentiated cells generated from iPS cells have the same characteristics as the original and intended cells in tissues."

The initial protocol for reprogramming somatic cells into iPS cells was published in 2006 and used retrovirus vector–mediated expression of four transcription factors (Oct4, Sox2, Klf4 and c-Myc).[1] The protocol was inefficient—less than 1% of the original somatic cell population reverted to the pluripotent state. Moreover, the method used a vector that inserts into the host cell genome, and the resulting cells appeared to be only partially reprogrammed when compared with ESCs.

Most research in iPS cell technologies has since focused on improving the reprogramming step by moving away from vectors that integrate into the host cell genome. Now, there are multiple nonintegrating methods for reprogramming that use transfectable DNA vectors, protein delivery and RNA delivery (*see* **Table 1, "Methods for stem cell reprogramming"**).

The move away from integrating vectors typically decreases reprogramming efficiency and increases complexity. One exception is a set of

Table 1. Methods for stem cell reprogramming. Current approaches to reprogramming somatic cells into induced pluripotent stem (iPS) cells fall into four major categories, each using a different set of vectors and/or molecules to deliver reprogramming factors into the cells. *Sources: Anokye-Danso, F.* et al. Cell Stem Cell **8**, 376–388 (2011). *Ho, R.* et al. J. Cell Phys. **226**, 868–878 (2011). *Nishimura, K.* et al. J. Biol. Chem. **286**, 4760–4771 (2011). *Stadtfeld, M. & Hochedlinger, K.* Genes Dev. **24**, 2239–2263 (2010). *BioCentury Archives*

Delivery/ Expression system	Description	Pros	Cons
Category: Integrating/nonexcisable			
Retrovirus	Somatic cells are transduced with retrovirus encoding genes for reprogramming factors	Average efficiency Transgenes for reprogramming factors are silenced after reprogramming into iPS cell state	Genomic integration Transgene silencing may be incomplete and interfere with subsequent differentiation steps
Lentivirus	Somatic cells are transduced with lentivirus encoding genes for reprogramming factors	Average efficiency Transgenes are silenced after reprogramming	Genomic integration Transgene silencing is less efficient than retrovirus
Inducible lentivirus	Somatic cells are transduced with lentivirus encoding inducible genes for reprogramming factors Transgene expression in infected cells is induced with inert drugs (for example, doxycycline)	Average efficiency Controlled transgene expression	Genomic integration
Secondary inducible lentiviral system	Primary populations of iPS cells are generated with an inducible lentivirus system Primary iPS cells are then differentiated into somatic cells that still carry the inducible transgenes Inert drug is then used to induce transgene expression in differentiated somatic cells to generate secondary iPS cells	Average to very high efficiency depending on cell type Controlled transgene expression No direct delivery of virus to secondary iPS cell population Capable of reprogramming cells that are difficult to transduce	Genomic integration Requires additional steps to differentiate and screen cells
Category: Integrating/excisable			
Lentivirus with floxed transgenes	Somatic cells are transduced with a lentivirus encoding excisable genes for reprogramming factors Virus-transduced transgenes are excised from the cell genome with a Cre recombinase when they are no longer needed	Average efficiency Transgenes removed	Requires additional steps to screen and analyze cells Short sequences from vector still remain in genome
Transposon	Transposon (for example, piggyBac) encoding genes for reprogramming factors are introduced into somatic cell genome using transposase Transposase also used to excise transposon from cell genome when transgenes are no longer needed	Average efficiency All vector sequences removed	Requires additional steps to screen and analyze cells
Category: Nonintegrating/DNA based			
Adenovirus	Somatic cells are transduced with an adenovirus encoding genes for reprogramming factors	No genomic integration under normal circumstances	Low efficiency Potential for vector DNA to integrate with host cell genome is low but still exists Requires additional steps to screen and analyze cells for possible vector integration
Plasmid	Somatic cells are transfected with a plasmid encoding genes for reprogramming factors		
Episome	Somatic cells are transfected with episomes encoding genes for reprogramming factors		
Minicircle	Somatic cells are transfected with a minicircle encoding genes for reprogramming factors		
Category: Nonintegrating/DNA free			
Protein	Reprogramming factors delivered directly into somatic cells Delivered as purified recombinant proteins or as whole-cell extracts from embryonic stem cells (ESCs), genetically engineered human cells or bacteria	No genomic integration	Low efficiency Need for steady supply of reprogramming factors can become expensive
Sendai virus	Somatic cells are transduced with Sendai virus encoding genes for reprogramming factors Vector is RNA and thus will not integrate into host cell genome Vector replicates in host cell cytoplasm	High efficiency No genomic integration Reprogramming factors produced in high quantities	Reprogrammed cells need to be continuously passaged to remove virus-encoded transgenes
Modified mRNA	Somatic cells are transfected with modified mRNAs encoding reprogramming factors	High efficiency No genomic integration	Requires multiple rounds of transfection
MicroRNA[A]	Somatic cells are transfected with miRNAs	Reprograms somatic cells without exogenous, transcription factor–based reprogramming agents May have high efficiency May be able to reprogram somatic cells that are refractory to reprogramming with standard reprogramming factors	Method still needs to be replicated and validated with an existing nonintegrating method for miRNA delivery Mechanism of reprogramming still needs to be defined

[A]Study used integrating lentivirus vector to deliver miRNAs, but nonintegrating vectors for delivering miRNA into cells already exist and have been used to increase iPS cell reprogramming efficiency with transcription factors.

modified mRNAs from **ModeRNA Therapeutics**, which have higher reprogramming efficiencies than integrating vectors.[2] However, this method requires multiple rounds of transfection to reprogram the cells, whereas integrating vectors like retroviruses and lentiviruses only need one round.

"What you really want is a derivation method that is easy to use and also high efficiency, and one that doesn't leave a genetic trace in the reprogrammed cells," said George Daley, director of the stem cell transplantation program at **Children's Hospital Boston** and scientific cofounder of iPS cell company **iPierian Inc.** "Efficiency makes it easier to generate the iPS cells, and high-efficiency methods may be needed for cells that are refractory to reprogramming. The quality of the generated iPS cells could also be related to efficiency. For example, if you use low-efficiency methods, it may be more difficult to pick out the high-quality iPS cells from your culture."

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>
> ***—George Daley, Children's Hospital Boston***

iPierian is using its iPS cell technology platform to aid drug discovery research.

"In order to choose between all of these different reprogramming methods, we ask a few simple questions," said Nuwaysir. "Is the method efficient enough to reprogram the sample of choice? Does it generate clean, pristine, footprint-free colonies? Is the method easy to perform? Is it reproducible? Is it amenable to automation and industrialization? From the CDI perspective, the only method that meets all these criteria today is episomal reprogramming. What makes this method even more attractive is that we can implement it from a standard blood draw sample."

CDI generates iPS cells using a type of transfectable DNA sequence called an episome.

Immature differentiation protocols

In addition to ramping up iPS cell yields, another area in need of improvement is the development of differentiation protocols used to turn iPS cells into the desired somatic cell types.

"Much of the progress in this field has been in finding better ways to create iPS cells, but it remains challenging to develop protocols to differentiate such cells," said Marius Wernig, an assistant professor of pathology in the Institute for Stem Cell Biology and Regenerative Medicine at **Stanford University**. "It is relatively easy to turn somatic cells into iPS cells, but scientists are finding that it is actually very challenging to generate mature, differentiated cells from iPS cells, and the available protocols take a lot of time—often two to three months. For example, iPS-derived blood and heart cells express gene products more reminiscent of fetal cell types even after extensive differentiation periods."

"One of the key reasons companies are not replacing their trusted assays with those involving iPS cell–derived cells is that these cells appear to be most similar to embryonic or fetal tissue and thus do not possess the physiological properties found in adult primary cells," added Singer. "Until differentiation or maturation protocols are developed that can solve this issue, industry may be slow to adopt iPS cell–derived cells for use in disease research and pipelines."

"The protocols for generating specialized cells and tissues don't yet exist for all cell types," Daley told *SciBX*. "Researchers are still struggling to develop protocols that can generate disease-relevant tissues from iPS cells. With the exception of those used to generate motor neurons, most other protocols for generating specialized cells from iPS cells may not be creating cells that fully recapture the desired cell phenotype."

Wernig added that there is an alternative cellular reprogramming approach called direct lineage reprogramming, whereby somatic cells are directly converted from one cell type into another without first reverting to a pluripotent state.

"This shortcut approach has now been demonstrated to generate neurons, heart cells, blood cells and liver cells. It is also much faster and may generate cells with a more homogeneous and mature phenotype," he said. However, he added that the cells used for direct lineage reprogramming are not as scalable as iPS cells.

"While somatic cells like human fibroblasts can be expanded in culture to some degree, they do eventually lose the ability to divide. So if one is thinking about generating cells in high throughput, this could be a significant disadvantage," said Wernig.

On the other hand, he noted that direct lineage reprogramming would be much easier to scale at the patient level, as generating iPS cells is slower, more laborious and shows considerable line-to-line variability.

Modeling relevance

Although there have been many reports of iPS cell–derived disease models,[3] researchers contacted by *SciBX* said it remains to be proven whether such models accurately capture the relevant disease phenotypes and biological processes that will aid the discovery of new drugs.

Ann Tsukamoto, EVP of R&D at adult stem cell company **StemCells Inc.**, gave an example. "Investigators have shown that iPS cell–derived neurons from patients with Parkinson's disease have defects that are characteristic of the disease, which is great. Now, the question that needs to be answered is: Will these models help scientists to better understand the disease and allow one to screen for new drug candidates, and will the identified candidates be better than those identified using standard cell lines?"

StemCells is running a Phase I/II trial of its human neural stem cells (HuCNS-SCs) for spinal cord injury (SCI). Early next year, the company also expects to report data from its ongoing Phase I trial of the cells in four patients with connatal Pelizaeus-Merzbacher Disease (PMD), a fatal myelination disorder in children.

Tsukamoto added that hepatocytes derived from iPS cells and ESCs "don't really behave like the hepatocytes we derive from prospectively purified adult liver stem cells, which behave more similarly to actual human hepatocytes. So far, we haven't seen iPS-derived cells that are able to perform with the same level of activity as cells with an adult phenotype."

Wernig said it will be important to show companies that cells derived from iPS cells or via direct lineage reprogramming can be incorporated into a high throughput assay that can be used to see whether candidate molecules rectify a particular disease phenotype

"Ultimately we will want to be able to show that we can use these iPS-derived cellular models to discover new drugs," Daley told *SciBX*.

Therapeutic viability

Because iPS cells are genetically matched to the patient from whom they are derived and can be expanded indefinitely, they theoretically could serve as a rich source of autologous cells for use in therapy. However, the use of cells derived from iPS cells in the therapeutic setting may be more challenging than originally thought.

Indeed, no company has disclosed an iPS cell–derived therapeutic in its pipeline.

On top of the hurdles associated with the use of iPS cell–derived somatic cells in the R&D setting, use of such cells as therapeutics faces additional roadblocks with respect to safety, efficacy and identifying the indications in which the cells could be used.

Earlier this year, multiple research groups discovered that iPS cells contain more mutations than ESCs.[4–6] This month, a group at the **University of California, San Diego** showed that the reprogrammed cells, despite being genetically matched, can still elicit an immune response from the host, thus calling into question whether these cells will actually be nonimmunogenic.[7]

"In the therapeutic setting, I think iPS cells are a dead end—the reason being that it is currently too challenging to establish that these cells are safe," said Florent Gros, a managing director at the Novartis Venture Funds unit of **Novartis AG**. "You don't know what abnormalities are being introduced into the cells during reprogramming, so there is going to be that implied risk of cancer, which cannot be disproved. The regulatory path for traditional stem cell therapeutics already is very difficult, and this reprogramming process creates an additional layer of risk. So from a venture capitalist's standpoint, this is creating an additional risk that no VCs would want to fund at this time."

Gros also said emerging technologies, such as lineage-specific pluripotent cells, could make iPS cell technologies obsolete from a therapeutic standpoint by the time the safety concerns are addressed.

Ding was more sanguine about the prospects of iPS cells as therapeutics. "If the iPS-derived cells are to be used for clinical applications, we will need to see many additional studies in disease models to show these cells have a clear therapeutic effect. I think ultimately iPS-derived cells will have therapeutic applications, but right now the focus should be on developing better protocols for creating the iPS cells and differentiating them into the relevant cell types and, most importantly, finding out what are the diseases where patients would benefit from the use of such cells," he said.

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