induced pluripotent stem cells

a model for transforming drug discovery

Induced pluripotent stem (iPS) cells have the potential to transform drug discovery by providing physiologically relevant cells for toxic compound identification, target validation, compound screening, and tool discovery. The technology for generating iPS cells is advancing rapidly, as is the repertoire of cell types that can be differentiated. Tissue-specific cells derived from iPS cells are currently being evaluated by the pharmaceutical industry for their utility in identifying cardiotoxic and hepatotoxic compounds as therapeutically relevant systems for modelling cardiovascular diseases, neurodegenerative disorders and metabolic disorders, as well as for generating patient-specific cell types. In order to fully capitalise on the rapidly evolving science of iPS cell technology, pharma will need to leverage the expertise found in academia and biotechnology companies to apply iPS-derived cells in an industrial setting. This review will summarise the potential of these cells as well as highlight many of the challenges that remain.

By Dr Dwight Morrow and Dr Julie Holder

Stem cells are a distinct self-replenishing cell population whose primary function is to generate progeny that then develop into terminally differentiated cell types, such as a cardiomyocytes, neurons or photoreceptors. Tissue-specific adult stem cells, or progenitors, are committed to producing tissue or lineage-specific cells, whereas totipotent or pluripotent stem cells can give rise to any of the 200+ cell types of the body. There are two types of pluripotent stem cells defined by their tissue origin: 1) embryonic stem (ES) cells obtained from early embryos, typically at the blastula stage, and 2) induced pluripotent stem (iPS) cells derived through a reprogramming process whereby terminally differentiated somatic cells are reprogrammed or induced to a pluripotent state (**Figure 1**).

Successful generation of human iPS cells was reported independently in 2007 by the research groups of Drs James Thomson and Shinya Yamanaka[1,2]. Both groups successfully identified a minimum number of nuclear factors that could reprogramme terminally differentiated fibroblasts to pluripotent cell lines by exogenously expressing distinct yet overlapping sets of genes: Yu and colleagues used Oct4, Sox2, Nanog and Lin28 by lentiviral gene transfer, while Yamanaka and colleagues employed Oct4, Sox2, Klf4 and c-Myc via retroviral gene transfer. These landmark studies were a significant advance over whole nucleus reprogramming[3] and have had several important consequences. Induced pluripotent stem cell technology does not require human embryos and thus circumvents the ethical issues associated with

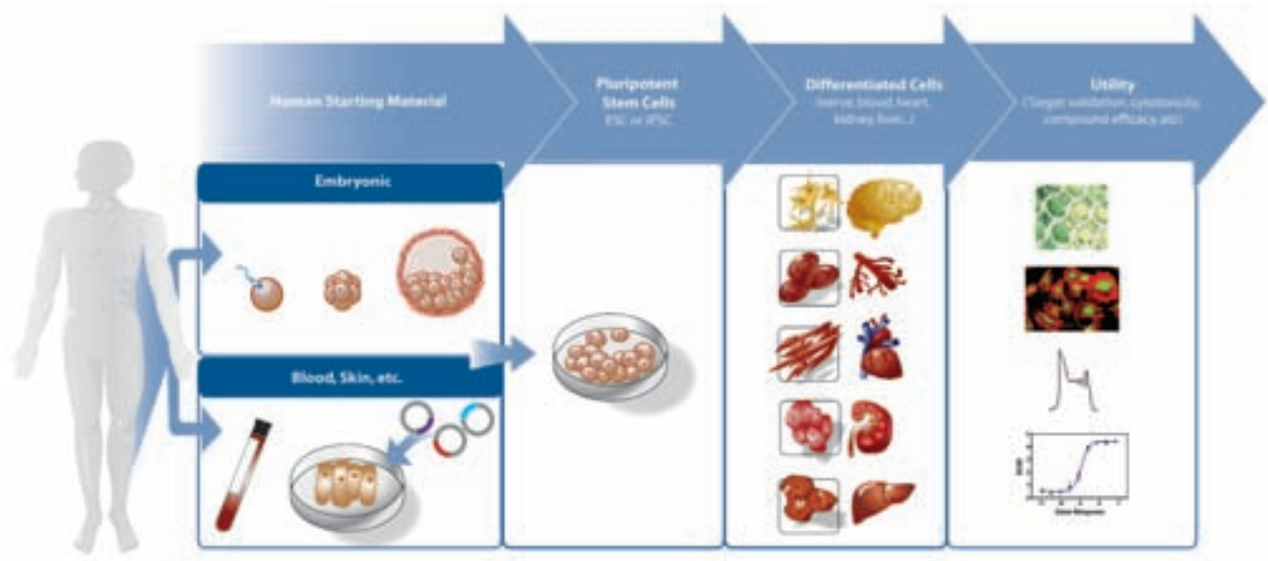

Figure 1
Pluripotent stem cell generation and utility. Embryonic stem cells are isolated from early stage embryos, whereas induced pluripotent stem (iPS) cells are reprogrammed from terminally differentiated cells types such as those found in blood and skin. Pluripotent stem cell populations are maintained and propagated indefinitely in culture. Experimental intervention causes differentiation to terminal cell types, which can then be used for a variety of investigational endpoints

embryonic stem cells. Of more utility is the potential to introduce population diversity into early and basic research programmes, as iPS cells can be generated from any individual. This iPS cell capability can provide researchers with tissue from virtually any genotype, ranging from the broad spectrum of 'healthy' individuals to clinical and disease cohorts to individuals exhibiting specific side-effects and/or idiosyncratic toxicities. Induced pluripotent stem cells will advance the concept of personalised medicine closer to reality.

iPS cell generation: Several ways to reprogramme a cell

As noted above, the initial iPS cell derivations utilised four-independent factors delivered via viral vectors to reprogramme the starting fibroblast populations. Since those seminal reports, reprogramming of cells has been successfully accomplished with a variety of delivery systems utilising variations of the initial four-factor cocktail, related transcription factor family members, endogenously expressed factors, small molecules, and soluble factors[4,5]. The use of non-genomic, small molecule factors is an attractive method for generating iPS cells, but the specificity with which they faithfully recapitulate transcriptional pathways is unknown, and it is unclear whether they can replace traditional reprogramming factors[4].

The potential to use autologous iPS cells as therapeutic medicines holds tremendous promise. Cellular physiological integrity is, however, paramount and will require delivery systems that do not cause oncogenic transformation or alter gene function. Lentiviral- and transposon-based systems, in which the inserted transgene is subsequently excised using the Cre recombinase/lox site system or transposases respectively[6,7], remain problematic, but recent technologies that do not require genome integration look more promising. These technologies include episomal vectors[8], recombinant proteins[9], viral RNA vectors[10], and synthetic modified mRNAs[11] (**Table 1**).

Another important consideration for reprogramming is the parental tissue source. The most popular starting material is fibroblasts, though other cell types have been successfully used, including keratinocytes[12], mesenchymal cells[13], adipose stem cells[14], and melanocytes[15]. While to date there are no reports of cell types refractory to reprogramming efforts, a more desirable source of starting material may be human peripheral blood, which can be readily obtained through routine and relatively non-invasive clinical procedures. Efforts to develop such methods have yielded iPS cells derived from human CD34+ blood stem cells and T lymphocytes[16-18]. Of particular note is a recent report demonstrating efficient human iPS cell derivation from T-lymphocytes collected in as little as 1ml of whole blood[19].

There is considerable work ahead in the characterisation of iPS cells and their progeny. For example, recent studies indicate that iPS cells carry epigenetic memory of their source tissue and exhibit a propensity to differentiate into their parental cell type[20,21]. However, this epigenetic memory

blank

References

1 Yu, J, Vodyanik, MA, Smuga-Otto, K, Antosiewicz-Bourget, J, Frane, JL, Tian, S, Nie, J, Jonsdottir, GA, Ruotti, V, Stewart, R, Slukvin, II and Thomson, JA. (2007). Induced pluripotent stem cell lines derived from human somatic cells. Science 318(5858): 1917-20.

2 Takahashi, K, Tanabe, K, Ohnuki, M, Narita, M, Ichisaka, T, Tomoda, K and Yamanaka, S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131(5):861-72.

3 Simonsson, S, Gurdon, JB. Changing cell fate by nuclear reprogramming. (2005). Cell Cycle, 4(4):513-5.

4 Maherali, N and Hochedlinger, K. Guidelines and techniques for the generation of induced pluripotent stem cells. (2008). Cell Stem Cell 3(6):595-605.

5 Kiskinis, E and Eggan, K. (2010). Progress toward the clinical application of patient-specific pluripotent stem cells. J Clin Invest 120(1):51-9.

6 Soldner, F, Hockemeyer, D, Beard, C, Gao, Q, Bell, GW, Cook, EG, Hargus, G, Blak, A, Cooper, O, Mitalipova, M, Isacson, O and Jaenisch, R. (2009). Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 136(5):964-77.

7 Woltjen, K, Michael, IP, Mohseni, P, Desai, R, Mileikovsky, M, Hämäläinen, R, Cowling, R, Wang, W, Liu, P, Gertsenstein, M, Kaji, K, Sung, HK and Nagy, A. (2009). piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 458(7239):766-70.

8 Yu, J, Hu, K, Smuga-Otto, K, Tian, S, Stewart, R, Slukvin, II and Thomson, JA. (2009). Human induced pluripotent stem cells free of vector and transgene sequences. Science 324(5928):797-801.

Continued on page 35

appears to be transitory and can be erased through repeated passaging, additional reprogramming, or with chromatin modifying drugs. Repeated passaging may be the most straightforward approach to erasing epigenetic memory, as it is a necessary step in any scale up of iPS cells.

Stem cell differentiation and demonstrations of utility

Terminally differentiated cells *in vivo* are the end result of a complex set of chemical interactions, positional cues, environmental effects, and cell-to-cell communications, many of which have proven difficult to reproduce under *in vitro* conditions. Some cell types, such as cardiomyocytes, develop spontaneously in tissue culture dishes (albeit at very low frequencies) from stem cells while other cell types, such as hepatocytes, require significant experimental intervention[22-24]. Once a protocol has been elucidated, initial derivation processes and robust cell type markers can be used for method optimisation and production of industrialised quantities of cells.

What is the promise?

There are three major areas where iPS cell technology is beginning to have or will have a major impact on *in vitro* aspects of drug discovery: safety assessment, small molecule screening, and *in vitro* disease models.

Safety assessment

Finding novel drugs that are safe and efficacious is challenging. Attrition from candidate selection to drug launch is estimated at 95%, with 20% of that due to toxicity issues (lack of efficacy accounts for the greatest single cause of attrition[24,5], therefore iPS-derived cells may have a significant impact on drug discovery if they can be used in more physiological assays for toxicity screening.

Cardiomyocytes: Cardiovascular toxicity remains the single most important targeted organ resulting in an estimated 28% of drug withdrawals[26]. Multiple assay systems are currently employed to identify cardiotoxic drugs. These systems are based predominantly on cell lines such as human HEK-293s or hamster CHOs for measuring drug effects

REPROGRAMMING SYSTEMS			
Integrating vectors			
Delivery system	**Reprogramming vector**	**Genomic disposition**	**Reference**
Retroviral	DNA	Integrated	Takahashi et al[2]
Lentiviral	DNA	Integrated	Yu et al[1]
Lentiviral/Cre-lox	DNA	Excisable	Soldner et al[6]
Transposon/Transposase	DNA	Excisable	Woltjen et al[7]
Episomal vectors			
Delivery system	**Reprogramming vector**	**Vector disposition**	**Reference**
Co-culture	Small molecules	Transient (replacing one or more transcription factors)	Lyssiotis et al[49]
Episomal Vectors	Plasmid DNA	Degraded / kicked out	Yu et al[8]
Recombinant Protein	Protein	Degraded	Kim et al[9]
Sendai Virus	RNA	Degraded / kicked out	Fusaki et al[10]
Synthetic nucleotides	Synthetic mRNA	Degraded / kicked out	Warren et al[11]

Table 1: Methods for delivery of iPS cell reprogramming factors

on ion channels (including hERG) and transporters; isolated animal tissues including rabbit wedge for ECG and QT elongation studies; or whole animal experiments using rats, dogs, or monkeys for ECGs, hemodynamic studies, and pathological evaluation of repeat dose studies to identify cardiotoxic compounds.

Recent progress in manufacturing cardiomyocytes has been made using pluripotent stem cell technology. In 2009, Cellular Dynamics International (CDI) introduced the first human iPS cell-derived product, iCell™ Cardiomyocytes, while GE/Geron corporation released embryonic stem cell-derived cardiomyocytes in 2010. These human cells have the potential to reduce or replace the assay systems and animal models mentioned above. Both platforms are a pan-cardiac population consisting of ventricular, atrial and nodal cells. Extensive characterisation of these cells has demonstrated molecular, electrophysiological, and cardiotoxic function similar to native heart cells[27] (Ma et al, in preparation). In addition to providing large numbers of reproducible cardiomyocytes for use in traditional toxicity testing, the *in vivo* functionality of pure populations of iPS cell-derived cardiomyocytes will enable the development of potentially more targeted, sensitive, and relevant biochemical and electrophysiological cardio-specific testing paradigms.

Hepatocytes: Liver toxicity is another major cause of drug withdrawal from the market. Current systems for measuring hepatotoxicity include primary human hepatocytes (PHH) or human hepatocyte cell lines such as HepG2s. Both of these have major draw backs. Primary human hepatocytes must be shipped either fresh immediately after isolation or frozen, and they display batch-to-batch variation. Additionally, they are not stable in culture for more than a few days and lose P450 expression. Yet PHH remain the gold standard. Cell lines such as HepG2 express little to no P450 enzymes, the major drug metabolising enzymes, making these cell lines inappropriate model systems[28]. What is needed is a reliable cell supply with batch-to-batch consistency. In addition, these cells must express appropriate markers and enzymes including the P450 enzymes and Phase I and Phase II xenobiotic metabolising enzymes, thus demonstrating intrinsic hepatocyte metabolism including oxygen consumption and ATP utilisation, exhibiting appropriate cell morphology and polarisation, and having the ability to identify known hepatotoxic compounds. Stem cell-derived hepatocytes have been reported to exhibit many, but not yet all, of these phenotypic and functional characteristics[24,29-30].

Small molecule screening

Traditional small molecule screens are performed using purified protein or recombinant cell lines over-expressing the target of interest. The industry uses such systems because they are easy to develop and configure to their platforms, are relatively inexpensive, and have a track record of success (Macarron et al, in preparation). Recombinant systems are not ideal, however. The target of interest is over-expressed in a non-native cell where splice variants or post-translational modifications may not occur; iPS cells have the potential to change that. A recent example comes from a paper by McNeisch et al[31] where neurons expressing a-amino-3-hydroxyl-5-methyl-4-isoxazoleproprionate (AMPA) subtype glutamate receptors were generated from mouse iPS cells. This is a particularly challenging target because there are four subtypes, multiple RNA splice variants, and interactions with transmembrane regulatory proteins, all giving rise to a significant number of potentially expressed receptors. McNeisch et al[31] successfully generated a sufficient quantity of cells to run a high-throughput screen of several million compounds and identified novel small molecule AMPA potentiators. Such an approach might readily be applied to other targets including ion channels, GPCRs, and receptor tyrosine kinases where full functionality requires multimers of subtypes, chaperones, and protein complexes.

***In vitro* disease models**

Induced pluripotent stem cell technology provides a unique opportunity to generate 'disease phenotypes in a dish' for use as *in vitro* model systems and substrates for small molecules screens. While it is unlikely that an *in vitro* system can adequately represent all aspects of a multi-faceted disease, the ability to generate any human tissue cells from any genotype or disease provides unparalleled access for studies. There are an increasing number of examples where iPS cells have been derived from patients, but as of now only a few show measurable phenotypic endpoints (**Table 2**). The notable examples include iPS cell-derived motor neurons from patients with spinal muscular atrophy (SMA), where the derived neurons demonstrated a significantly reduced level of survival motor neuron 1 (SMN1) gene expression, and appropriate response to drugs known to increase SMN1 gene expression accurately mimicked the phenotype observed in SMA patients[32]. Another example comes from Lee et al[33] who studied familial dysautonomia (FD), a fatal neurodegenerative disease of sensory and autonomic neurons. iPS cell-derived

Continued from page 34

9 Kim, D, Kim, CH, Moon, JI, Chung, YG, Chang, MY, Han, BS, Ko, S, Yang, E, Cha, KY, Lanza, R and Kim, KS. (2009). Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell (6):472-6.
10 Fusaki, N, Ban, H, Nishiyama, A, Saeki, K and Hasegawa, M. (2009). Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. Proc Jpn Acad Ser B Phys Biol Sci 85(8):348-62.
11 Warren, L, Manos, PD, Ahfeldt, T, Loh, YH, Li, H, Lau, F, Ebina, W, Mandal, PK, Smith, ZD, Meissner, A, Daley, GQ, Brack, AS, Collins, JJ, Cowan, C, Schlaeger, TM and Rossi, DJ. (2010). Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell 7(5):618-30.
12 Aasen, T, Raya, A, Barrero, MJ, Garreta, E, Consiglio, A, Gonzalez, F, Vassena, R, Bilic, J, Pekarik, V, Tiscornia, G, Edel, M, Boué, S and Izpisúa Belmonte, JC. (2008). Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nat Biotechnol 26(11):1276-84.
13 Park, IH, Arora, N, Huo, H, Maherali, N, Ahfeldt, T, Shimamura, A, Lensch, MW, Cowan, C, Hochedlinger, K and Daley, GQ. (2008). Disease-specific induced pluripotent stem cells. Cell 134(5):877-86.
14 Sun, N, Panetta, NJ, Gupta, DM, Wilson, KD, Lee, A, Jia, F, Hu, S, Cherry, AM, Robbins, RC, Longaker, MT and Wu, JC. (2009). Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells. Proc Natl Acad Sci U S A 106(37):15720-5.

Continued on page 37

Table 2: Examples of 'disease phenotypes in a dish' by iPS-derived terminal cell types

DISEASE PHENOTYPES IN A DISH			
Cardiac			
Disease	**Source**	**Expressed phenotype**	**Reference**
Long QT Type 1	Patient iPS cells	Prolonged action potential, reduced IKs current	Moretti et al[35]
LEOPARD Syndrome	Patient iPS cells	Enlarged cell size, greater sarcomeric organisation, signal localisation	Carvajal-Vergara et al[36]
Pompe Disease	Patient iPS cells	lack of acid alpha glucosidase activity, glycogen filled lysosomes	Raval et al[37]
Hypertrophy	Exogenous factors	Enlarged cells size, sarcomeric organisation and ANNP mRNA expression	Foldes et al[39]; Kattman et al, unpublished
Hepatic			
Disease	**Source**	**Expressed phenotype**	**Reference**
Alpha1-antitrypsin deficiency	Patient iPS cells	Aggregation of misfolded protein	Rashid et al[38]
Familial hypercholesterolemia	Patient iPS cells	Deficient LDL receptor-mediated cholesterol uptake	Rashid et al[38]
Glycogen storage disease type 1a	Patient iPS cells	Elevated lipid and glycogen accumulation	Rashid et al[38]
Neuronal			
Disease	**Source**	**Expressed phenotype**	**Reference**
Spinal muscular atrophy (SMA)	Patient iPS cells	Reduced levels of SMN1; response to compounds	Ebert et al[32]
Familial Dysautonomia (FD)	Patient iPS cells	Decreased neurogenic differentiation	Lee et al[33]
Parkinson Disease (PD)	Genetic engineering	Decreased number of dopaminergic neurons	Schneider et al[50]
Huntington's Disease (HD)	Transgenic monkey iPS	Intracellular aggregates and intranuclear inclusions	Chan et al[34]
Misc			
Disease	**Origin**	**Cell phenotype**	**Citation**
Dyskeratosis congenita	Patient iPS	Telemere shortening	Agarwal et al[51]

neural crest progenitor cells from an FD patient displayed the expected abnormal splicing of IKBKAP, exon skipping and a general reduction of protein. These neurons displayed a reduced ability to migrate, a reduction in the number of focal adhesion points, and the expected and appropriate

response to drugs known to reduce the level of mutant IKBKAP splice form. Other diseases where cellular phenotypes have been observed include Huntington's disease[34]; Parkinson's disease; Long QT syndrome[35]; Leopard syndrome[36]; Pompe disease[37]; hepatic glycogen storage disease[38]; and ubiquitous aspects of cardiac hypertrophic phenotypes in both ES and iPS cell-derived cardiomyocytes[39]; (Kattman et al, unpublished).

What needs to change for Pharma to implement iPSC-derived models?

Even though significant progress has been made, it is likely that full adoption and general acceptance will require comparative studies, scale-up and pilot screens. There remains insufficient data demonstrating that iPS cells are superior to existing primary animal cells or standard lab cell lines, though protocols for generating terminally mature, differentiated human cell types are progressing rapidly. Significant progress has been made in creating insulin-producing beta-cells[40-41], ventricular cardiomyocytes[42], P450-expressing hepatocytes[43], specific neuronal populations[44], and retinal photoreceptors[45], to name just a few. Once these protocols are established, technology for scale up will be required that maintains a cell's phenotype, shows batch-to-batch consistency and reproducibility, and is cost-effective for the industry. Given the intensive research efforts ongoing in academic labs to improve protocols and in biotechnology companies to scale cells, there is reason for optimism that iPS cells will be used in the lab either alongside or as replacements for traditional cell lines.

What's the future vision for iPS cells?

iPS cells may have a profound impact on drug discovery, either in the generation of previously unobtainable cellular disease model systems for small molecule screening; mechanism of action studies; or highly predictable, animal-free systems for determining drug safety. One can envisage a screen of genetic diversity panels of cardiomyocytes or hepatocytes to identify rare responders, analyse drug effects on complex 3D organ systems, or perform 'clinical trials' using iPS-derived diseased cells where safety, efficacy, dosage studies, and the effect of genetics could be studied before initiating Phase I studies in patients. Another potential application will be use of iPS-derived cells and/or genetically-modified iPS cells in transplantation where there may be immunological advantages for organ engraftment. Proof of concept is now well-documented in pre-clinical models of retinal dystrophy[46], in reversal of liver damage[47], and in diabetic mouse models[48]. Full adoption of stem cell technologies will require generation of iPS cells that are safe, stable and efficacious. The use of iPS cells for transplantation, potentially coupled with gene therapy, may usher in the era of true personalised medicine for patients with high unmet needs.

Acknowledgements

The authors would like to thank Blake Anson and Joleen Rau from Cellular Dynamics International, Madison, WI, for extensive discussions, helpful advice and critical review of the document; as well as Jason Gardner and Aaron Chuang (Stem Cell Discovery Performance Unit, GlaxoSmithKline) for their insight and comments. DDW

Dr Julie Holder *has been involved in early discovery and development and late stage drug discovery throughout her GSK career. Julie is currently Director of Preclinical in the Stem Cell Discovery Performance Unit at GSK and has direct experience working with Mesenchymal Stem Cells (MSC) and extensive experience of compound development. She holds a PhD in Biochemistry from Leeds University, UK.*

Dr Dwight Morrow *is Director of Discovery in the Stem Cell Discovery Performance Unit at GSK and has extensive experience in cell-based assays, high throughput screening, and compound profiling (SAR). He received his PhD in Molecular Biology from Case Western Reserve University and did his post-doctoral work at Johns Hopkins University School of Medicine.*

Continued from page 35

15 Utikal, J, Maherali, N, Kulalert, W and Hochedlinger, K. (2009). Sox2 is dispensable for the reprogramming of melanocytes and melanoma cells into induced pluripotent stem cells. J Cell Sci 122(Pt 19):3502-10.
16 Loh, YH, Agarwal, S, Park, IH, Urbach, A, Huo, H, Heffner, GC, Kim, K, Miller, JD, Ng, K and Daley, GQ. (2009). Generation of induced pluripotent stem cells from human blood. Blood 113(22):5476-9.
17 Loh, YH, Hartung, O, Li, H, Guo, C, Sahalie, JM, Manos, PD, Urbach, A, Heffner, GC, Grskovic, M, Vigneault, F, Lensch, MW, Park, IH, Agarwal, S, Church, GM, Collins, JJ, Irion, S and Daley, GQ. (2010). Reprogramming of T cells from human peripheral blood. Cell Stem Cell 7(1):15-9.
18 Seki, T, Yuasa, S, Oda, M, Egashira, T, Yae, K, Kusumoto, D, Nakata, H, Tohyama, S, Hashimoto, H, Kodaira, M, Okada, Y, Seimiya, H, Fusaki, N, Hasegawa, M and Fukuda, K. (2010). Generation of induced pluripotent stem cells from human terminally differentiated circulating T cells. Cell Stem Cell 7(1):11-4.
19 Brown, ME, Rondon, E, Rajesh, D, Mack, A, Lewis, R, Feng, X, Zitur, LJ, Learish, RD and Nuwaysir, EF. (2010). Derivation of induced pluripotent stem cells from human peripheral blood T lymphocytes. PLoS One 5(6):e0011373.
20 Kim, K, Doi, A, Wen, B, Ng, K, Zhao, R, Cahan, P, Kim, J, Aryee, MJ, Ji, H, Ehrlich, LI, Yabuuchi, A, Takeuchi, A, Cunniff, KC, Hongguang, H, McKinney-Freeman, S, Naveiras, O, Yoon, TJ, Irizarry, RA, Jung, N, Seita, J, Hanna, J, Murakami, P, Jaenisch, R, Weissleder, R, Orkin, SH, Weissman, IL, Feinberg, AP and Daley, GQ. (2010). Epigenetic memory in induced pluripotent stem cells. Nature. 467(7313):285-90.

Continued on page 38

Continued from page 37

21 Polo, JM, Liu, S, Figueroa, ME, Kulalert, W, Eminli, S, Tan, KY, Apostolou, E, Stadtfeld, M, Li, Y, Shioda, T, Natesan, S, Wagers, AJ, Melnick, A, Evans, T and Hochedlinger, K. (2010). Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. Nat Biotechnol 28 (8):848-55.

22 Kehat, I, Kenyagin-Karsenti, D, Snir, M, Segev, H, Amit, M, Gepstein, A, Livne, E, Binah, O, Itskovitz-Eldor, J and Gepstein, L. (2001). Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. J Clin Invest 108 (3):407-14.

23 He, JQ, Ma, Y, Lee, Y, Thomson, JA and Kamp, TJ. (2003). Human embryonic stem cells develop into multiple types of cardiac myocytes: action potential characterization. Circ Res 93(1):32-9.

24 Sullivan, GJ, Hay, DC, Park, IH, Fletcher, J, Hannoun, Z, Payne, CM, Dalgetty, D, Black, JR, Ross, JA, Samuel, K, Wang, G, Daley, GQ, Lee, JH, Church, GM, Forbes, SJ, Iredale, JP and Wilmut, I. (2010). Generation of functional human hepatic endoderm from human induced pluripotent stem cells. Hepatology 51(1):329-35.

25 Kola, I and Landis, J. (2004). Can the pharmaceutical industry reduce attrition rate? Nat Rev Drug Dis 3:711-6.

26 Gwathmey, JK, Tsaioun, K and Hajjar, RJ. (2009). Cardionomics: a new integrative approach for screening cardiotoxicity of drug candidates. Expert Opin. Drug Metab. Toxicol 5(6):647-60.

27 Peng, S, Lacerda, AE, Kirsch, GE, Brown, AM and Bruening-Wright, A. (2010). The action potential and comparative pharmacology of stem cell-derived human cardiomyocytes. J Pharmacol Toxicol Methods 61(3):277-86.

28 Donato, MT, Lahoz, A, Castell, JV and Gómez-Lechón, MJ. (2008). Cell Lines: A tool for In Vitro Drug Metabolism Studies. Curr Drug Metab 9:1-11.

29 Sancho-Bru, P, Roelandt, P, Narain, N, Pauwelyn, K, Notelaers, T, Shimizu, T, Ott, M and Verfaillie, C. (2010). Directed differentiation of murine-induced pluripotent stem cells to functional hepatocyte-like cells. J Hepatology, 54(1): 98-107

30 Guguen-Guillouzo, C, Corlu, A, Guillouzo, A. (2010). Stem cell-derived heptocytes and their use in toxicology. Toxicology 270:3-9.

31 McNeish, J, Roach, M, Hambor, J, Mather, RJ, Weibley, L, Lazzaro, J, Gazard, J, Schwarz, J, Volkmann, R, Machacek, D, Zawadzke, L, O'Donnell, C and Hurst, R. (2010). High-throughput screening in embryonic stem cell-derived neurons identifies potentiators of a-amino-3-hydroxyl-5-methyl-4-isoxazolepropionate-type glutamate receptors. J Biol. Chem. 285(22):17209-17.

32 Ebert, AD, Yu, J, Rose, FF, Mattis, VB, Lorson, CL, Thomson, JA and Svendsen, CN. (2009). Induced pluripotent stem cells from a spinal muscular atrophy patient. Nature 457:277-81.

33 Lee, G, Papapetrou, EP, Kim, H, Chambers, SM, Tomishima, MJ, Fasano, CA, Ganat, YM, Menon, J, Shimizu, F, Viale, A, Tabar, V Sadelain, M, and Studer L. (2009). Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. Nature 461(7262):402-6.

34 Chan, AWS, Cheng, P-H, Neumann, A and Yang, J-J. Reprogramming Huntington monkey skin cells into pluripotent stem cells. (2010). Cellular Reprogramming 12(5):509-17.

35 Moretti, A, Bellin, M, Welling, A, Jung, CB, Lam, JT, Bott-Flügel, L, Dorn, T, Goedel, A, Höhnke, C, Hofmann, F, Seyfarth, M, Sinnecker, D, Schömig, A and Laugwitz, KL. (2010). Patient-specific induced pluripotent stem-cell models for long-QT syndrome. N Engl J Med 363(15):1397-409.

36 Carvajal-Vergara, X, Sevilla, A, D'Souza, SL, Ang, YS, Schaniel, C, Lee, DF, Yang, L, Kaplan, AD, Adler, ED, Rozov, R, Ge, Y, Cohen, N, Edelmann, LJ, Chang, B, Waghray, A, Su, J, Pardo, S, Lichtenbelt, KD, Tartaglia, M, Gelb, BD and Lemischka, IR. (2010). Patient-specific induced pluripotent stem-cell-derived models of LEOPARD syndrome. Nature 465(7299):808-12.

37 Raval, KK, Koonce, CH, Zhang, J, Yu, J, Kamp, TJ and Thomson, JA. (2010). A Human Induced Pluripotent Stem Cell-derived Cardiomyocyte Model of Pompe Disease. Circulation 122: A19502.

38 Rashid, ST, Corbineau, S, Hannan, N, Marciniak, SJ, Miranda, E, Alexander, G, Huang-Doran, I, Griffin, J, Ahrlund-Richter, L, Skepper, J, Semple, R, Weber, A, Lomas, DA and Vallier, L. (2010). Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. J Clin Invest 120(9):3127-36.

39 Foldes, G, Mioulane, M, Wright, JS, Liu, AQ, Novak, P, Merkely, B, Gorelik, J, Schneider, MD, Ali, NN and Harding, SE. (2010). Modulation of human embryonic stem cell-derived cardiomyocyte growth: A testbed for studying human cardiac hypertrophy? J Mol Cell Cardiol, doi:10.1016/j.yjmcc.2010.10.029

40 Zhang, D, Jiang, W, Liu, M, Sui, X, Yin, X, Chen, S, Shi, Y and Deng, H. (2009). Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells. Cell Research 19:429-38.

41 Borowiak, M and Melton DA. (2009). How to make β cells? Curr Opin Cell Biol. 21:727-32.

42 Kuzmenkin, A, Liang, H, Xu, G, Pfannkuche, K, Eichhorn, H, Fatima, A, Luo, H, Saric, T, Wernig, M, Jaenisch, R and Hescheler, J. (2009). Functional characterization of cardiomyocytes derived from murine induced pluripotent stem cells in vitro. FASEB, 23:4168-80.

43 Song, Z, Cai, J, Liu, Y, Zhao, D, Yong, J, Duo, S, Song, X, Guo, Y, Zhao, Y, Qin, H, Yin, X, Wu, C, Che, J, Lu, S, Ding, M and Deng, H. (2009). Efficient generation of hepatocyte-like cells from human induced pluripotent stem cells. Cell Research, 19(11):1233-42.

44 Hwang, D, Kim, DS and Kim, DW. (2010). Human ES and iPS cells as cell sources for the treatment of Parkinson's disease: current state and problems. J Cell Biochem 109(2):292-301.

45 Parameswaran, S, Balasubramanian, S, Babai, N, Qiu, F, Eudy, JD, Thoreson, WB and Ahmad, I. (2010). Induced pluripotent stem cells generate both retinal ganglion cells and photoreceptors: Therapeutic implications in degenerative changes in glaucoma and age-related macular degeneration. Stem Cells 28:695-703.

46 Carr, A-J, Vugler, AA, Hikita, ST, Lawrence, JM, Gias, C, Chen, LL, Buchholz, DE, Ahmado, A, Semo, M, Smart, MJK, Hasan, S, da Cruz, L, Johnson, LV, Clegg, DO and PJ. (2009). Protective effects of human iPS-derived retinal pigment epithelium cell transplantation in the retinal dystrophic rat. PLoS One 4(12):e0008512.

47 Espejel, S, Roll, GR, McLaughlin, KJ, Lee, AY, Zhang, JY, Laird, DJ, Okita, K, Yamanaka, S and Willenbring, H. (2010). Induced pluripotent stem cell-derived hepatocytes have the functional and proliferative capabilities needed for liver regeneration in mice. J Clin Invest 120(9): 3120-6.

48 Alipio, Z, Liao, W, Roemer, EJ, Waner, M, Fink, LM, Ward, DC and Ward, Y. (2010). Reversal of hyperglycemia in diabetic mouse models using induced-pluripotent stem (iPS)-derived pancreatic beta-like cells. Proc Natl Acad Sci USA 107(30):13426-31.

49 Lyssiotis, CA, Foreman, RK, Staerk, J, Garcia, M, Mathur, D, Markoulaki, S, Hanna, J, Lairson, LL, Charette, BD, Bouchez, LC, Bollong, M, Kunick, C, Brinker, A, Cho, CY, Schultz, PG and Jaenisch, R. (2009) Reprogramming of murine fibroblasts to induced pluripotent stem cells with chemical complementation of Klf4. Proc Natl Acad Sci U S A 106(22):8912-7.

50 Schneider, BL, Seehus, CR, Capowski, EE, Aebischer, P, Zhang, SC and Svendsen, CN. (2007). Over-expression of alpha-synuclein in human neural progenitors leads to specific changes in fate and differentiation. Hum Mol Genet 16(6):651-66.

51 Agarwal, S, Loh, YH, McLoughlin, EM, Huang, J, Park, IH, Miller, JD, Huo, H, Okuka, M, Dos Reis, RM, Loewer, S, Ng, HH, Keefe, DL, Goldman, FD, Klingelhutz, AJ, Liu, L and Daley, GQ. (2010). Telomere elongation in induced pluripotent stem cells from dyskeratosis congenita patients. Nature 464(7286):292-6.

Tolar, J, Park, IH, Xia, L, Lees, CJ, Peacock, B, Webber, B, McElmurry, RT, Eide, CR, Orchard, PJ, Kyba, M, Osborn, MJ, Lund, TC, Wagner, JE, Daley, GQ and Blazar, BR. (2010). Hematopoietic differentiation of induced pluripotent stem cells from patients with mucopolysaccharidosis type I (Hurler syndrome). Blood. Oct 29. doi:10.1182/blood-2010-05-287607. [Epub ahead of print].