

# Induced pluripotent stem cells: advances to applications

Timothy J Nelson<sup>1</sup>  
Almudena Martinez-Fernandez<sup>1</sup>  
Satsuki Yamada<sup>1</sup>  
Yasuhiro Ikeda<sup>2</sup>  
Carmen Perez-Terzic<sup>1</sup>  
Andre Terzic<sup>1</sup>

<sup>1</sup>Marriott Heart Disease Research Program, Division of Cardiovascular Diseases, Mayo Clinic, Rochester, Minnesota, USA; <sup>2</sup>Department of Molecular Medicine; Mayo Clinic, Rochester, Minnesota, USA

**Abstract:** Induced pluripotent stem cell (iPS) technology has enriched the armamentarium of regenerative medicine by introducing autologous pluripotent progenitor pools bioengineered from ordinary somatic tissue. Through nuclear reprogramming, patient-specific iPS cells have been derived and validated. Optimizing iPS-based methodology will ensure robust applications across discovery science, offering opportunities for the development of personalized diagnostics and targeted therapeutics. Here, we highlight the process of nuclear reprogramming of somatic tissues that, when forced to ectopically express stemness factors, are converted into *bona fide* pluripotent stem cells. Bioengineered stem cells acquire the genuine ability to generate replacement tissues for a wide-spectrum of diseased conditions, and have so far demonstrated therapeutic benefit upon transplantation in model systems of sickle cell anemia, Parkinson's disease, hemophilia A, and ischemic heart disease. The field of regenerative medicine is therefore primed to adopt and incorporate iPS cell-based advancements as a next generation stem cell platforms.

**Keywords:** iPS, regenerative medicine, individualized medicine, stem cell therapy

## Introduction

Stem cell technology has systematically advanced from purely theoretical to applied biomedical science with significant progress towards practical applications anticipated in the upcoming decade. Naturally derived stem cells, including embryonic, umbilical cord blood and adult stem cells, contribute to organ development *in utero* and tissue renewal throughout adulthood.<sup>1-11</sup> Beyond natural sources that are limited by stem cell availability, immune intolerance, and lineage specification, the latest platform of recently developed bioengineered stem cells is rapidly enriching the armamentarium of regenerative medicine. This overview highlights state of the art bioengineered stem cell technology, referred to as induced pluripotent stem cells (iPS), and underscores the emerging advances in iPS-based therapeutic applications.

## Advances in bioengineered stem cell technology

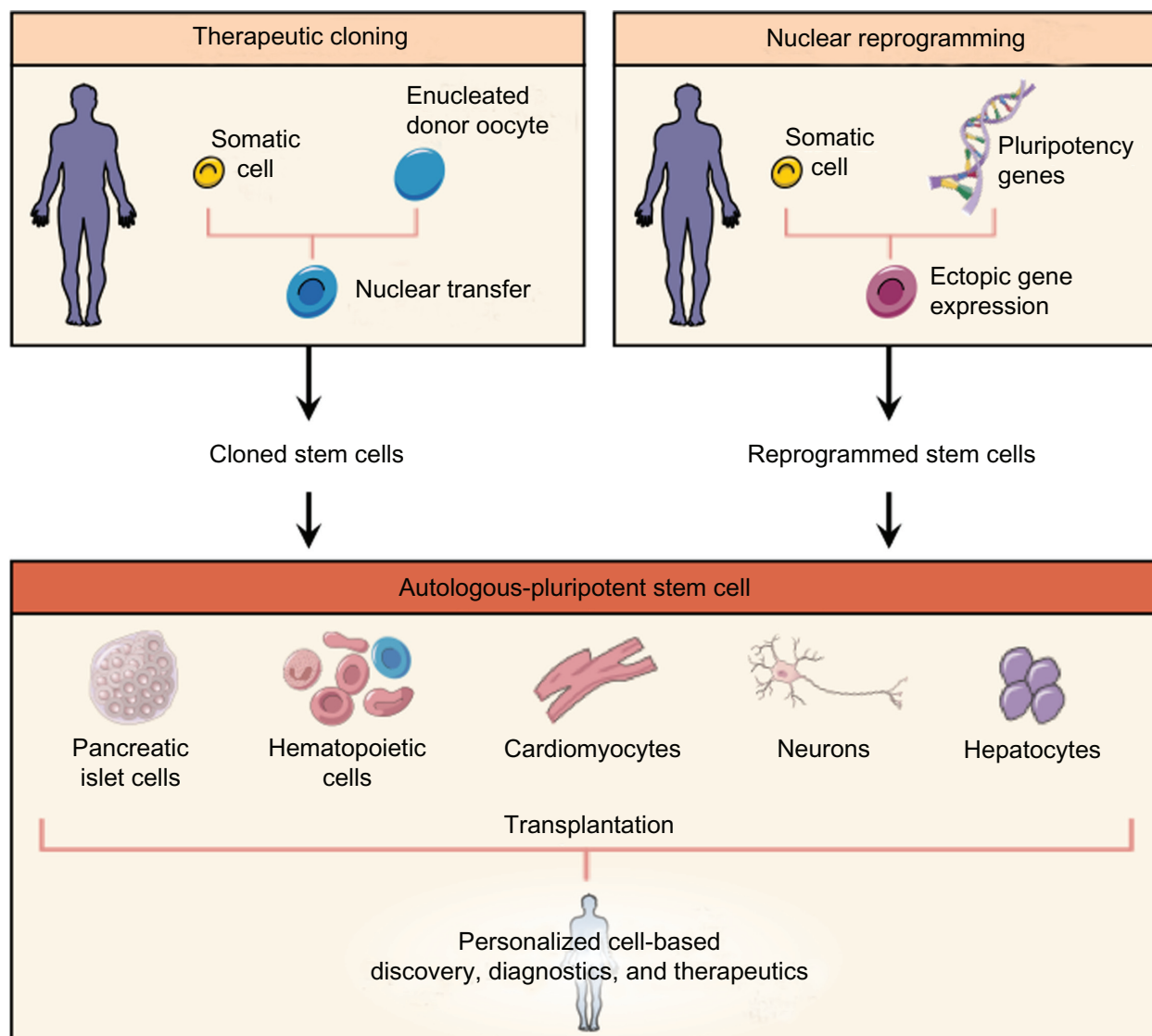
By exploiting the ability to reprogram ordinary self-derived tissue sources, the innovation of bioengineered stem cells offers an unlimited supply of progenitor cells for virtually all cell types and tissues of the adult body (Figure 1). Through control of the epigenetic environment within common cell types, nuclear reprogramming reverses cell fate, converting mature cells back to the embryonic ground state.<sup>12</sup> Advancement of nuclear reprogramming has materialized through the pioneering work of somatic cell nuclear transfer techniques that established the conserved ability of transacting environment, within the mammalian oocytes, to reprogram somatic cell nuclei to an undifferentiated state.<sup>13,14</sup>

Correspondence: Andre Terzic  
Mayo Clinic, 200 First Street SW,  
Rochester, MN 55905, USA  
Tel +1 507 284 5514  
Fax +1 507 266 9936  
Email [terzic.andre@mayo.edu](mailto:terzic.andre@mayo.edu)

Somatic cell nuclear transfer (SCNT), defined as therapeutic cloning, transplants the nuclear content of a somatic cell into an enucleated donor egg to engineer a blastocyst genetically identical to the parental source and derive pluripotent embryonic-like stem cells (Figure 1). In this way, SCNT has resulted in cloned embryonic stem cells from mammalian somatic cell biopsies.<sup>15–18</sup> However, SCNT still requires an embryonic host environment to direct the reprogramming of somatic cells. The search for factors sufficient to induce complete nuclear reprogramming has provided the more recent breakthroughs for successful embryo-independent iPS technologies.

## Science of nuclear reprogramming

Nuclear reprogramming of ordinary somatic tissue through the ectopic introduction of stemness factors is a streamlined approach to coerce an embryonic stem cell-like phenotype.<sup>19–22</sup> The transcription factors sets, Oct4, Sox2, c-Myc, and Klf4 or alternatively Oct4, Sox2, Nanog, and Lin28,<sup>23</sup> are sufficient to reprogram somatic cells through a sequential reversal into a pluripotent phenotype (Figure 1). The process of reprogramming requires controlled, stoichiometric expression of transgenes for a transient period of time.<sup>24–30</sup> Multiple sources of tissue such as ordinary fibroblasts,<sup>31</sup>



**Figure 1** Bioengineering stem cells. Therapeutic cloning with the advent of somatic cell nuclear transfer has allowed bioengineering processes to create pluripotent stem cells derived with the genetic identity of a somatic cell nucleus. The technique requires a natural embryonic host environment to induce reprogramming of the transplanted, non-stem cell nucleus. Full conversion enables the production of cloned blastocysts that are capable of providing embryonic stem cells for therapeutic purposes. Alternatively, nuclear reprogramming has been streamlined to be efficient with a minimal set of stemness related genes that can be introduced ectopically in order to recapitulate full reprogramming without the requirements of any embryonic tissue source. Collectively, autologous pluripotent stem cells are generated that have acquired the fate of stem cells and are able to give rise to all mature tissues and cell types. Bioengineered stem cells offer unprecedented opportunities for discovery, diagnostics, and therapeutics to advance personalized cell-based platforms.

keratinocytes,<sup>32</sup> hematopoietic lineages,<sup>33</sup> or adipose tissue<sup>34</sup> have been successfully reprogrammed. Ectopic stemness factors are sufficient to induce telomere elongation,<sup>35</sup> histone modifications,<sup>36</sup> secondary gene expression profiles,<sup>37</sup> and cellular metamorphosis that collectively re-establish a self-stabilizing phenotype.<sup>38</sup> Reprogramming occurs typically within weeks, following exposure to trans-acting factors that can be delivered to the nucleus either by plasmids, viruses, or bioengineered proteins. Thus, transgene expression initiates a sequence of reprogramming events that eventually transforms a small fraction of cells (<0.5%) to acquire an imposed pluripotent state characterized by a stable epigenetic environment indistinguishable from the blastocyst-derived natural stem cell *milieu*. The converted pluripotent ground state results in the maintenance of the unique developmental potential with the ability to differentiate into all germ layers (Figure 1). Thereby, iPS cells should largely eliminate the concern of stem cell shortage, immune rejection of non-autologous sources, and inadequate capacity for lineage specification.<sup>39-41</sup> Moreover, iPS-based technology will facilitate the production of patient-specific cell line panels that closely reflect the genetic diversity of a population enabling the discovery, development and validation of diagnostics together with therapeutics tailored for each individual.<sup>42</sup>

Induced pluripotent stem cell platforms bypass the need for embryo extraction to generate genuine pluripotent stem cells from self-derived, autologous sources. In the mouse, bioengineering strategies have yielded iPS cells sufficient for complete *de novo* embryogenesis as the highest evidence of pluripotent stringency.<sup>43,44</sup> In humans,<sup>23,26,28</sup> iPS cells have ensured comprehensive multi-lineage tissue differentiation by demonstrating the ability to give rise to all three germ layers in teratoma formation (Table 1). Self-derived iPS cells are recognized within the transplanted hosts as native tissue due to their autologous status and thus require protection from dysregulated growth in the absence of a defensive immune system. Optimization of bioengineered stem cells will likely produce specialized properties that improve stress tolerance, streamline differentiation capacity, and increase engraftment/survival to improve regenerative potential.

## Theoretical models of reprogramming

There are two proposed models that describe the mechanism of the reprogramming process: an “elite model” in which a small number of partially preprogrammed progenitor cells are capable of responding to transgenic stemness factors or alternatively, a “stochastic model” in which virtually any ordinary cell type can be reprogrammed with the proper

**Table 1** Pluripotent stringency criteria

Pluripotent stringency	
Mouse model systems	Human model systems
<i>In vitro</i> morphology and gene expression	<i>In vitro</i> morphology and gene expression
<i>In vivo</i> teratoma formation	<i>In vivo</i> teratoma formation
<i>In utero</i> chimeric embryogenesis	Not applicable
Tetraploid aggregation and germline transmission	Not applicable

combination of conditions depending on both the nature and environment of the target cell.<sup>22</sup> Both being plausible and supported by documented observations, the “stochastic model” has been further strengthened by evidence presented that parental sources not contaminated by progenitor cells, such as mature lymphoid cell types validated according to V(D)J recombination, are capable of dedifferentiating into stable pluripotent stem cells.<sup>45,46</sup> The data supports the model that cell fate is indeed fully reversible even from mature tissue sources upon exposure to the proper intracellular and extracellular environments.

## Original iPS technology

Gene delivery to somatic cells through retroviral or lentiviral vectors (Table 2) provided the initial strategy for ectopic expression, and establishes the technological basis of nuclear reprogramming.<sup>19,23,24,32,47-52</sup> The potential for oncogenesis due to insertional mutagenesis that is inherent to stable genomic integration has been identified as a limitation. However, it is important to recognize that distinct advantages of the retroviral-based vector systems enabled critical insight into the fundamental mechanisms of nuclear reprogramming. Retroviral and lentiviral systems have built-in sequences within the vector systems that silence the transcriptional machinery upon successful pluripotent induction. Therefore, persistent exposure to ectopic gene expression through these vectors is inhibited at the time of pluripotency re-induction, enabling an essential observation,

**Table 2** Strategies for nuclear reprogramming through ectopic gene delivery

Pluripotent induction	
Genomic modification	Genomic modification-free
Retrovirus delivery	Cre recombinase
Lentivirus delivery	Transposon-transposase systems
Stable integration-drug selection	Adenovirus delivery
Homologous gene targeting	Plasmid-episome transduction

in that successful self-maintenance of the pluripotent ground state is possible without long-term transgene expression. Thereby, next generation vectors and gene delivery systems for transient expression of stemness related genes have been designed to improve safety and ultimately efficacy of nuclear reprogramming (Table 2). The feasibility study of genomic modification free strategies was achieved by nonintegrating viral vector systems, such as adenovirus,<sup>53</sup> and confirmed by repeated exposure to extra-chromosomal plasmid-based transgenes.<sup>54</sup> Importantly, these reports established the evidence that expression of stemness related factors was required for only a limited timeframe – defined by the ability of progeny to develop autonomous self-renewal, establishing nuclear reprogramming as a bioengineered process that resets a sustainable pluripotent cell fate independent of permanent genomic modifications. The inefficiency of nonintegrated technologies has, however, hindered broader applicability and provoked the search for more efficient methodologies.

## Optimization of iPS technology

The innovative advances that propel iPS-based products towards clinical applications is dependent on genome modification-free approaches equipped for high efficiency delivery of transgenes and subsequent nuclear reprogramming.<sup>55,56</sup> One of these emerging approaches has utilized short sequences of mobile genetic elements that can integrate transgenes into host cell genomes and yet provide a genetic tag to “cut and paste” flanked genomic DNA sequences.<sup>57</sup> The prototypic piggyBac (PB) system couples enzymatic cleavage with sequence specific recognition using a transposon/transposase interaction to ensure high efficiency removal of flanked DNA without any footprint. Importantly, this technology achieves a traceless transgenic approach in which nonnative genomic sequences, that are transiently required for nuclear reprogramming, can be removed upon induction of pluripotency. Using the PB transposition system with randomly integrated stemness-related transgenes, recent studies have demonstrated that disposal of ectopic genes could be efficiently regulated upon induction of self-maintaining pluripotency according to expression of the transposase enzyme without infringement on genomic stability.<sup>56</sup> This state of the art system allows safe integration and removal of ectopic transgenes, and advances the technology by improving the efficiency of iPS production utilizing a minimally invasive strategy. Furthermore, the security of genetically unmodified interventions can be achieved with non-integrating episomal vectors.<sup>58</sup> Collectively these recent strategies (Table 2) accelerate translation towards clinical

applicability with progenitor cells that have acquired the capacity of pluripotency without compromise to the genomic stability of the parental cell source.

Additionally, advances in bioengineering technology have produced high stringency iPS cells with only proteins in the absence of any genetic or DNA material.<sup>59,60</sup> The protein only approach has successfully induced reprogramming with either whole cell extract enriched in four stemness factors used in combination with pharmacological induction of cell permeability or with stemness factors modified by a cell permeating poly-arginine tag.<sup>59</sup> Although the reprogramming efficiency is reduced compared to original genetic based methodologies, there are emerging strategies that complement the influence of stemness factors exposure within somatic cells, namely, small molecules targeting histone modifications have improved the overall reprogramming efficiencies<sup>61</sup> along with the latest discovery that the tumor suppressor gene p53 is a roadblock that spontaneously inhibits the reprogramming process.<sup>62–66</sup> Thereby, transient knock-down of p53 according to small interfering RNA (siRNA) strategies targeting the breakdown of mRNA or overexpression of MDM-2, to increase p53 protein degradation, have proven to successfully increase the overall efficiency one to two orders of magnitude with up to 20% of selected cells undergoing *bona fide* reprogramming.<sup>62–66</sup> Together, these rapid advancements in nuclear reprogramming have brought bioengineered pluripotent stem cell platforms closer to the milestones required for possible clinical applications.

## Therapeutic applications for bioengineered stem cells

Regenerative medicine aims to provide novel solutions for patients suffering from a spectrum of chronic degenerative diseases often triggered by a specific underlying genetic predisposition. Due to progressive cellular destruction and loss of functional tissues, degenerative diseases are largely responsible for chronic disabilities suffered throughout a lifespan. This creates an ever growing need for new therapies to apply a curative paradigm to repair underlying pathophysiology with corrupted cellular architecture. The emergence of regenerative medicine platforms expands the therapeutic options by establishing new approaches to address disease management needs unmet by traditional palliative strategies. In this way, stem cell-based regenerative medicine is expected to drive the evolution of medical sciences from palliation, which mitigates symptoms, to curative therapy aimed at treating the root cause of degenerative and genetic diseases.<sup>67,68</sup> Uniquely, stem cell populations demonstrate an aptitude to differentiate into



lineage specific progenitors, and form new tissue.<sup>69</sup> Cell-based strategies that promote, augment, and reestablish repair are at the core of translating the science of stem cell biology into the practice of regenerative medicine.<sup>70–76</sup>

The major impediments from the discovery to the application of stem cell technologies, have been based on two formidable challenges. First, immune intolerance between stem cells and the host environment inherent to allogeneic stem cell sources; and second the inability to secure definitive tissue specific differentiation from stem cells for *in situ* repair. With the advent of iPS technology, these limitations are addressed by the pluripotent potential of bioengineered stem cells that are derived from autologous sources (Figure 1). Thereby, the ability to reproducibly generate unlimited self-derived progenitors that avoid immune intolerance is a unique feature. Furthermore, all lineages of the adult body have become viable targets for replacement, utilizing iPS-based technology. Finally, iPS cells enable the ability to genetically repair sequence defects through homologous recombination, which then produces healthy stem cells devoid of the original disease causing genetic impairment. These defining characteristics of iPS cells thus offer a new trajectory for advancing regenerative medicine; yet they also present new challenges that have only partially been addressed with previous natural stem cell sources. The unlimited differentiation potential of iPS is similar to embryonic stem cells, and thus the risk of dysregulated growth and teratoma formation requires stringent safeguards. Ensuring proper differentiation of pluripotent stem cells has been addressed in embryonic stem cells by either growth factor guidance of lineage-specific differentiation or physical selection of established lineage-specific progenitors.<sup>77,78</sup> However, beyond the common challenges of natural pluripotent stem cells, iPS cells may also contain genetic modification as a consequence of the strategy used for reprogramming or spontaneously acquired cytogenetic abnormalities due to extensive *in vitro* manipulation. The long-term implications of nuclear reprogramming have yet to be determined as this technology is in the early stages of development.

The broad scope of therapeutic potential for iPS has been demonstrated in proof of principle studies for 4 diverse conditions to date (Table 3), namely sickle cell anemia, Parkinson's disease, hemophilia A, and ischemic heart disease.<sup>79–82</sup> Efficient *in vitro* differentiation of the tissue-specific lineage was the first required milestone for each of these conditions. The validated iPS clones were demonstrated to produce hematopoietic lineages, neural precursor cells giving rise to neuronal and glial cell types, and functional cardiac tissue prior to therapeutic application. Upon transplantation of

**Table 3** Models of disease treated with iPS-based interventions

iPS-based therapy	
Disease condition	Therapeutic outcome
Sickle cell disease	Hematopoiesis, functional physiological improvement
Parkinson's disease	Dopamine production, symptomatic improvement
Hemophilia A	Decreased clotting time, survival benefit
Ischemic heart disease	Improved cardiac performance, <i>in situ</i> tissue repair

iPS progeny into target organs ranging from fetal brain to adult post-ischemic heart tissue, progenitor cells migrated into microenvironments and differentiated *in situ* into target tissues. Collectively, these experimental models of diseases provide a proof-of-principle for therapeutic benefit of iPS-based strategies.

### Sickle cell anemia

Sickle cell anemia is an inherited disease that affects millions of individuals worldwide, often producing life threatening symptoms. The disease is based on inadequate red blood cell production in the bone marrow, which is limited to replenishing circulating blood cells every 120 days. However, sickle cell anemia causes fragile red blood cells that are unable to survive more than 20 days. Thus, the bone marrow is unable to keep up with the high-demand of continuous cell production that ultimately results in low oxygen carrying capacity, accumulation of waste products, and risk of hypoxia throughout the body. This common disease has no known cure and patients are managed for symptomatic relief. A humanized mouse model for sickle cell anemia was used to determine the repair potential of progenitor cells derived from autologous iPS cells.<sup>79</sup> Diseased mice were the source of starting tissues that were reprogrammed into iPS clones. These acquired stem cells then underwent gene correction of the sickle hemoglobin gene through gene-specific targeting. Upon transplantation with hematopoietic progenitors obtained *in vitro*, the pathognomonic features of the disease were averted. Specifically, kidney defects due to red blood cell destruction in renal tubules with reduction in renal blood flow, and the systemic deterioration as demonstrated by decreased body weight and increased respiratory rate were rescued upon iPS therapy.<sup>79</sup> Thus, the first therapeutic application of iPS technology illustrated the advantages of both regeneration of a degenerative disease as well as gene-specific correction of an inheritable defect.

## Parkinson's disease

Parkinson's disease is a debilitating neurodegenerative disease that affects 1–2 individuals per 1,000 due to the loss of dopaminergic neurons in a specialized region of the substantia nigra, that projects from the basal ganglia to the striatum, and is responsible for regulation of body movement. Muscle rigidity, resting tremor, and a generalized slowing of physical movements characterize the disease that is chronic and progressive in most patients. Standard of care is guided by the principle goal of increasing the dopamine concentration within the brain through daily titration of medications. Utilizing iPS technology in an animal model of Parkinson's disease, the goal was to determine therapeutic efficacy with bioengineered stem cells that acquired the ability to differentiate into dopamine-producing progeny.<sup>80</sup> Electrophysiological recordings and morphological characterization demonstrated successful engraftment of transplanted iPS-derived neurons with functional neuronal activity. Notably, iPS progeny demonstrated characteristics of midbrain neurons with dopamine production.<sup>80</sup> The presence of these *de novo* cells enabled the improvement of symptoms in a model of Parkinson's disease, with little risk of tumor formation from the engrafted cells. These results established the therapeutic reparative potential of nuclear reprogramming for neurodegenerative diseases.

## Hemophilia A

Hemophilia A is a common inheritable disease that affects the levels of a single protein required for normal clotting of the blood. The disease that affects 1 in 5,000 males is caused by mutations within the Factor VIII (FVIII) gene and leads to decreased protein levels and subsequently life-threatening bleeding. Gene therapy attempts have failed for multiple reasons not limited to immune rejection of the recombinant protein. Utilizing validated iPS cells derived from a mouse model, endothelial progenitor cells were produced through spontaneous differentiation according to a standardized method. The resulting progeny expressed cell-specific markers, including FVIII protein, prior to transplantation into the liver of immunodeficient hemophilia A mice. Chimeric cohorts circumvented life-threatening bleeding, in dramatic contrast to vulnerable diseased cohorts.<sup>81</sup> As predicted, increased FVIII protein levels were associated with iPS treatment and beneficial outcome. Thereby, this study further established the evidence for successful iPS based therapy in the context of a genetic disorder, demonstrating the feasibility of achieving a targeted outcome.

## Ischemic heart disease

Ischemic heart disease results when the arteries that carry oxygenated blood to the heart muscle are restricted or blocked. The heart muscle and vasculature are then unable to sufficiently rejuvenate themselves, which collectively culminates into massive tissue destruction with loss of billions of cells in the setting of acute infarction that eventually leads to decreased functional performance of the heart. The subsequent lack of blood flow then directly affects the rest of the body and precipitates overt heart failure symptoms. This disease is estimated to affect 1 in 100 people and limits functional activity in more than 14 million individuals in the United States with increasing prevalence throughout the world. Nuclear reprogramming provides an emerging strategy to produce *de novo* cardiac tissues from patient-specific somatic sources. In proof of principle studies, fibroblasts were transduced with human stemness factors OCT3/4, SOX2, KLF4, and c-MYC and converted into pluripotent stem cells that acquired the ability to contribute to normal embryonic heart development.<sup>82</sup> Upon intramyocardial delivery into adult infarcted hearts, cardiogenic iPS progeny properly engrafted without disrupting host tissues.<sup>82</sup> Notably, the parental fibroblasts that had not undergone the reprogramming process lacked the ability to engraft and when transplanted into post-ischemic hearts were associated with progressive heart dilation, with worsening heart function over the 4-week follow-up period.<sup>82</sup> Importantly, iPS-based transplantation restored post-ischemic cardiac performance with evidence of increased left ventricular thickness, and improved electrical stability following *in situ* regeneration of cardiac, smooth muscle, and endothelial tissue throughout the 4-week follow-up period. Furthermore, cardiogenic iPS clones are able to contribute to healthy adult chimeric animals with normal cardiac function.<sup>83</sup> Thereby, nonreparative fibroblasts reprogrammed by human stemness factors have demonstrated the potential for *in situ* regeneration of heart smooth muscle tissue following injury (such as acute myocardial infarction) to establish iPS-derived progeny in the treatment of heart disease.

## Clinical perspective

Regenerative medicine, built on emerging discoveries of stem cell biology,<sup>57,67,70</sup> has begun to define a new perspective of future clinical practice. Regenerative medicine and stem cell biology integrate multiple disciplines of medicine and surgery to establish a universal paradigm of curative goals based on scientific discovery and clinical translation. Building on the foundation of transplant medicine with further advances in

delivery systems,<sup>84</sup> regenerative medicine will continue to expand and implement new technologies to treat diseases at earlier stages. Individualized treatment applications for regenerative medicine will first require quantification of the inherent reparative potential of the patient to determine the scope of benefit from a targeted stem cell therapy.

Bioengineered nuclear reprogramming offers a revolutionary strategy for embryo-independent derivation of autologous pluripotent stem cells from an ordinary adult source which remain incompletely validated when compared to the gold standard embryonic stem cell counterparts.<sup>85</sup> Applying this technology, iPS progeny have to date attained similar differentiation capacity previously demonstrated only by natural embryonic stem cells, which now present new challenges to ensure reproducibility of safe and effective reprogramming throughout the bioengineering process. Furthermore, adult somatic cells from multiple tissue sources may provide variations in the overall efficiencies for iPS bioengineering as indicated recently by the degree of heterogeneity even within individual primary fibroblast cultures.<sup>86</sup> Therefore, continued mapping of the innate characteristics of the starting somatic source and the reprogrammed iPS-derived progeny should pave the way to optimize outcome. Thus, the uniqueness of iPS cells has established a new paradigm for personalized therapeutics across a diverse spectrum of chronic degenerative diseases, including incurable genetic disorders (Table 3). With “on demand” tissue repair now possible, regenerative medicine is entering a new era of research and discovery focused on applying optimization strategies to facilitate the acceleration of novel products and services to improve the value of patient care.

## Acknowledgments

Supported by National Institutes of Health (R01HL083439, T32HL007111, R01HL085208, R56AI074363), American Heart Association, American Society for Clinical Pharmacology and Therapeutics, Caja Madrid Graduate Program, Marriott Individualized Medicine Program, Marriott Heart Disease Research Program, and Mayo Clinic.

## Disclosures

The authors have no conflicts of interest that are directly relevant to the content of this review.

## References

- Nagy A, Gócza E, Diaz EM, et al. Embryonic stem cells alone are able to support fetal development in the mouse. *Development*. 1990;110:815–821.
- Nelson TJ, Martinez-Fernandez A, Terzic A. KCNJ11 knockout morula re-engineered by stem cell diploid aggregation. *Philos Trans R Soc Lond B Biol Sci*. 2009;364:269–276.
- Yamada S, Nelson TJ, Behfar A, et al. Stem cell transplant into preimplantation embryo yields myocardial infarction-resistant adult phenotype. *Stem Cells*. 2009;27:1697–1705.
- Quaini F, Urbanek K, Beltrami AP, et al. Chimerism of the transplanted heart. *N Engl J Med*. 2002;346:5–15.
- Torella D, Ellison GM, Méndez-Ferrer S, Ibanez B, Nadal-Ginard B. Resident human cardiac stem cells: role in cardiac cellular homeostasis and potential for myocardial regeneration. *Nat Clin Pract Cardiovasc Med*. 2006;3(Suppl 1):S8–S13.
- Kubo H, Jaleel N, Kumarapeli A, et al. Increased cardiac myocyte progenitors in failing human hearts. *Circulation*. 2008;118:649–657.
- Copelan EA. Hematopoietic stem-cell transplantation. *N Engl J Med*. 2006;354:1813–1826.
- Nelson TJ, Behfar A, Yamada S, Martinez-Fernandez A, Terzic A. Stem cell platforms for regenerative medicine. *Clin Trans Sci*. 2009;2:222–227.
- Klimanskaya I, Rosenthal N, Lanza R. Derive and conquer: sourcing and differentiating stem cells for therapeutic applications. *Nat Rev Drug Discov*. 2008;7:131–142.
- Morrison SJ, Spradling AC. Stem cells and niches: Mechanisms that promote stem cell maintenance throughout life. *Cell*. 2008;132:598–611.
- Surani MA, McLaren A. Stem cells: a new route to rejuvenation. *Nature*. 2006;443:284–285.
- Jaenisch R, Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. *Cell*. 2008;132:567–582.
- Beyhan Z, Iager AE, Cibelli JB. Interspecies nuclear transfer: Implications for embryonic stem cell biology. *Cell Stem Cell*. 2007;1:502–512.
- Henderson JT. Lazarus's gate: challenges and potential of epigenetic reprogramming of somatic cells. *Clin Pharmacol Ther*. 2008;83:889–893.
- Hall VJ, Stojkovic M. The status of human nuclear transfer. *Stem Cell Rev*. 2006;2:301–308.
- Yang X, Smith SL, Tian XC, et al. Nuclear reprogramming of cloned embryos and its implications for therapeutic cloning. *Nat Genet*. 2007;39:295–302.
- Byrne JA, Pedersen DA, Clepper LL, et al. Producing primate embryonic stem cells by somatic cell nuclear transfer. *Nature*. 2007;450:497–502.
- French AJ, Adams CA, Anderson LS, et al. Development of human cloned blastocysts following somatic cell nuclear transfer with adult fibroblasts. *Stem Cells*. 2008;26:485–493.
- Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126:663–676.
- Yamanaka S. Pluripotency and nuclear reprogramming. *Philos Trans R Soc Lond B Biol Sci*. 2008;363:2079–2087.
- Yamanaka S. A fresh look at iPS cells. *Cell*. 2009;137:13–17.
- Yamanaka S. Elite and stochastic models for induced pluripotent stem cell generation. *Nature*. 2009;460:49–52.
- Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007;318:1917–1920.
- Meissner A, Wernig M, Jaenisch R. Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells. *Nat Biotechnol*. 2007;25:1177–1181.
- Maherali N, Sridharan R, Xie W, et al. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell*. 2007;1:55–70.
- Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131:861–872.
- Yamanaka S. Strategies and new developments in the generation of patient-specific pluripotent stem cells. *Cell Stem Cell*. 2007;1:39–49.
- Park IH, Lerou PH, Zhao R, Huo H, Daley GQ. Generation of human-induced pluripotent stem cells. *Nat Protoc*. 2008;3:1180–1186.
- Park IH, Arora N, Huo H, et al. Disease-specific induced pluripotent stem cells. *Cell*. 2008;134:877–886.
- Nelson TJ, Martinez-Fernandez AJ, Yamada S, et al. Induced pluripotent reprogramming from promiscuous human stemness-related factors. *Clin Transl Sci*. 2009;2:118–126.

31. Takahashi K, Okita K, Nakagawa M, Yamanaka S. Induction of pluripotent stem cells from fibroblast cultures. *Nat Protoc.* 2007;2:3081–3089.
32. Aasen T, Raya A, Barrero MJ, et al. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat Biotechnol.* 2008;26:1276–1284.
33. Loh Y, Agarwal S, Park I, et al. Generation of induced pluripotent stem cells from human blood. *Blood.* 2009;113:5476–5479.
34. Sun N, Panett NJ, Gupt DM, et al. Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells. *Proc Natl Acad Sci U S A.* 2009;106:15720–15725.
35. Marion RM, Strati K, Li H, et al. Telomeres acquire embryonic stem cell characteristics in induced pluripotent stem cells. *Cell Stem Cell.* 2009;4:141–154.
36. Deng J, Shoemaker R, Xie B, et al. Targeted bisulfite sequencing reveals changes in DNA methylation associated with nuclear reprogramming. *Nat Biotechnol.* 2009;27:353–360.
37. Mikkelsen TS, Hanna J, Zhang X, et al. Dissecting direct reprogramming through integrative genomic analysis. *Nature.* 2008;454:49–55.
38. Silva J, Nichols J, Theunissen TW, et al. Nanog is the gateway to the pluripotent ground state. *Cell.* 2009;138:722–737.
39. Nishikawa S, Goldstein RA, Nierras CR. The promise of human induced pluripotent stem cells for research and therapy. *Nat Rev Mol Cell Biol.* 2008;9:725–729.
40. Nakagawa M, Koyanagi M, Tanabe K, et al. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol.* 2008;26:101–106.
41. Park IH, Zhao R, West JA, et al. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature.* 2008;451:141–146.
42. Waldman SA, Terzic A. Therapeutic targeting: a crucible for individualized medicine. *Clin Pharmacol Ther.* 2008;83:651–654.
43. Zhao XY, Li W, Lv Z, et al. iPS cells produce viable mice through tetraploid complementation. *Nature.* 2009;461:86–90.
44. Boland MJ, Hazen JL, Nazor KL, et al. Adult mice generated from induced pluripotent stem cells. *Nature.* 2009;461:91–94.
45. Hanna J, Markoulaki S, Schorderet P, et al. Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency. *Cell.* 2008;133:250–264.
46. Utikal J, Polo JM, Stadtfeld M, et al. Immortalization eliminates a roadblock during cellular reprogramming into iPS cells. *Nature.* 2009;460:1145–1148.
47. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature.* 2007;448:313–317.
48. Aoi T, Yae K, Nakagawa M, et al. Generation of pluripotent stem cells from adult mouse liver and stomach cells. *Science.* 2008;321:699–702.
49. Huangfu D, Osafune K, Maehr R, et al. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nat Biotechnol.* 2008;26:1269–1275.
50. Eminli S, Utikal J, Arnold K, et al. Reprogramming of neural progenitor cells into induced pluripotent stem cells in the absence of exogenous Sox2 expression. *Stem Cells.* 2008;26:2467–2474.
51. Kim JB, Zaehres H, Wu G, et al. Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. *Nature.* 2008;454:646–650.
52. Feng B, Jiang J, Kraus P, et al. Reprogramming of fibroblasts into induced pluripotent stem cells with orphan nuclear receptor Esrrb. *Nat Cell Biol.* 2009;11:197–203.
53. Stadtfeld M, Nagaya M, Utikal J, et al. Induced pluripotent stem cells generated without viral integration. *Science.* 2008;322:945–949.
54. Okita K, Nakagawa M, Hyenjong H, et al. Generation of mouse induced pluripotent stem cells without viral vectors. *Science.* 2008;322:949–953.
55. Kaji K, Norrby K, Paca A, et al. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. *Nature.* 2009;458:771–775.
56. Woltjen K, Michael IP, Mohseni P, et al. piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature.* 2009;458:766–770.
57. Nelson TJ, Terzic A. Induced pluripotent stem cells: reprogrammed without a trace. *Regen Med.* 2009;4:333–355.
58. Yu J, Hu K, Smuga-Otto K, et al. Human induced pluripotent stem cells free of vector and transgene sequences. *Science.* 2009;324:797–801.
59. Zhou H, Wu S, Joo JY, et al. Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell.* 2009;4:381–384.
60. Kim D, Kim CH, Moon JI, et al. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. *Cell Stem Cell.* 2009;6:472–476.
61. Shi Y, Despons C, Do JT, Hahm HS, Schöler HR, Ding S. Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. *Cell Stem Cell.* 2008;3:568–574.
62. Banito A, Rashid ST, Acosta JC, et al. Senescence impairs successful reprogramming to pluripotent stem cells. *Genes Dev.* 2009;23:2134–2139.
63. Hong H, Takahashi K, Ichisaka T, et al. Suppression of induced pluripotent stem cell generation by the p53-p21 pathway. *Nature.* 2009;460:1132–1135.
64. Marión RM, Strati K, Li H, et al. A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. *Nature.* 2009;460:1149–1153.
65. Li H, Collado M, Villasante A, et al. The Ink4/Arf locus is a barrier for iPS cell reprogramming. *Nature.* 2009;460:1136–1139.
66. Kawamura T, Suzuki J, Wang YV, et al. Linking the p53 tumor suppressor pathway to somatic cell reprogramming. *Nature.* 2009;460:1140–1144.
67. Nelson TJ, Behfar A, Terzic A. Stem cells: biologics for regeneration. *Clin Pharmacol Ther.* 2008;84:620–623.
68. Waldman SA, Terzic MR, Terzic A. Molecular medicine hones therapeutic arts to science. *Clin Pharmacol Ther.* 2007;82:343–347.
69. Segers V, Lee RT. Stem-cell therapy for cardiac disease. *Nature.* 2008;451:937–942.
70. Nelson TJ, Behfar A, Terzic A. Strategies for therapeutic repair: The “R<sup>3</sup>” regenerative medicine paradigm. *Clin Transl Sci.* 2008;1:168–171.
71. Daley GQ, Scadden DT. Prospects for stem cell-based therapy. *Cell.* 2008;132: 544–548.
72. Rosenthal N. Prometheus’s vulture and the stem-cell promise. *N Engl J Med.* 2003;349:267–274.
73. Leri A, Kajstura J, Anversa P, Frishman WH. Myocardial regeneration and stem cell repair. *Curr Probl Cardiol.* 2008;33:91–153.
74. Dimmeler S, Zeiher AM, Schneider MD. Unchain my heart: the scientific foundations of cardiac repair. *J Clin Invest.* 2005;115:572–583.
75. Chien KR, Domian IJ, Parker KK. Cardiogenesis and the complex biology of regenerative cardiovascular medicine. *Science.* 2008;322:1494–1497.
76. Menasche P. Cell-based therapy for heart disease: a clinically oriented perspective. *Mol Ther.* 2009;17:758–766.
77. Behfar A, Perez-Terzic C, Faustino RS, et al. Cardiopoietic programming of embryonic stem cells for tumor-free heart repair. *J Exp Med.* 2007;204:405–420.
78. Nelson TJ, Faustino RS, Chiriac A, Crespo-Diaz R, Behfar A, Terzic A. CXCR4<sup>+</sup>/FLK-1<sup>+</sup> biomarkers select a cardiopoietic lineage from embryonic stem cells. *Stem Cells.* 2008;26:1464–1473.
79. Hanna J, Wernig M, Markoulaki S, et al. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science.* 2007;318:1920–1923.
80. Wernig M, Zhao JP, Pruszak J, et al. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson’s disease. *Proc Natl Acad Sci U S A.* 2008;105:5856–5861.
81. Xu D, Alipio Z, Fink LM, et al. Phenotypic correction of murine hemophilia A using an iPS cell-based therapy. *Proc Natl Acad Sci U S A.* 2009;106:808–813.
82. Nelson TJ, Martinez-Fernandez A, Yamada S, et al. Repair of acute myocardial infarction with human stemness factors induced pluripotent stem cells. *Circulation.* 2009;120:408–416.



83. Martinez-Fernandez A, Nelson TJ, Yamada S, et al. iPS Programmed without c-MYC yield proficient cardiogenesis for functional heart chimerism. *Circ Res*. 2009;105:648–656.
84. Bartunek J, Sherman W, Vanderheyden M, et al. Delivery of biologics in cardiovascular regenerative medicine. *Clin Pharmacol Ther*. 2009;85:548–552.
85. Belmonte JC, Ellis J, Hochedlinger K, Yamanaka S. Induced pluripotent stem cells and reprogramming: seeing the science through the hype. *Nat Rev Genet*. 2009;10:878–883.
86. Byrne JA, Nguyen HN, Reijo Pera RA. Enhanced generation of induced pluripotent stem cells from a subpopulation of human fibroblasts. *PLoS ONE*. 2009;4(9):e7118.

### Stem Cells and Cloning: Advances and Applications

Dovepress

#### Publish your work in this journal

Stem Cells and Cloning: Advances and Applications is an international, peer-reviewed, open access journal. Areas of interest in stem cell research include: Embryonic cell stems; Adult stem cells; Blastocysts; Cordblood stem cells; Stem cell transformation and culture; Therapeutic cloning; Umbilical cord blood and bone marrow cells; Laboratory,

animal and human therapeutic studies; Philosophical and ethical issues related to stem cell research. This journal is indexed on CAS. The manuscript management system is completely online and includes a quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/stem-cells-and-cloning-advances-and-applications-journal>