Autologous Induced Pluripotent Stem Cell–Based Cell Therapies: Promise, Progress, and Challenges

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The promise of human induced pluripotent stem cells (iPSCs) lies in their ability to serve as a starting material for autologous, or patient-specific, stem cell–based therapies. Since the first publications describing the generation of iPSCs from human tissue in 2007, a Phase I/IIa clinical trial testing an autologous iPSC-derived cell therapy has been initiated in the U.S., and several other autologous iPSC-based therapies have advanced through various stages of development. Three single-patient in-human transplants of autologous iPSC-derived cells have taken place worldwide. None of the patients suffered serious adverse events, despite not undergoing immunosuppression. These promising outcomes support the proposed advantage of an autologous approach: a cell therapy product that can engraft without the risk of immune rejection, eliminating the need for immunosuppression and the associated side effects. Despite this advantage, there are currently more allogeneic than autologous iPSC-based cell therapy products in development due to the cost and complexity of scaling out manufacturing for each patient. In this review, we highlight recent progress toward clinical translation of autologous iPSC-based cell therapies. We also highlight technological advancements that would reduce the cost and complexity of autologous iPSC-based cell therapy production, enabling autologous iPSC-based therapies to become a more commonplace treatment modality for patients.

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Keywords: autologous • cell therapies • cell therapy • induced pluripotent stem cells (iPSCs) • regenerative medicine

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I. INTRODUCTION

Pluripotent stem cells (PSCs) can multiply indefinitely and differentiate into all cell types in the body, making them the ideal starting material for developing a range of cell therapies (Yamanaka, 2020). The ability to generate induced PSCs (iPSCs) from easily harvested human tissue was first reported in 2007 (Takahashi et al., 2007; Yu et al., 2007). Since then, iPSCs have been generated from a range of readily sourced tissues, including blood, urine, and hair follicles (Drozd et al., 2015; Ohmine et al., 2011; Petit et al., 2012; Raab, Klingenstein, Liebau, & Linta, 2014). iPSCs are an attractive alternative to human embryonic stem cells (ESCs), which are sourced from human embryos and thus controversial (Ilic & Ogilvie, 2017). However, the real promise of iPSCs lies in their ability to enable the development of autologous, or patient-specific,
stem cell–based therapies. Autologous cell therapies may facilitate long-term engraftment without the need for immunosuppression, providing safer treatments for patients (Hatzimichael & Tuthill, 2010; Majhail, Mau, Denzen, & Arneson, 2013; Osborn, Hallett, Schumacher, & Isacson, 2020). Since the discovery of iPSCs, three in-human transplants of autologous iPSC-derived cells have occurred, with the first transplant taking place in 2014 (Mandai et al., 2017; Schweitzer et al., 2020; Takagi et al., 2019) (Clinical Trial Identification number: jRCTa050190117). None of the patients in these trials has suffered serious adverse events, despite not undergoing immunosuppression, an encouraging outcome for the field of personalized regenerative medicine.

In contrast to autologous cell therapies, which are patient specific, allogeneic iPSC-based cell therapies use donor-derived tissues as a starting material. A greater number of allogeneic iPSC-derived cell therapies are being developed than autologous therapies (Martín-Ibáñez & Sareen, 2020). The advantage of allogeneic treatments is that many doses can be manufactured simultaneously from a single batch of iPSCs and banked. The production process can therefore be “scaled up” and achieve economies of scale that reduce the cost of each dose (Simaria et al., 2014). This also allows for “off-the-shelf” distribution, simplifying logistics and minimizing the time to patient (Sullivan et al., 2018; Yamanaka, 2020). Minimizing the time to patient is critical for certain acute indications where the patient requires immediate care. The disadvantage of an allogeneic approach is the potential for immune rejection. Patients must undergo immunosuppression, which is associated with a higher risk of infection and cardiovascular defects (Miller, 2002). One strategy to reduce immune rejection is to create human leukocyte antigen (HLA)-matched haplobanks, in which donor iPSC lines are generated from individuals with the most common HLA haplotypes to match a range of patients (Hanatani & Takasu, 2020; Sullivan et al., 2018). A 100-line HLA-matched bank would cover 78% of European-Americans. However, it would only cover 52% of Hispanics and <50% of African-Americans (Gourraud, Gilson, Girard, & Peschanski, 2011), making it harder for patients from racial and ethnic minority groups to receive treatment. Although HLA matching reduces the risk of an immune reaction, it does not eliminate the need for immunosuppression. Even “immune-privileged” sites, such as the brain or eye, would require short-term immunosuppression for transplantations of HLA-matched cells (Carson, Doose, Melchior, Schmid, & Ploix, 2006; Osborn et al., 2020; Taylor, 2016).

Another approach to circumventing immune rejection in iPSC-derived transplants is creating universal, or immunocloaked, cells. Generation of these cell lines requires multiple rounds of gene editing; critical genes are deleted to avoid T-cell invasion, whereas others are left intact or knocked in to avoid NK-cell invasion, whereas others are left intact or knocked in to avoid NK-cell invasion (Gornalusse et al., 2017; Meissner, Strominger, & Cowan, 2015; Xu et al., 2019; Yamanaka, 2020). These approaches could enable coverage of the entire human population with significantly fewer iPSC lines than an HLA-matched haplobank, with the off-the-shelf convenience of a standard allogeneic approach. However, this approach is earlier in its development and therefore farther away from clinical use. The multiplexed gene editing required for this approach comes with safety risks, and any cell products will need to be carefully assessed for off-target effects (Zhang, Tee, Wang, Huang, & Yang, 2015). At this point, the safety of autologous iPSC-derived cell transplants has been validated to a greater extent, both in animal models and in humans (Schweitzer et al., 2020; Sharma et al., 2019; Takagi et al., 2019).

Autologous therapies are considered safer than allogeneic from an immune rejection standpoint (Eliopoulos, Stagg, Lejeune, Pommey, & Galipeau, 2005; Kitala et al., 2016; Sohn et al., 2015; Song et al., 2020). Still, the immunogenicity of autologous iPSCs is not without controversy. At least one study described the rejection of autologous iPSCs transplanted into mice (Zhao, Zhang, Rong, & Xu, 2011). Deuse et al. (2009) proposed that this immunogenicity could occur due to de novo mutations in mitochondrial DNA (mtDNA), potentially warranting screening of mtDNA in the development of autologous cell therapy products. However, most studies have not found rejection of autologous iPSCs or iPSC-derived cells in animal models (Araki et al., 2013; Guha, Morgan, Mostoslavsky, Rodrigues, & Boyd, 2013; Osborn et al., 2020; Schweitzer et al., 2020). Human trials involving transplantation of autologous iPSC-derived cells, although limited in number, have resulted in no serious adverse effects, with the most extended follow-up being 4 years post-transplant (Mandai et al., 2017; Schweitzer et al., 2020; Takagi et al., 2019).
Figure 1  Progression of autologous iPSC-derived cell therapies toward the clinic. Since the first publications demonstrating generation of iPSCs from human tissue (Takahashi et al., 2007; Yu et al., 2007), three in-human transplants of autologous iPSC-derived cells have taken place (Mandai et al., 2017; Schweitzer et al., 2020), and one Phase I/IIa clinical trial has been initiated in the U.S. (Sharma et al., 2019).

An autologous iPSC-based approach may be the safest path toward developing PSC-based cell therapies for specific indications and patient populations (Miller, 2002; Tepperman et al., 2010). These include patients with chronic progressive diseases, patients who are not covered by major HLA-matched haplobanks, and patients who would do poorly under immunosuppression (Gourraud et al., 2011; Miller, 2002; Papapetrou, 2016; Tepperman et al., 2010). With advancements in the enabling technology landscape and relevant regulatory frameworks, autologous iPSC-derived cell therapies will have the potential to benefit a larger fraction of regenerative medicine patients (Jha, Farnoodian, & Bharti, 2020; Martín-Ibáñez & Sareen, 2020). This review covers the breadth of autologous iPSC-derived cell therapies in development (Section II) and describes logistical complexities and other factors to be considered when moving forward (Section III). Although there are still obstacles to overcome, bringing autologous iPSC-derived cell therapies to patients presents a promising path toward treating a wide range of diseases.

II. PROGRESS IN AUTOLOGOUS iPSC-BASED CELL THERAPY DEVELOPMENT

Since the first in-human transplant of autologous iPSC-derived cells in 2014, there has been a growth in recent efforts to translate autologous iPSC-based therapies to the clinic (Fig. 1). This section and Table 1 provide a sampling of the autologous iPSC-based cell therapies currently in development and is not an exhaustive list (Table 1).

A. Indications for In-Human Autologous iPSC-Derived Cell Transplants

i. iPSC-derived retinal pigment epithelial cells for age-related macular degeneration

iPSC-derived retinal pigment epithelial cells (RPEs) were the first autologous iPSC-derived cell type to be transplanted into a human, accomplished in 2014 by Masayo Takahashi’s team at the RIKEN Center for Developmental Biology in Japan (Mandai et al., 2017; Schweitzer et al., 2020). This transplant was performed to treat age-related macular degeneration (AMD), a chronic disease characterized by the degeneration of RPE cells and the leading cause of vision loss among individuals over age 60 (Sharma, Bose, Maminishkis, & Bharti, 2020). There are three main reasons why RPEs were the first iPSC-derived transplant: (1) the eye is relatively easy to image and access surgically; (2) RPEs were the first ocular cell type to be differentiated from iPSCs, in 2009, just 2 years...
| Organ     | Indication                                   | Cell product                                      | Reprogramming starting material and chosen technique | Stage                  | Investigator, institution                                      | Country |
|-----------|----------------------------------------------|---------------------------------------------------|-----------------------------------------------------|------------------------|---------------------------------------------------------------|---------|
| Eye       | Dry age-related macular degeneration         | Retinal pigment epithelial cell sheet on PLGA scaffold | CD34+ cells, episomal plasmids                       | Phase I/IIa            | Kapil Bharti, NIH/NEI                                           | USA     |
| Eye       | Wet age-related macular degeneration         | Retinal pigment epithelial cell sheet             | Fibroblasts, episomal plasmids                       | 1 patient dosed (2014) | Masayo Takahashi, RIKEN                                        | Japan   |
| Brain     | Parkinson’s disease                          | Dopaminergic progenitor cells                     | Fibroblasts, episomal plasmids                       | 1 patient dosed (2018) | Kwang-Soo Kim, McLean Hospital/Harvard Medical School          | USA     |
| Brain     | Parkinson’s disease                          | Dopaminergic neurons                              | Fibroblasts, chosen technique not specified in publication | Preparing for IND   | Jeanne Loring, Aspen Neuroscience                              | USA     |
| Brain     | Parkinson’s disease                          | Dopaminergic neurons                              | Chosen technique for in-human studies not specified in publication | Non-human primate studies published, preparing for IND | Ole Isacson and Penny Hallett, Neuroregeneration Research Institute at McLean Hospital/Harvard Medical School | USA     |
| Blood     | Thrombocytopenia                             | Platelets                                         | Peripheral blood mononuclear cells, episomal plasmids | 1 patient received multiple doses (2019-2020) | Koji Eto, Kyoto University                                    | Japan   |
| Skin      | Recessive dystrophic epidermolysis bullosa   | Gene-edited keratinocytes and fibroblasts         | Fibroblasts, RNA                                    | Mouse studies in progress, preparing for IND | Dennis Roop, Gates Center for Regenerative Medicine; Ganna Bilousova and Igor Kogut, University of Colorado School of Medicine | USA     |
Table 1 sample of autologous iPSC-derived cell therapies in development, continued

| Organ | Indication                                | Cell product                                | Reprogramming starting material and chosen technique | Stage                        | Investigator, institution                                           | Country       |
|-------|-------------------------------------------|---------------------------------------------|-----------------------------------------------------|------------------------------|--------------------------------------------------------------------|---------------|
| Skin  | Recessive dystrophic epidermolysis bullosa | Gene-edited keratinocytes and fibroblasts   | Fibroblasts, episomal plasmids                      | Mouse studies published, preparing for IND | Angela Christiano, Columbia University                           | USA           |
| Skin  | Recessive dystrophic epidermolysis bullosa | Gene-edited keratinocytes and fibroblasts   | Fibroblasts and keratinocytes, excisable lentiviral approach for reprogramming, excisable AAV approach for gene editing | Mouse studies published, preparing for IND | Anthony Oro, Stanford University                                   | USA           |
| Skin  | Hair loss                                 | Neural crest–derived dermal papillae        | Peripheral blood mononuclear cells, Sendai virus    | Mouse studies published, preparing for IND | Alexey Terskikh, Sanford Burnham Prebys Medical Discovery Institute/Stemson Therapeutics | USA           |
| Muscle| Muscular dystrophies                      | Myogenic stem cells                         | Peripheral blood mononuclear cells                 | Received orphan drug designation from FDA, preparing for IND | Douglas Falk, Vita Therapeutics                                   | USA           |
| Eye   | Retinal degenerative disorders (e.g., retinitis pigmentosa) | Photoreceptor precursors                    | Fibroblasts, Sendai virus                          | Mouse studies published     | Budd Tucker, University of Iowa                                    | USA           |

Table 1 includes a sample of the autologous iPSC-based cell therapies in development. The reprogramming starting materials and chosen reprogramming techniques shown here are based on available publications and may change as programs advance toward the clinic.
after the landmark publication demonstrating iPSC generation (Buchholz et al., 2009); and (3) previous surgical experiments had shown proof of concept supporting an autologous cell therapy for AMD. In these experiments, RPEs from the healthy region of an eye of an AMD patient were removed and transplanted into the degenerated region of the same eye (Van Meurs et al., 2004; Van Zeeburg, Maaijwee, Missotten, Heimann, & Van Meurs, 2012). These were complex surgeries requiring significant skill and precision that ultimately resulted in the partial recovery of vision.

In their groundbreaking study, Takahashi’s team transplanted an iPSC-RPE sheet measuring 1.3 × 3 mm under the fovea of the right eye of a 77-year-old female patient with neovascular ("wet") AMD (Mandai et al., 2017). The iPSCs were reprogrammed from fibroblasts using non-integrating episomal vectors, differentiated to RPEs with a 6-week process using a combination of directed and spontaneous differentiation methods, and then purified and expanded over a period of 6 weeks (Kamao et al., 2014; Mandai et al., 2017; Osaka, Ikeda, Sasai, & Takahashi, 2009; Osaka, et al., 2009). Over a period of 8 weeks, selected RPE colonies were seeded onto a collagen-coated transwell to form the RPE sheet, which was lifted from the transwell by treatment with collagen IV. From skin tissue collection to shipment of RPE cell sheets, the process took ∼10 months (Mandai et al., 2017). A 4-year follow-up demonstrated that the iPSC-derived RPE sheet had survived and displayed normal morphology (Takagi et al., 2019). The patient experienced no serious adverse events, demonstrating a promising safety profile for iPSC-RPE transplants. Although there was no improvement in vision, the patient’s vision remained stable since the surgery, despite continually decreasing in the years before surgery.

Efforts to bring an autologous iPSC-RPE cell therapy to patients have been continued by Kapil Bharti’s team at the National Eye Institute of the NIH. Bharti’s team developed clinical-grade iPSC-derived RPE patches on a biodegradable poly-(lactic-co-glycolic) acid (PLGA) scaffold. The PLGA scaffold—unique to Bharti’s approach—serves several purposes, including enabling the RPEs to form a confluent, correctly polarized monolayer and providing structural integrity for easier transplantation. The patches were successfully transplanted into the Royal College of Surgeons (RCS) rat and laser-induced RPE injury pig models (Sharma et al., 2019). The iPSCs were generated from CD34+ cells isolated from patients’ peripheral blood using a clinical-grade episomal reprogramming protocol (Mack, Kroboth, Rajesh, & Wang, 2011). They were then differentiated to mature RPEs via an 11-week triphasic monolayer directed differentiation protocol. The RPE progenitors were enriched and seeded onto a PLGA scaffold to mature for the final 35 days. Imaging 10 weeks post-transplantation confirmed that the PLGA scaffold had efficiently degraded. The iPSC-RPE patch integrated into both the rat and the pig models, even demonstrating functionality by phagocytosing photoreceptor outer-rod segments and preventing degeneration of the overlying photoreceptors in the pig models (Sharma et al., 2019). Having established the feasibility and efficacy of the iPSC-RPE on a PLGA scaffold in a large animal model, in 2020, the NIH team initiated Phase I/IIa clinical trials to test the safety of autologous transplantation of the iPSC-RPE in 20 dry-AMD patients (National Clinical Trial number: NCT04339764). This clinical trial marks the first and, at the time of this publication, the only Phase I/IIa clinical trial in the U.S. to test autologous iPSC-derived cells in patients and simultaneously opens the door to other scaffold-supported transplant approaches using iPSC-derived cells.

### ii. iPSC-derived dopaminergic neurons for Parkinson’s disease

The second autologous iPSC-derived cell therapy transplanted into a human consisted of iPSC-derived midbrain dopaminergic (mDA) progenitor cells (mDAPs), transplanted into the putamen of a 69-year-old man with a 10-year history of Parkinson’s disease (PD) (Schweitzer et al., 2020). PD is a progressive neurodegenerative disease affecting an estimated 7 to 10 million patients worldwide (Dorsey, Sherer, Okun, & Bloem, 2018). Degeneration of dopaminergic neurons leads to motor symptoms such as tremors and bradykinesia. By the time symptoms have become prominent enough to lead to a diagnosis, ~60% of the mDA neurons have degenerated (Engelender & Isacson, 2017; Osborn et al., 2020). Today’s cell therapy development for PD builds on a 30+ year history of experimentation, in which mDA neurons from fetuses were transplanted into PD patients (Freed et al., 1992; Hagell et al., 2000; Hallett et al., 2014; Kefalopoulou et al., 2014; Kordower et al., 1998; Li et al., 2016; Lindvall et al., 1990; Mendez et al., 2005; Piccini et al., 1999; Redmond, Vinuela, Kordower, & Isacson, 2008). Long-term follow-ups and
data collection are essential for assessing the safety and efficacy of such transplants, as the cells may take months or years to integrate into the patient’s neural pathways (Barker, Barrett, Mason, & Björklund, 2013; Politis & Piccini, 2012; Politis et al., 2010). Hence, the value of studies such as those conducted by Politis et al. assessing safety and efficacy in patients 15+ years post-transplantation (Politis & Piccini, 2012; Politis et al., 2010). Although the patients followed still suffered from some of the non-motor symptoms of PD related to regulation of sleep and emotion, the relief from motor symptoms was long lasting. Positive improvements were measured by PET neuroimaging of dopamine uptake and reduced scores on the Unified Parkinson’s Disease Rating Scale (UPDRS). Moreover, postmortem imaging of brain tissues of patients who had received fetal tissue transplants 4 to 14 years earlier was consistent with these clinical results, showing that the transplanted tissue was healthy and non-atrophied and expressed important dopamine transporters (DATs) (Hallett et al., 2014). Although these findings are promising, fetal transplants are limited by ethical and practical concerns and require patients to undergo immunosuppression. An autologous iPSC-based approach to mDA cell therapy development would address these challenges.

In 2018, autologous iPSC-derived mDAPs were transplanted first into the left hemisphere of a 69-year-old PD patient and then into the right hemisphere 6 months later (Schweitzer et al., 2020). This study was led by Kwang-Soo Kim at McLean Hospital (Harvard Medical School) in collaboration with Bob Carter’s and Jeff Schweitzer’s teams at Massachusetts General Hospital. The iPSCs were generated from fibroblasts harvested from a skin biopsy and differentiated to mDAPs using a good manufacturing practice (GMP)-grade 28-day protocol (Song et al., 2020). On day 9, the cells were treated with quercetin to remove any undifferentiated iPSCs. The patient-specific iPSC-mDAPs and allogeneic human ESC-derived mDAPs were transplanted into the allogeneic humanized mice and patient-humanized mice, both groups without immunosuppression. PET neuroimaging showed an increase in DAT binding sites (Hallett et al., 2015). Postmortem analysis of the brain at 2 years post-surgery showed survival of the engrafted dopaminergic neurons and expression of important markers. Isacson and Hallett’s team also demonstrated functional improvement post-transplantation in non-human primate models with 8 years of chronic PD as part of preclinical studies toward developing an autologous iPSC-based cell therapy for PD (Osborn et al., 2020). They were recently awarded a $6M grant from the NIH to support their in-human studies, which will use autologous iPSCs as a starting material for the cell therapy product.

Jeanne Loring of The Scripps Research Institute co-founded Aspen Neuroscience in 2018, which raised a $70M Series A to develop an autologous iPSC-derived neuron replacement therapy for PD (Loring, 2018). Aspen Neuroscience generates iPSCs from dermal fibroblasts using a proprietary artificial
cells, and differentiated into platelets 5 months after platelets 3 months later, (Madrid et al., 2016).

B. Indications in Earlier (Preclinical) Stages of Development

i. iPSC-derived keratinocytes and fibroblasts for epidermolysis bullosa

Inherited epidermolysis bullosa (EB) is a group of rare, incurable, life-threatening skin diseases characterized by severe blistering and scarring (Fine, 2010). The chronic skin wounds are similar to thermal or chemical burns. The severity of symptoms varies across and within subtypes, with some resulting in death in early infancy. Much of the available treatment focuses on pain management, but several in vitro and in vivo studies using mouse models support a cell-based therapeutic approach (Bilousova & Roop, 2014; Jacków et al., 2019; Sebastiano et al., 2014; Shinkuma, 2015; Tolar et al., 2014; Torkelson et al., 2019; Umegaki-Arao et al., 2014; Vanden Oever, Twaroski, Osborn, Wagner, & Tolar, 2018; Wenzel et al., 2014). The skin is an ideal organ for developing cell-based therapies: similar to the eye, it is easy to access and easy to monitor. Each subtype of EB is associated with various genetic mutations, which would need to be corrected in an autologous cell therapy (Fine, 2010). Several research groups are developing autologous gene-edited iPSC-derived cell therapies for EB (Jacków et al., 2019; Roop, Bilousova, & Kogut, 2019; Sebastiano et al., 2014).

Angela Christiano’s team at Columbia University used episomal vectors to derive iPSCs from the fibroblasts of two patients with recessive dystrophic EB (RDEB). RDEB is a severe subtype characterized by mutations affecting type VII collagen production (Jacków et al., 2019). Jacków and colleagues tested both plasmid and ribonucleoprotein-based CRISPR-Cas9 approaches for gene editing and differentiated the gene-corrected iPSCs to both keratinocytes and fibroblasts using 60-day and ~30-day protocols, respectively. The iPSC-derived keratinocytes and fibroblasts were used to generate 3D human skin equivalents (HSEs). When grafted onto immunodeficient mice, the HSEs showed functional restoration of collagen expression and normal
dermal morphology. No tumors were seen 9 months after grafting, a promising result toward developing a safe cell-based therapy.

Anthony Oro’s team at Stanford University was awarded a $5M grant from the California Institute for Regenerative Medicine (CIRM) to translate an autologous gene-edited iPSC-based cell therapy for RDEB to the clinic (Oro, n.d.). In their 2014 publication, Oro’s team used an excisable lentiviral reprogramming method to generate GMP-grade iPSCs from patient-specific skin biopsies (Sebastiano et al., 2014). The mutated gene was corrected using an excisable adeno-associated viral (AAV)-based approach. An ~110-day protocol was used to differentiate the gene-corrected iPSCs into keratinocytes, which successfully formed sheets of skin in vitro. The iPSC-derived keratinocytes were also able to rebuild mature skin when grafted onto immunocompromised mice, and the grafts survived for 3 weeks post-transplantation. Funding from the CIRM grant will be used to perform additional in vivo studies in preparation for a pre-IND meeting with the FDA.

Ganna Bilousova’s and Igor Kogut’s teams at the University of Colorado School of Medicine, in collaboration with Dennis Roop, director of the Gates Center for Regenerative Medicine, are also translating an autologous iPSC-based therapy into the clinic for RDEB. Kogut and colleagues developed a clinically relevant RNA-based technology capable of reprogramming ~90% of individually plated human primary fibroblasts, unprecedentedly high considering that reprogramming efficiencies are typically fractions of a percent or in the single digits (Kogut et al., 2018). Bilousova and colleagues were the first to publish a demonstration of the differentiation of mouse iPSCs into keratinocytes, a critical step in advancing iPSC-derived cell therapies for EB into the clinic. The University of Colorado team is currently developing composite skin grafts from patient-specific iPSC-derived keratinocytes and fibroblasts for transplantation (Roop et al., 2019). Reprogramming and gene correction are performed simultaneously using the proprietary RNA technology. The resulting gene-edited iPSCs are differentiated into keratinocytes and fibroblasts (Kogut et al., 2018; Roop et al., 2019). The University of Colorado team is currently exploring the feasibility of a spray-based applicator from AVITA Medical for delivering iPSC-derived keratinocytes and fibroblasts in mouse models of RDEB (Roop et al., 2019; Sood et al., 2015).

### ii. iPSC/neural crest–derived dermal papillae for alopecia

Hair loss, or alopecia, is a significant problem that affects nearly 20% of the population. Studies measuring the impact of hair loss on quality of life reveal long-lasting negative effects, including low self-confidence, heightened self-consciousness, and an increased prevalence of depression (Marks et al., 2019; Schmitt, Ribeiro, Souza, Siqueira, & Beber, 2012; Williamson, Gonzalez, & Finlay, 2001). Follicular unit extraction (FUE), in which individual follicular units are harvested from one region of a patient’s scalp and transplanted into a different section of the same patient’s scalp, provides a minimally invasive solution for hair transplantation (Rassman et al., 2002). This procedure offers validation of an autologous cell therapy–based approach to hair loss. However, patients with severe hair loss may not have sufficient follicles for FUE transplantation. An autologous iPSC-based approach solves this problem, making it possible to generate large volumes of patient-specific hair follicles for treating patients suffering from severe hair loss. Stemson Therapeutics, based on technology developed in Alexey Terskikh’s lab at Sanford Burnham Prebys Medical Discovery Institute, is pursuing such an approach (Gnedeva et al., 2015). Gnedeva and colleagues developed a two-phase 28-day protocol for differentiating ESCs and iPSCs to adherent hair-inducing dermal papillae (DP) cells via a neural crest (NC) cell intermediate stage (Gnedeva et al., 2015). Suspensions of $5 \times 10^5$ ESC-DP cells were combined with suspensions of $5 \times 10^5$ epidermal cells and successfully transplanted into immunodeficient mice, demonstrating an ability to induce hair follicle formation in vivo, an exciting step toward bringing iPSC-derived hair transplants to patients.

### iii. iPSC-derived myogenic stem cells for muscular dystrophies

Muscular dystrophies are a group of genetic disorders characterized by muscle wasting: weakening and shrinking of the muscles due to loss of muscle mass (Emery, 2002). Muscle cell transplants into mouse models of muscular dystrophy date back to 1989, and the positive results support a cell therapy approach (Partridge, Morgan, Coulton, Hoffman, & Kunkel, 1989; Tedesco et al., 2012). However, heterologous myoblast transplantation into humans has not resulted in functional improvements; these results are partially due to immune rejection, indicating...
that an autologous approach may be more promising (Skuk & Tremblay, 2003; Sun, Serra, Lee, & Wagner, 2020). The various dystrophies are characterized by specific genetic mutations, meaning that an autologous approach would require gene editing (Emery, 2002; Ghaoui et al., 2015). Vita Therapeutics, founded out of Johns Hopkins University and based on technology developed by Gabsang Lee at Johns Hopkins School of Medicine and Kathryn Wagner at the Kennedy Krieger Institute, is developing gene-edited cell therapies to regenerate diseased and lost muscle tissue (U.S. Patent No. 15/781,709, 2016). Using a combination of iPSC technology and genome editing, patient-specific cells are differentiated into myogenic stem cells capable of dividing asymmetrically to generate additional satellite cells and myoblasts (Choi et al., 2016; Sun et al., 2020). The myoblasts can then fuse, effectively regenerating muscle fibers (Sun et al., 2020). Vita Therapeutics aims to target specific types of muscular dystrophies, including limb-girdle muscular dystrophies (LGMDs), the most heterogeneous of the muscular dystrophies, and recently received orphan drug designation from the FDA to initiate a clinical study evaluating the use of iPSC-derived myogenic stem cells for Duchenne muscular dystrophy (DMD), which is the most common form of muscular dystrophy and typically affects young boys (Emery, 2002; Sun et al., 2020).

IV. iPSC-derived photoreceptor precursors for retinal degenerative blindness

Retinal degenerative disorders, such as retinitis pigmentosa, Stargardt disease, and Leber congenital amaurosis, are characterized by incurable blindness caused by the dysfunction or loss of light-sensing photoreceptors (den Hollander, Roepman, Koenekoop, & Cremers, 2008; Hartong, Berson, & Dryja, 2006; Tsang & Sharma, 2018). In many cases, the neurons of the inner layer of the retina—whose job is to connect the photoreceptors to the brain—remain intact after photoreceptor degeneration, encouraging the pursuit of a photoreceptor transplantation-based cell therapy approach (Cideciyan et al., 2007; Mullins et al., 2012). At least two studies have demonstrated technical feasibility and safety, if not efficacy, of cadaveric photoreceptor transplantation into human patients with retinitis pigmentosa (Berger, Tezel, Del Priore, & Kaplan, 2003; Kaplan, Tezel, Berger, Wolf, & Del Priore, 1997). Several studies have demonstrated transplanted PSC-derived photoreceptor precursors’ ability to restore retinal function and improve vision in animal models of retinal degeneration (Lamba, Gust, & Reh, 2009; Pearson et al., 2012; Tucker et al., 2011). An autologous approach could avoid some of the problems leading to poor long-term survival post-transplantation, which may be due in part to immune responses (Santos-Ferreira, Borsch, & Ade, 2017; West et al., 2010). Budd Tucker’s lab at the University of Iowa developed a current GMP (cGMP)-grade protocol for generating patient-specific iPSCs and photoreceptor precursors to support the development of an autologous approach. Fibroblast samples were obtained from 35 patients with inherited retinal degeneration and expanded under cGMP-compliant conditions (Wiley et al., 2016). Sendai virus was used to generate iPSC clones from the patient samples. The iPSCs were differentiated using an ~16-week 3D suspension-based protocol, resulting in retinal organoids composed primarily of post-mitotic photoreceptor precursor cells. iPSC-derived photoreceptor precursors from eight different patients were transplanted into immunocompromised mice. The transplants resulted in no tumor formation and demonstrated no pluripotent cells in the final cell population, an important concern for cell therapy products (Wiley et al., 2016).

III. CHALLENGES AND CONSIDERATIONS IN BRINGING AUTOLOGOUS iPSC-DERIVED CELL THERAPIES TO PATIENTS

With several clinical trials planned or underway, the past 10 years have seen significant progress in developing autologous iPSC-based cell therapies (see Section II). However, an autologous iPSC-derived cell therapy has yet to reach the market, and the majority of iPSC-based cell therapy development efforts are utilizing an allogeneic approach (Martin-Ibanez & Sareen, 2020). For autologous iPSC-based therapies to become a commonplace treatment modality, safety and scalability are crucial. Section III discusses areas where progress would enable autologous iPSC-derived cell therapies to become more widely used. The topics covered are shortening the manufacturing timeline (A), ensuring safety and quality of the final cell product (B), and using scalable manufacturing processes (C).
Figure 2  Manufacturing timeline for autologous iPSC-derived cell therapy products. The manufacturing process consists of reprogramming and differentiation and takes on the order of months. The reprogramming portion of the process can range from 2 to 6 months in length and includes (i) patient sample collection, (ii) patient sample QC, (iii) expansion of patient cells, (iv) reprogramming, (v) colony expansion and reprogramming vector clearance, and (vi) iPSC QC. The steps with the most potential to shorten the manufacturing timeline are (iii) expansion of patient cells and (v) colony expansion and reprogramming vector clearance. In some cases, (vi) iPSC QC can be performed in parallel to (v) colony expansion and reprogramming vector clearance or in parallel to (vii) differentiation. The downstream portion of the process includes (vii) differentiation, (viii) final product QC, and (ix) transplantation. The differentiation timeline is highly cell-type and technique dependent, with differentiation protocols ranging in length from 4 days (Ng et al., 2020) to several months (Wiley et al., 2016). EPCs, epithelial progenitor cells.

A. Manufacturing Timeline

It takes several months for patient-extracted somatic cells to be reprogrammed and differentiated to produce a cell therapy for patients. This review focuses on accelerating the timelines for reprogramming somatic cells, as the timeline for differentiation is highly cell-type and application dependent and as rapid differentiation techniques have been discussed at length elsewhere (Buchholz et al., 2013; Busskamp et al., 2014; Ehrlich et al., 2017; see Current Protocols article; Fernandopulle et al., 2018; Frega et al., 2017; Ng et al., 2020; Yang et al., 2017). Optimizing the manufacturing timeline will ensure that patients receive their treatments in a timely fashion. This subsection highlights areas of innovation that could compress the autologous iPSC manufacturing timeline.

Timeline for reprogramming somatic cells to iPSCs

The first step in developing an autologous iPSC-based therapy is to create an iPSC MCB for each patient. Automated manufacturing technologies and automation-compatible QC assays hold promise for a faster and more streamlined manufacturing process. However, the biology and reprogramming method limit how rapidly primary tissues can be reprogrammed to produce a validated autologous iPSC MCB. The three most used reprogramming methods use viral vectors (retroviral and Sendai virus), episomal vectors, and mRNA. The field is moving away from retroviral methods due to the potential for permanent, undesired integration of the reprogramming material into the genome (Okita, Ichisaka, & Yamanaka, 2007). Non-integrating, “footprint-free” approaches, such as those using Sendai virus, episomal vectors, and mRNA, are an attractive alternative (Schlaeger et al., 2015).

Figure 2 shows the manufacturing process from somatic cell extraction to reprogramming, differentiation, and transplantation. The current process of reprogramming takes ~4 months; the exact time depends on the
chosen somatic cell source and reprogramming technique. The six steps of reprogramming are as follows: i) patient sample collection, ii) patient sample QC, iii) expansion of patient cells, iv) reprogramming, v) colony expansion and reprogramming vector clearance, and vi) iPSC QC.

Within this process, shortening steps iii and v will have the biggest impact on the manufacturing timeline.

The sourced patient cells must be expanded to get sufficient starting material for reprogramming. The expansion of patient cells (step iii) can take \(\sim 7\) days for peripheral blood CD34+ cells or \(>28\) days for adherent cells. This difference in required expansion time is reflective of differences in the number of extracted cells and the proliferation rate for the two cell types (Loh et al., 2009; Takahashi et al., 2007). Peripheral blood CD34+ cells have advantages as a starting material: shorter expansion timelines and higher reprogramming efficiencies (Eminli et al., 2009). At this time, regulatory agencies do not have specific recommendations regarding patient cell type; the choice of starting material is at the discretion of cell therapy developers (Jha et al., 2020).

The process of reprogramming (step iv) takes \(\sim 28\) days, independent of the starting cell type or the chosen reprogramming vector (Ban et al., 2011; Fusaki, Ban, Nishiyama, Saeki, & Hasegawa, 2009; Loh et al., 2009; Okita et al., 2011; Takahashi et al., 2007; Warren et al., 2010). Reprogrammed colonies emerge during this time. Colonies are then expanded, and the reprogramming vector is cleared from the cells over time (step v). Each reprogramming vector has a different clearance rate. For replication-competent episomal and viral methods, the expansion and vector clearance stage can take up to 110 days due to the exogenous reprogramming material’s slow elimination rate (Fusaki et al., 2009; Nanbo, Sugden, & Sugden, 2007). On average, each cell division dilutes the episomal plasmids with Epstein-Barr virus (EBV) replicon by 5%, with significant differences between clones (Nanbo et al., 2007). Sendai virus can take up to 20 passages to be diluted out (Fusaki et al., 2009), making it challenging to predict the manufacturing time. It may be possible to streamline the cell division–dependent elimination rate of episomal plasmids and viral vectors by using automation to optimize passage ratios, media changes, and cell density management (Schorl & Sedivy, 2007).

An mRNA-based approach to reprogramming may have the greatest impact in shortening reprogramming timelines. The mRNA is cleared from the cells within 48 to 72 hr, producing footprint-free iPSCs 60 days faster than episomal or viral vector-based techniques (Warren et al., 2010). However, the transient nature of the mRNA-induced transcription factor expression requires 4 to 8 rounds of mRNA delivery to somatic cells every 48 hr (Kogut et al., 2018; Warren et al., 2010). The mRNA is delivered to somatic cells by electroporation or lipofection-based methods, causing significant stress to the cells and negatively affecting the viability and reprogramming efficiency. To date, only adherent cells, most commonly fibroblasts, have been reprogrammed with mRNAs (Warren & Lin, 2019). New intracellular delivery methods are emerging that might allow for repeated mRNA delivery into blood cells, such as non-viral gene delivery systems from AveXis (O’Dea et al., 2017), SQZ Bio (Ding et al., 2017), Kytopen (Garcia, Ge, Moran, & Buie, 2016), and Cellino (Saklayen et al., 2017). There is a growing interest in developing approaches for mRNA-based reprogramming of blood cells to provide greater options for cell therapy developers.

B. Safety and Quality Control

Autologous therapies are considered safer than allogeneic from an immunogenicity perspective (see Section I). Autologous therapies do not suffer from the immensely high QC barrier of allogeneic therapies. There is no risk of introducing infectious agents from one patient into another, as long as sterility and identity chain are maintained during manufacturing. Autologous cell therapies do not have to go through the expensive screening processes that allogeneic donor tissues must undergo for disease-transmitting agents such as viruses (Zika, West Nile, SARS, HIV, HBV, HCV, vaccinia, HTLV, and others), parasites (Treponema pallidum), and transmissible spongiform encephalopathies. Certain safety risks relevant to iPSCs, such as genomic instability, need to be assessed regardless of whether the approach is autologous or allogeneic. Developing standardized regulatory guidelines and validating each autologous iPSC MCB using standardized QC assays can mitigate these safety risks. Standardized QC measurements are also useful for assessing the reproducibility of a manufacturing process, which is an essential consideration for autologous iPSC-based cell therapies, given that the manufacturing process needs to be repeated.
for each patient. Automated systems for scalable manufacturing (Section IIIC) can play a role in enhancing the reproducibility of a process, as can thoughtful selection of the iPSC culture parameters (this subsection). This subsection describes the currently used QC standards for iPSC manufacturing, relevant tools for assessing key iPSC characteristics, and the status of regulatory guidelines for iPSC-derived products.

i. Developing a reproducible iPSC-based process

Achieving reproducibility has been identified as a critical goal in the iPSC field, and a lack thereof has been a barrier to broader adoption of iPSCs, keeping innovators from realizing iPSCs’ full therapeutic potential. The culture factors affecting iPSC outcomes are growth factors, extracellular matrix (ECM) proteins, steroids, hormones, small molecules (agonist/antagonist), shear flow, mechanical strain/stiffness of substrates, ions (such as $\text{Ca}^{2+}$, $\text{Mg}^{2+}$, and $\text{Cl}^-$), electromagnetic fields, and a host of other parameters (Murphy, McDevitt, & Engler, 2014). In this regard, small molecules can induce efficient and rapid differentiation and enable cells to handle the stress of single-cell seeding strategies such as use of Rho-associated protein kinase (ROCK) inhibitors (Maldonado, Luu, Ramos, & Nam, 2016).

One of the key elements of the cellular microenvironment that shapes differentiation choices is the ECM, typically used to coat the tissue culture plate (substrate) during cell culture. Recent studies have implicated various biomaterial properties as critical elements of the stem cell environment; substrate mechanical stiffness (Engler, Sen, Sweeney, & Discher, 2006; Iwashita et al., 2019; Lee, Abdeen, Zhang, & Kilian, 2013), nanometer-scale topography (Dalby et al., 2007; McMurray et al., 2011; Yim, Darling, Kulangara, Guijak, & Leong, 2010), and chemical functionality (Benoit, Schwartz, Durney, & Anseth, 2008; Saha et al., 2011) each impact stem cell expansion and function. Although many of these earlier studies used inconsistent and poorly reproducible mesenchymal stromal cell (MSC) models, they demonstrate that biomaterials, in principle, can provide an environment that may help control iPSC behavior. Many current iPSC protocols use Matrigel or Geltrex to deliver a physiological ECM substrate for cell adhesion. Both products are related basement membrane matrices made up of secreted ECM proteins, including laminin, collagen IV, heparin sulfate proteoglycans, and entactin/nidogen, purified from murine Engelbreth-Holm-Swarm (EHS) tumor cells. These products are biochemically complex, with over 2000 different protein components. They have an unfortunate but well-documented track record of high variability and lack of reproducibility from batch to batch (Nguyen et al., 2017). Therefore, the selection of custom ECM materials to promote the optimal survival, potency, and differentiation efficiency of iPSC-derived target cells is a product development goal worthy of pursuit on the long, hard road from the bench to the clinic.

ii. Quality control

For both autologous and allogeneic approaches, enabling safe delivery of iPSC-derived therapies will require streamlined, rapid QC testing for safety, identity, potency, and purity. Although most of the QC tests developed for a diverse set of cell therapies may translate to the iPSC-derived workflow, there are special considerations to be factored into the QC plan for generating therapeutic cells from iPSCs.

The key to developing a robust and reproducible process for the generation of multipotent cells from iPSCs will be to generate and validate suitable analytics to accurately track and measure the critical quality attributes (CQAs) that will govern the efficiency and consistency of the differentiation method. However, before this, the developer often needs to address the key variables of cell counts and viability. Counting viable cells seems to be one of the simplest and most important steps in developing any cell therapy; it is one of the most fundamental measures for cell-based therapeutics yet is often overlooked or underestimated. The National Institute of Standards and Technology (NIST) and the FDA held a workshop on cell counting in April 2017 with over 50 small and medium-sized enterprises (SMEs) from industry, with the conclusion that for each counting purpose (process and cell type), an appropriate cell counting method needs to be designed, validated, and implemented (Arcidiacono et al., 2018). The ideal instrument needs to robustly determine accurate, reproducible, and validated (21 CFR Part 11 compliant) cell counts for iPSCs and other intermediate cell types in the process with similar size and despite varied clumping characteristics, which can skew results. Instruments such as the ChemoMetec NucleoCounter NC-200 use image cytometry and specialized assays for aggregated cells to...
overcome these constraints. There is no subjective evaluation of what constitutes a live cell visually, as with traditional counting. Typical errors introduced during pipetting and staining are avoided by a built-in pipet, which is part of the counting cassette. This system uses the permeable dyes acridine orange and DAPI to respectively stain the total and dead cell populations. Our initial but thorough evaluation of the NC-200 compared to a typical lab cell counter using trypan blue (Bio-Rad TC20) has demonstrated the NC-200 to be superior and highly accurate for our purposes. Using these types of novel instruments well suited to GMP manufacturing QC will allow the autologous iPSC developer with limited cell numbers to standardize cell counts for the entire process, from iPSCs to final cell type, and enable a solid foundation for all the cell-based QC assays, such as flow cytometry and enzyme-linked immunosorbent assays (ELISAs) for secreted factors, to demonstrate multipotency and specific differentiation potential.

Genomic (chromosomal) instability is an endemic source of concern for iPSC lines (Henry, Hawkins, Boyle, & Bridger, 2019). Many iPSC lines have been found to contain aneuploidies and pose a serious risk to patients if they are used as input material to generate medically useful cells via controlled differentiation. In fact, the first autologous iPSC clinical trial at RIKEN to restore vision ran into safety concerns when chromosomal abnormalities were detected in iPSCs generated for the second patient (Garber, 2015). There were genetic copy-number alterations at the chromosomal level that were not detected in the patient’s original fibroblast sample. The trial was halted until the safety concerns could be addressed. Experiences such as this have taught developers that iPSC chromosomal health needs to be tested and validated at multiple steps along the process workflow. This can be seen as a “digital karyotyping” revolution to replace the standard visual karyotyping that produces beautiful images of striped chromosomes but is otherwise slow and difficult to integrate into future-proof QC plans. In contrast, the recent development of rapid handheld next-generation sequencing tools such as the Oxford Nanopore MinION device has allowed QC for genomic instability to be brought inline during manufacturing, in between cell line splits, and for the identity of the cells to be validated at important steps along the workflow. Combined with cloud-based systems to store, compile, and analyze digital karyotyping data, such as the FIND Genomics system, with a structural variant detection resolution of 5 Mb or greater, such low-cost, high-data QC tests can be standardized and integrated into regular QC testing (Lu & Sanjana, 2019).

### iii. Moving toward standardized regulatory guidelines

Autologous iPSC-derived cells have undergone extensive QC validation before being transplanted into patients (Mandai et al., 2017; Schweitzer et al., 2020). However, there is currently no pre-defined regulatory path for autologous iPSC-based cell therapies; for instance, there is no regulatory guidance regarding the choice of starting tissue for iPSC generation (Jha et al., 2020). Development of a standardized regulatory roadmap will require collaboration between subject matter experts and regulatory agencies. Several international bodies, such as the Global Alliance for iPSC Therapies (GAiT) and Alliance for Regenerative Medicine (ARM) are working together with regulatory agencies to reach consensus on CQAs for iPSC-derived cell therapy products (Sullivan et al., 2018). Table 2 summarizes the current QC standards for iPSC manufacturing but, considering the fast pace of progress in the field, these standards are bound to evolve quickly. These guidelines will require frequent reassessment as the field’s scientific understanding grows and the enabling technology landscape evolves. Though a well-defined regulatory path for autologous iPSC-derived cells still needs to be developed, several regulatory agencies have introduced new pathways to expedite the review process for regenerative medicine products. Examples of these are the SAKIGAKE in Japan, PRIME in Europe, and RMAT in the U.S., aiming to improve the regulatory review process for regenerative medicines (Nagai, 2019).

### C. Scalable Manufacturing

Autologous iPSC-derived cell therapies require repeating the manufacturing process for each patient. Enabling cost-effective autologous iPSC-derived cell therapy manufacturing will require some form of a “process-in-a-box” approach, which will allow scaling up via multiple units each housing one patient’s cells. Even though the “expand-the-bioreactor-volume” approach to scaling up allogeneic cell therapy processes may seem more familiar and comparable to biologics manufacturing, autologous iPSC-derived cell therapy processes are well on their way to being scaled out. The process of generating
| Attribute               | Assay       | Method                                                                 | Timing     | Notes                                                                 |
|------------------------|-------------|------------------------------------------------------------------------|------------|----------------------------------------------------------------------|
| Safety                 | Mycoplasma  | USP<63>, Ph.Eur.2.6.7 and JP17<G3>, qualified qPCR or culture (broth/agar or Vero cell inoculation/DNA stain method) | Release    | Rapid mycoplasma assays such as real-time qPCR (e.g., MycoSEQ) can deliver results in <4 hr versus 28 days for compendial culture-based methods |
| Endotoxin              | USP<85>, Ph.Eur.2.6.14, JP17<4.01> | Release                                                                |            | Availability of rapid systems such as Endosafe nexgen-PTS allows testing to be performed in-line (at the source) versus at a core QC lab |
| Bacteriology           | USP<71> and <61>, Ph.Eur.2.6.27 and 2.6.1, JP17<4.05> and <4.06> | In-process, release                                                   |            | Rapid sterility testing methods are now available (e.g., Milliflex), which reduce the time from the compendial from 14 to ≤5 days |
| Viral testing          | USP<1237>, Ph.Eur.2.6.16, JP17<G3> | Release                                                                |            | Based on risk assessment of starting and raw materials; iPSCs for autologous use have a different viral risk profile than those slated for allogeneic clinical applications |
| Residual vector testing| Appropriate specific assay such as RT-PCR or immunochemistry with vector-specific antibodies | Release                                                                |            | To ensure a detectable absence of vectors (such as Sendai virus) used in iPSC generation |

(Continued)
| Attribute            | Assay                        | Method                                                                 | Timing                      | Notes                                                                                                                                 |
|----------------------|------------------------------|------------------------------------------------------------------------|-----------------------------|---------------------------------------------------------------------------------------------------------------------------------------|
| Identity             | Karyotyping                  | Chromosome counting, G-banding, array analysis, NanoString (digital karyotyping), fluorescence in situ hybridization (FISH) | In-process, release         | Can use compliant cloud-based tracking systems to ensure a chain of identity, such as FIND Genomics                                                                                     |
|                      |                              |                                                                        |                              |                                                                                                                                                                                                   |
|                      | Short tandem repeat (STR)    | Simple and automated process using capillary electrophoresis (CE) of fragments amplified from microsatellite loci with variable numbers of repeats and analysis by PCR | Release                     | Used to match donor to recipient, existing familiarity, and user base with instruments due to use in forensic analysis                                                               |
|                      |                              |                                                                        |                              |                                                                                                                                                                                                   |
|                      | Single nucleotide polymorphism (SNP) | SNP chip-based copy number variation (CNV) analysis                        | Release                     | Can be queried against database of known CNVs (dgv.tcag.ca/)                                                                                                                                   |
|                      | Genomic stability           | RNA-seq-based intensive genetic quality test, whole genome sequencing (WGS), whole exome sequencing (WES), e.g., Illumina transcriptome human gene expression panel | Release                     | Targeted panels can measure expression levels of >20,000 human RefSeq genes                                                                                                                     |
| Potency              | Pluripotency by differentiation | Confirm differentiation into progeny cells of the three germ layers, e.g., neuron (ectoderm), heart (mesoderm), and liver (endoderm) | Release                     | May include embryoid body and/or organoid formation and assessment. Teratoma formation is not currently required as a potency assay.                                                     |
|                      | Pluripotency by gene expression | Expression of pluripotency markers SSEA4, TRA1-60, OCT4, Nanog, and other genes, USP <1027>, Ph.Eur.2.7.24 | Release                     | Real-time qPCR assays such as TaqMan hPSC Scorecard and genome-wide assays such as PluriTest-compatible PrimeView can be used to assess many markers in parallel |
|                      | Viability, apoptosis, and cell count | Manual (USP <1046>, Ph.Eur.2.7.29 using dye exclusion, e.g., trypan blue), or automated cell counter/flow cytometry, e.g., MACSQuant or NC-200 | Typical release criterion is a viability of >70% post-thaw |                                                                                                                                                                                                   |

*aTable 2 lists the current typical QC assays for GMP-grade autologous iPSCs (Huang et al., 2019; Jo et al., 2020; Sullivan et al., 2018; Yaffe, Noggle, & Solomon, 2016).*
iPSC MCBs is gradually becoming more standardized. Optimization of media components, replacement of recombinant proteins in the media with small molecules that target the same pathways, and generally streamlining the process to produce more target cells of the desired CQAs with shorter process times will all work together to reduce the cost of goods and the complexity of iPSC manufacturing. The automation, closed systems, and data-centric processes described in this subsection are also expected to play a key role in making autologous iPSC-based cell therapy manufacturing more scalable, cost effective, and efficient.

**Automation, closed systems, and big data**

A typical iPSC-based process will undergo an evolution from a manual, academic protocol to a robust process in a closed, automated system customized to meet the previously determined CQAs and lead to desired clinical outcomes with the final output cell types. Automation and closed systems are considered to be essential for realizing the long-term commercial success of iPSC-derived therapies because one of the primary bottlenecks is the inability to migrate the manufacturing process from a manual, high-risk academic bench protocol to an industrial-scale, GMP-compliant manufacturing system operating in a “factory of the future” to be able to handle patient demand and confidently manage risks to continuity and sustainability. As commonly stated by manufacturing experts, the two main risks for a typical cell therapy process are human error and lack of process understanding (including understanding of CQAs), causing unexpected outcomes; both risks can be effectively managed by housing an evolved process in the appropriate closed, automated system (Leong, Nankervis, & Beltzer, 2019).

The Miltenyi CliniMACS Prodigy instrument is a fully integrated GMP-compliant closed system for the medium-scale manufacturing of cell therapies. This instrument has successfully been used at hundreds of sites globally for clinical manufacturing of suspension cells such as T cells and CD34+ stem cells but initially was not configured to handle adherent cells such as iPSCs and NC cells. Recently, a proof-of-concept study highlighted the upgraded ability of the Prodigy instrument to successfully handle the GMP-compliant differentiation of iPSC-derived mesencephalic dopaminergic (mesDA) progenitor cells, which can be identified based on the integrin-associated protein (IAP) marker CD47 (Lehnen et al., 2017). Another suitable instrument is the Quantum Cell Expansion System (QES) from Terumo Blood and Cell Technologies, based on a large-surface-area hollow-fiber bioreactor membrane. The QES system manufactured iPSCs at large scale using a laminin matrix, with the in-line measurement of lactate providing a consistent readout of cell metabolism and final yield (Paccola Mesquita, Hochman-Mendez, Morrissey, Sampaio, & Taylor, 2019).

Success in this field will be enabled by putting together expert teams of diverse and highly technical stakeholders from industry sponsors, contract development and manufacturing organizations (CDMOs), and academic labs, as well as clinicians and process engineers. As a recent example, in September 2019, the iPSC-derived Cortical neurons (iCone) Consortium, comprising MaSTherCell SA (a CDMO dedicated to the industrialization and production of cell and gene therapies), Ncardia (an industry sponsor), the Pierre Vanderhaeghen Lab at Université Libre de Bruxelles (an academic collaborator), and the “Laboratoire de Chimie Analytique Pharmaceutique” led by Philippe Hubert at Université de Liège (an analytical collaborator), reported their initial success with a new 3D mid- to large-scale process for the production of human iPSC-derived cortical neurons in stirred-tank bioreactors using the Eppendorf DASbox® bioreactor platform (Sumen, 2019).

Lastly, the potential of big data solutions to improve the iPSC manufacturing process cannot be underestimated. AI and machine learning (ML) approaches have leveraged new algorithms, less expensive cloud computing power, and the expansion of various sources of genomic, proteomic, and imaging data to bring new insights to those of us working at the cutting edge to develop CQAs and streamline our processes. Going forward, big data solutions coupled to efficient laboratory information management system/electronic lab notebook (LIMS/ELN) systems will provide new insights while integrating different streams of data using promising computing approaches such as convolutional neural networks (CNNs), recurrent neural networks (RNNs), and generative adversarial networks (GANs) coupled with unsupervised deep learning to extract and relate features to outcomes. These systems are being used to develop new therapies and uncover novel ways for iPSC-derived autologous therapies to address unmet medical needs and provide succor to those suffering from debilitating diseases.
IV. CONCLUSION

The promise of iPSCs lies in their ability to serve as a standardizable starting material for the development of autologous, or patient-derived, cell therapies. In contrast to the allogeneic approach, autologous therapies would treat a range of diseases without the need for immunosuppression. An autologous process could be used to treat all patients, not just those who are not covered by HLA haplombs. Since the first groundbreaking in-human study in 2014, in which autologous iPSC-derived RPE cells were transplanted into the eye of a patient suffering from AMD, two additional patients have received autologous iPSC-derived transplants for other disease indications. None of the patients received immunosuppression, and no serious adverse events were reported for any of these studies, further confirming the promise of autologous iPSC-derived cell therapies to treat patients without subjecting them to the side effects of immunosuppression. The autologous iPSC-derived cell therapy landscape has grown significantly in the past decade, with advancements in the enabling technology landscape and the development of well-defined QC standards, autologous iPSC-derived therapies can provide relief to the many individuals suffering from incurable degenerative diseases.

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Marinna Madrid: Conceptualization, Data curation, Writing-original draft, Writing-review & editing, Cenk Sumen: Conceptualization, Data curation, Writing-original draft, Writing-review & editing, Suvi Aivio: Data curation, Writing-original draft, Writing-review & editing, Nabiha Saklayen: Conceptualization, Writing-review & editing.

CONFLICT OF INTEREST

The authors M.M., S.A., and N.S. are employees at Cellino. The author C.S. is an employee at Stemson Therapeutics.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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