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Illustrating the potency of current Good Manufacturing Practice–compliant induced pluripotent stem cell lines as a source of multiple cell lineages using standardized protocols

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Abstract

Background aims. We have previously reported the generation of a current Good Manufacture Practice (cGMP)-compliant induced pluripotent stem cell (iPSC) line for clinical applications. Here we show that multiple cellular products currently being considered for therapy can be generated from a single master cell bank of this or any other clinically compliant iPSC line *Methods.* Using a stock at passage 20 prepared from the cGMP-compliant working cell bank (WCB), we tested differentiation into therapeutically relevant cell types of the three germ layers using standardized but generic protocols. Cells that we generated include (i) neural stem cells, dopaminergic neurons and astrocytes; (ii) retinal cells (retinal pigment epithelium and photoreceptors); and (iii) hepatocyte, endothelial and mesenchymal cells. To confirm that these generic protocols can also be used for other iPSC lines, we tested the reproducibility of our methodology with a second clinically compliant line *Results.* Our results confirmed that well-characterized iPSC lines have broad potency, and, despite allelic variability, the same protocols could be used with minimal modifications with multiple qualified lines. In addition, we introduced a constitutively expressed GFP cassette in Chr13 safe harbor site using a standardized previously described method and observed no significant difference in growth and differentiation between the engineered line and the control line indicating that engineered products can be made using a standardized methodology *Conclusions.* We believe that our demonstration that multiple products can be made from the same WCB and that the same protocols can be used with multiple lines offers a path to a cost-effective strategy for developing cellular products from iPSC lines.

Key Words: *Good Manufacture Practice (cGMP), induced pluripotent stem cell (iPSC), neural stem cells, dopaminergic neurons, astrocytes, retinal cells, hepatocyte, endothelial and mesenchymal cells*

Introduction

Currently there are three major models of how induced pluripotent stem cell (iPSC) lines will be used for therapy: allogeneic, autologous and a hybrid human leukocyte antigen (HLA)-matched model that may be regarded as an intermediate between the allogeneic and autologous models. The allogeneic model is similar to the manufacture of most biologicals in that a few well-characterized iPSC lines will be selected and used for the manufacture of a final product in a centralized facility. Rejection of cell-based products can be managed with transient immune suppression with well-characterized drugs, or cells can be transplanted to sites where immune suppression will be minimal. However, the use of immunosuppression and the possibility of rejection at late stages or the possibility of sensitization precluding repeated transplants remains a concern. One strategy to reduce the requirement for immunosuppression is the autologous approach by which iPSC lines are made from the same individuals that require cell-based therapeutic intervention. These iPSCs are then used as starting material to manufacture a differentiated cell product that is transplanted into that same person [1]. This approach is expensive and difficult to implement under current regulations for a manufactured product and may not be applicable for older individuals (where iPSC lines may not be as easy to generate) or when acute intervention is required and a personalized line cannot be

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generated in time. Such personalized therapy may also not be applicable to individual with hereditary disorders without additional gene correction.

Given these issues and the prohibitive cost of personalized medicine, several groups have suggested a hybrid HLA-matching approach. This approach has the added advantage that products could be available off the shelf if HLA-matched cell donors are carefully selected and the bank size does not need to be too large [2]. This model could obtain the benefit of cost reduction and having well-characterized cells available on demand, while mitigating the use of immunosuppressive agents. An alternative approach also under consideration is to use HLA null or stealth cells [3–5] so that a single or few well-characterized liens can be used for all individuals without immunosuppression.

As efforts to enable iPSC-based therapy under these models begins, a common issue for all models has been the cost of manufacture of cell therapeutics. The high cost is mainly due to the long and often inefficient differentiation process. Current bioreactor technology is not optimally suited for growing adherent cells and the current Good Manufacture Practice (cGMP) suites are not designed for small-lot production that requires prolonged cell culture times. Small-scale manufacture as is seen for the generation of autologous products or some allogeneic products such as platelets cannot be tested with the same rigor as is expected for large lots [6]. Although promising alternative strategies for manufacture that include automation, three-dimensional methodologies and tissue printing techniques are being evaluated, these technologies do not necessarily reduce cost.

Our proposed approach can be combined with any of these manufacturing technologies and has the potential of significantly reducing the cost to manufacture multiple products from the same allogeneic iPSC line. iPSCs are unique in that they can make derivatives of all three germ layers and appear to be truly immortal as well. It is therefore not unreasonable to assume that a single iPSC line could be used to make retinal pigment epithelium (RPE), dopaminergic neurons or any other cell product that is moving forward for therapy. With gene editing techniques, it is also possible to remove the immune mismatch by targeting the HLA locus, as has been proposed for making universal cells [3–5].

Given the potential cost reduction, we decided to test this process empirically. We developed a clinically compliant line (NCL2) made by a standardized cGMP-compliant protocol that we have extensively characterized using comparability criteria that are under consideration by regulatory authorities [7]. We tested its ability to differentiate into several major cell types that are being considered for therapeutics using generic standardized protocols that have been previously tested with multiple lines. We showed that this cGMP-compliant iPSC line could be differentiated into neural derivatives, including dopaminergic neurons; RPE and photoreceptors; as well as hepatocyte, endothelial and mesenchymal lineages. In addition, we show that a genetically modified subclone generated by standard genome editing techniques behave similarly and theoretically could be used as therapy with minimal additional testing. We believe that this data provides important support for the idea that iPSC-derived cell-based therapy can truly become cost-effective in the future.

Methods

Human iPSC culture

The cGMP-compliant human iPSC line used in this study, NCL2, was generated from cord blood CD34 + cells by episomal vectors as previously described [7]. Cells were cultured on Matrigel in mTeSR medium (Stem Cell Technologies) with 10 μmol/L ROCK inhibitor Y-27632 and passaged with Accutase.

Generation of neural stem cell and dopaminergic differentiation

Generation of neural stem cell (NSC) from iPSC was as previously described [8]. Briefly, confluent NCL2 cells were detached via collagenase and cultured in suspension as embryoid bodies (EBs) in STEMPRO SFM medium (Life Technologies) supplemented with 100 nmol/L LDN193189 (Stemgent), 10 μmol/L SB431542 (Tocris), 2 μmol/L Purmorphamine (Stemgent), 3 μmol/L CHIR99021 (Stemgent), 100 ng/mL sonic hedgehog (Peprotech) and 100 ng/mL fibroblast growth factor (FGF)8 (Peprotech) for 8 days. EBs were directed toward neural lineages by the addition of FGF2 and allowed to attach in adherent cultures in NSC maintenance medium (XCell Science Inc.). After attachment, neural tube–like rosette structures were manually dissected and expanded in NSC maintenance medium.

Dopaminergic differentiation was carried out on culture dishes or glass cover slips coated with 2 mg/mL poly-L-ornithine (Sigma) and 10 mg/mL laminin (Life Technologies) using Dopaminergic Induction and Maturation medium (XCell Science Inc.). In brief, NSC was cultured in dopaminergic Induction medium supplemented with 200 ng/mL SHH with a medium change every other day for 8 days. Then cells were dissociated by Accutase (Life Technologies) and passaged onto new poly-L-ornithine/laminin treated dishes in complete dopaminergic maturation medium for another 15–20 days with medium change every other day until the time of analysis.

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