Chapter

Induced Pluripotent Stem Cells for Clinical Use

Valérie Vanneaux

Abstract

The use of induced pluripotent stem cells (iPSCs) represents a great promise in regenerative medicine. So far, several clinical trials are underway and preliminary results are promising with the human embryonic stem cells, their non-reprogrammed counterparts. The experience of the clinical use of iPSC derivatives is extremely limited because of several major safety concerns, but many technological advances in the field of iPSC generate high expectations in the near future to develop new clinical trials with an adapted level of patient safety. New guidelines and several recommendations are edited by researchers and regulatory agencies to guarantee the safety of the iPSC products in a clinical context for medical applications. In this chapter, we review the clinical trials with induced pluripotent stem cells and the main factors affecting the safe translation of iPSC to the clinic and how to overcome these issues by standardization and to control the quality of the clinical-grade iPSC products.

Keywords: clinical-grade induced pluripotent stem cells, regenerative medicine, clinical trials, quality control

1. Introduction

Since the discovery of induced pluripotent stem cells (iPSCs) by Yamanaka and Takahashi in 2006, many expectations have emerged, and iPSCs have opened up a world of possibilities for new cell-based therapies in regenerative medicine [1]. In the domain of pluripotent stem cells, iPSCs are considered as equivalent to embryonic stem cells (ESCs), because of two intrinsic key properties: their indefinite proliferative capacities while preserving pluripotency and their capacity to differentiate into all known cell types. However, in contrast to ESCs, iPSCs can be generated without any controversial ethical issues, thus favoring their use in clinical settings. Last but not least, in an autologous approach of cell-based therapy, by using the patient's own cells as source for iPSC generation, one circumvents all the issues related to the immunological compatibility between the donor and receiver. This largely explains the tremendous enthusiasm engendered by iPSC discovery in the sphere of regenerative medicine during the last decade. In this review article, we provide an overview of the launched clinical trials with iPSC and the ongoing efforts to understand the risk related to safety of iPSC-derived cells, highlighting some of the problems that have to be overcome.

2. Clinical trials with iPSC

After over a decade of research on iPSC, and due to fast-track facilitating procedure in Japan, several clinical studies were launched. While the first clinical trial based on the human ESC started in 2010, taking advantage of the acquired extensive knowledge of ESC biology, despite their relatively recent discovery, the first clinical study based on the iPSC-derived retinal pigmented epithelium was authorized and conducted at the RIKEN Institute in Japan in 2014 [2]. A sheet of autologous iPSC-derived retinal cells were transplanted in a patient with eye-related macular degeneration (AMD). In 2015, the RIKEN Institute decided to suspend the study due to safety concerns on the cells of the second recruited patient [3]. Nonetheless, regarding the first transplanted patient, a 25-month follow-up revealed neither serious events, nor clinical signs of rejection. Moreover, the macular degeneration progress was delayed in the treated eye compared to the untreated eye. This result corroborated all the results obtained previously in the course of the ESC-based clinical studies, where no adverse events related to transplanted cells were observed. Still this problem induced a shift in the approach from patient-specific autologous to highly securized allogeneic iPSC lines. This study was resumed in 2017 and until now five patients with AMD have been treated with allogeneic iPSC-derived cells.

Since then, several clinical studies based on allogeneic iPSCs have been developed and approved. Until mid-2019, there have been nine ongoing clinical studies based on iPSC, mostly nationally approved in Japan, with four of them being approved in the first months of 2019, with indications including Parkinson's disease, AMD, severe cardiac failure, aplastic anemia, spinal cord injury and corneal stem cell deficiency. Furthermore, two private companies—Cynata Therapeutics, an Australian stem cell and regenerative medicine company, and Fate Therapeutics, an American clinical-stage biopharmaceutical company—have developed a line of products based on allogeneic human iPSC-derived cells. In Australia and United Kingdom, Cynata Therapeutics just concluded a phase I study using CYP-001, an iPSC-derived mesenchymoangioblast precursor administered intravenously in 15 patients with graft-versus-host disease (GVHD) occurring after an allogeneic hematopoietic stem cell transplant [4]. Currently, all patients treated so far have demonstrated at least a partial response, while no treatment-related serious adverse events or safety concerns have been observed. The product development activities of CYP-001 will be done in a phase II study in 2019 by Fujifilm in collaboration with Cynata Therapeutics. On its part, Fate Therapeutics received a first approval from Food and Drug Administration (FDA) in November 2018 to transplant an off-the-shelf iPSC-derived Natural Killer cell, FT-500, as cancer immunotherapy to treat solid tumors and for a second cell product derived from a genetically engineered iPSC, FT-516, in February 2019, for the treatment of relapsed/refractory hematologic malignancies. For the first product FT-500, all the three patients with advanced solid tumors have been treated with multiple doses of FT-500, 100 million cells per dose, and it has been well tolerated with no dose-limiting toxicities or adverse events [5].

Even though the first clinical studies have already been started, technical advances in iPSC biology have revealed that several factors could affect their safety for a larger range of medical applications, and should be taken into account for short- and long-term follow-up of patients. Two of the major concerns related to iPSC-based products are their potential tumorigenicity and immunogenicity. The scientific community is still continuing to elucidate the biological mechanisms underlying iPSC's immunogenicity and tumorigenicity and how to manage or overcome them.

3. iPSC safety

3.1 Tumorigenicity

The potential risk of tumorigenicity to patients from both teratomas and malignant tumors could arise if transplanted cells are contaminated with undifferentiated iPSC, or if transplanted cells have been genetically modified and become unstable during the *in vitro* production steps.

The major concern related to iPSC-based tumorigenicity is the reprogramming method. In the original cocktail of transcription factors developed by Yamanaka, somatic cells are transduced by retroviral vectors that become integrated into the genome of the host cells. Two of these factors—*c-Myc* and *klf4*—are potent oncogenes [6]. Subsequently, reports of tumorigenicity after transplantation of iPSC or iPSC-derived cells are not surprising. Thereby, teratoma formation could be induced by the undesired activation/suppression of essential host genes proximal to integration sites or by residual expression of reprogramming factors in the derived cells in animal model [7, 8]. With hindsight, there is evidence for the necessity to select a non-integrative method for reprogramming, a higher rate of genomic alterations occurring when human iPSCs are generated with viral vectors, compared to mRNA [7, 9]. Numerous studies, focused on the choice of reprogramming factors and methods of delivery, have developed various novel strategies to enhance the efficiency of reprogramming and reduce the potential risk of tumorigenicity. To circumvent this risk, human iPSCs have been generated by several "integration-free" methods, based on the use of viral vectors (adenoviral vectors and Sendai virus-based vectors) or non-viral vectors (piggyBac system, minicircle vector, and episomal vectors). Originally, the four transcription factors needed for complete cell reprograming were *c-myc*, *klf4*, *oct4* and *sox2* [1]. The pro-tumorigenic transcription factor *c-myc* has been found to be unnecessary for the reprogramming process, but the overall efficiency is decreased without it. Several strategies have been developed with the use of different transcription factors and/or replacement of *c-myc*, or the use of direct protein delivery and synthesized mRNA [10–12].

Furthermore, the tumorigenicity risk is often linked to the genetic instability of iPSC. Random genomic alterations are frequently observed in human iPSCs showing their intrinsic instability, essentially due to the massive genome remodeling, and probably also resulting from various mechanisms such as replicative stress, reactivation of the telomerase and metabolism modification from the oxidative to the glycolytic state. Epigenetic modifications may also contribute to iPSC variation due to residual epigenetic memories of the starting cell type [13]. The incomplete resetting of the non-CpG methylation patterns during reprogramming leads to a biased differential potential in certain cell types depending on the donor cell source [14, 15]. However, it has been shown that their residual epigenetic memory diminishes with the *in vitro* expansion over a period of time [16, 17]. As just mentioned, the selection of the donor cell type is of importance. Many human somatic cell types have been successfully reprogrammed. However, even if the use of different transcription factors, delivery methods and culture conditions does not facilitate any comparison, it is well known that reprogramming efficiencies, kinetics and tumorigenicity vary between somatic cell types. Firstly, cell sources have to be permissive to avoid to turn to integrative methods and to the use of oncogenes. Some human, adult somatic cells, such as melanocytes, are known to naturally express endogenously reprogramming factors, for instance Sox 2, at sufficiently high levels [18, 19]. Moreover, some types of donor cells such as dermal fibroblasts and blood cells are easily accessible, but they might carry more mutational burdens

and chromosomal abnormalities, due to their frequent exposure to environmental stress factors, like ultraviolet rays, or due to the donor's age, thereby leading to increased tumorigenicity, and significant safety problems [20, 21]. With all these considerations of cell variability and tumorigenic potential in mind, reflection on the generation of homogeneous cell source and banking emerged.

Many approaches have been evaluated to address the tumorigenicity challenge by eliminating the pluripotent cells of the final product such as small molecule, genetic approach to introduce a suicide gene; miRNA switch; antibodies targeting a surface-specific antigen; phototoxic approach; live detection and quantification of the residual human iPSC [22]. For the suicide gene approach, the most widely used gene is herpes simplex virus thymidine kinase (HSV-TK) that phosphorylates ganciclovir (GCV) and induces apoptosis by inhibiting DNA synthesis. Many studies demonstrated its efficacy as safeguard to eliminate tumoral cells [23]. Until then, this genetic approach with an inducible suicide system may remain not necessary enough to induce tumor elimination because of potential acquired resistance to GCV due to variability of insertion location sites and to the uncontrolled number of inserted transgene [24]. Another study demonstrated the same mechanism of inducing apoptosis in 95% of iPSCs and iPSC-derived cells by transducing an inducible Caspase 9 [25]. Recently, with development of targeted genetic strategies such as gene-editing, researchers try to identify the location of "genomic safe harbors" (GSH), corresponding to the safest permissive loci for transgenes' insertion [26]. The already known GSH candidates could be AAVS1 (adeno-associated virus integration site 1), CCR5 (chemokine CC motif receptor 5), human ROSA26 and some extragenic loci. Recently, to predict the influence of gene integration on nearby genes, it has been suggested that the combination of several distinct approaches such as the analysis of the topologically associated domains of GSH candidates of chromosomes could reduce the risks associated with cell therapy [27]. Another targeted alternative, eliminating selectively residual pluripotent cells sparing precursors and differentiated cells, involves PluriSIns, pluripotent cells-specific inhibitors [28]. Alternatively, antibody, lectin or miRNA-mediated removal undesired cells were developed to suppress the pluripotent stem cells from the final product [29]. Lastly, a novel methodology using synthetic microRNA switch is developed to improve the purity of the final product even if the cell surface markers are not available to tag the relevant cells [30, 31].

3.2 iPSC immunogenicity

The immunogenicity of differentiated cells derived from iPSC is of clinical significance. At the beginning, because of the use of the patient's own cells, theoretically there is no risk of rejection after their transplantation. Some studies demonstrated no immune rejection of autologous iPSC-derived cells, but an activated immune response after the use of allogeneic iPS derived cells. Contrarily, immune rejection has been observed after autologous transplantation of iPSC-derived cells, suggesting that *in vitro* operations could also impact on the immunogenicity of the iPSC [32]. Moreover, the immune response to undifferentiated iPSC is different from their derivatives, emphasizing the need to perform similar comparative analyses in starting cell populations in order to predict immune tolerance after transplantation. Whereas autologous hiPSC-derived smooth muscle cells were highly immunogenic, autologous hiPSC-derived retinal pigment epithelial (RPE) cells were immune tolerated, suggesting a potential abnormal expression of some immunogenic antigens in smooth muscle cells [33]. These results demonstrated that the nature of the differentiated cells could trigger an immune response suggesting the importance of the differentiation protocol.

As mentioned earlier, because of their genomic instability, generation, amplification and differentiation of iPSC could induce a modified immune response of the iPSC *in vivo*. Concerning reprogramming, the RNA-based methods are relatively efficient and do not integrate in the genome, but they are also known to be highly immunogenic. Concerning cell type, it has been widely shown that iPSCs could be generated from a patient's own cells including fat cells, nerve cells, skin fibroblasts, cuticle cells, fetal foreskin cells, B cells, T cells, peripheral blood mononuclear cells, umbilical cord mesenchymal cells, chorionic mesenchymal cells and amniotic mesenchymal cells. But, some studies showed that the genetic memory of the cellular immunogenicity is conserved after reprogramming and differentiation. So, the selection of donor cell type/origin is crucial. As an example, iPSCs derived from less immunogenic cells, such as umbilical cord mesenchymal cells, generated less immunogenic neural derivatives than those from skin fibroblasts-derived iPSCs [34]. Recently, several researchers showed the less immunogenic potential of some iPSC-derived cells as cartilage and retinal pigment epithelium cells when they are implanted *in vivo*, arguing that some cell types are less immunogenic and should be preferred for clinical settings [35, 36].

Recently, a novel approach of "Universal" iPSC was developed to address the difficulty of immunogenicity of allogeneic iPSCs. Hypoimmunogenicity of iPSC was induced by inactivation of major histocompatibility complex class I and II genes and overexpression of CD47 enabled them to escape to immune rejection in fully HLA-mismatched allogeneic recipients. This strategy allowed the long-term survival of the transplanted cells without the use of immunosuppression. However, overexpression of CD47 is associated with malignant transformation, leading to include some suicide strategies as a safety concern [37]. These immune escape approaches open the door to the clinical use of allogeneic iPSC-derived cell products without immune rejection concerns and complications. However, their complex production process including a combination of several transduction and gene-editing operations could add many safety issues. Even though other vectors and gene-editing techniques [38, 39] could also be used to reduce the risks, the multiple genetic manipulations and additional expansions in culture require a reinforced control of the "Universal" iPSC quality for clinical settings.

4. iPSC for clinical use

4.1 Clinical-grade allogeneic iPSC line bank

The use of human iPSCs in medicinal applications requires the establishment of standardized and validated protocols that will allow large-scale, cost-effective cultivation procedure, while maintaining their quality. Implementation of good manufacturing practice (GMP)-compliant protocols for the generation and maintenance of human iPSC lines is crucial to increase the application safety and to fulfill the regulatory requirements to obtain clinical trials' approval. Many efforts to increase the overall iPSC stability, reproducibility and quality have been performed by (1) selecting the cell type that is easily accessible, less immunogenic, and permissive for reprogramming and presents the ability to be stored for longer periods of time; (2) improving reprogramming efficiency, which should be as high as possible without genomic integration-based delivery method and without using oncogene and (3) improving cultivation methods with xeno- and feeder-free products, with defined and scalable conditions for maintenance and differentiation of human iPSC such as automation, closed cell systems and validated protocols [40]. Moreover, selection of cell source is of importance. Demonstration of comparability, standardization and

validation of such systems is critical for iPSC-derived therapies. To circumvent and manage the safety risk of the iPSC for regenerative medicine, several groups worked at the early stage on the development of standardized clinical grade iPSC banks from allogeneic donors. Indeed, the use of highly defined iPSC as starting cells presents many advantages as overcoming the genetic variations inducing different immunogenicity, genetic instability, tumorigenicity, and differentiation outcomes. Moreover, generation of iPSC from each patient is costly and time-consuming. In this regard, several groups in the world have developed banking of allogeneic iPSC lines for clinical use with validated and standardized protocols. The possibility of creating off-the-shelf iPSC-based therapies has attracted not only academics but also industrial groups as Lonzo and Cellular Dynamics International, a Fujifilm company.

iPSC banks can provide a cost-effective mass-production strategy. Several groups have developed iPSC banks from selected HLA donors trying to cover the majority of the population [41, 42]. The Center for iPSC Research and Application (CiRA), in Kyoto University, started the iPS Cell Stock for Regenerative Medicine in 2013. Initially, based on the limited diversity of the Japanese population, CiRA wanted to generate clinical-grade iPSCs from samples of peripheral blood and umbilical cord blood from healthy selected donors that would cover 90% of Japanese population with only 50 iPSC lines [43]. This strategy is valuable for countries such as Japan, but could be difficult to expand to the worldwide population. It has been evaluated that a multiethnic iPSC bank of the 100 most common HLA types in each population would cover only 78% of European individuals, 63% of Asians, 52% of Hispanics and 45% of African Americans [44]. This probabilistic model highlights the necessity of a large-scale international collaboration for the constitution of haplobank of iPSC lines. Using HLA-homozygous donors limits the numbers of iPSC lines needed to cover a given population, but identification of the potential donors would need large screenings or the use of established data from cord blood banks. The potential development of "universal" iPSCs made of genetically modified cells offering an off-the-shelf product that is readily available could be an alternative to the iPSC bank using materials from HLA-homozygous donors. The "universal" iPSC could solve the problem of immune rejection profile of iPSC-derived cells by artificially expressing, for example, HLA molecule as HLA-E allowing iPSC-derived cells to escape T cell-mediated rejection and to be resistant to NK-cell lysis [37, 45].

Nevertheless, stochastic events potentially occurring during reprogramming, colony expansion, iPSC selection, differentiation, iPSC-derived cell expansion and purification, storage and transport could complicate efforts toward a standardized product. Consequently, it has to be taken into consideration that variation may exist within any iPSC bank, between iPSC and final product composed of iPSC-derived cells in the clinic. Such variability requires continual extensive genotypic, phenotypic and functional assessment and highlights the need of a global quality control confirming the iPSC and the iPSC-derived cells' quality whatever the manufacturer, the reprogramming method or the cell donors.

4.2 Quality control of clinical-based iPSC

Given the high variability across iPSC lines and their differentiated derivatives in terms of their epigenetic status, tumorigenic and immunogenic potential, differentiation capacity, batch variability and existence of heterogeneous populations and/or non-relevant cells such as contaminating cell, the clinical outcome of the cell replacement therapy, in terms of efficacy and safety with these iPSC-based products, highly relies on the acceptable quality and safety standards of these products. Because of dissimilarities between institutions on these criteria, agreement on the critical quality attributes (CQAs) of such lines and the assays that should be used

is required. The CQAs correspond to the chemical, physical and biological properties of the product. As well as the type of assay, they have to be defined within an appropriate limit, range or distribution to ensure quality and safety of the product. For cell therapy product and for clinical-grade iPSC, the CQAs include identity, microbiological sterility, genetic fidelity and stability, viability, characterization and potency. In the last few years, there was a common effort made on the banking and the quality control of the iPSC lines. After a series of workshop, adaptation to iPSC of the established recommendations and guidance realized by the International Stem Cell Banking Initiative (ISCBI) for human embryonic stem cell banking, has generated initial recommendations on the minimum dataset required to consider an iPSC line of clinical grade [46]. During these workshops, the researchers, industrial and regulation agencies pointed out the requirement of standardization and validation of process and quality and safety controls. For each criterion, one or several tests are required with regard to the recommended analytical methods. Global consensus recommends the performance of assays by accredited and licensed laboratories. When it is not available, in-house tests should be undertaken after validation and qualification, and comparability with other laboratories should be performed if possible.

The first mandatory test is to validate the identity of the iPSC line with the short tandem repeat (STR) analysis to genotype the original cells, the iPSC seeds and the master cell bank to ascertain the absence of switch or cross contamination of several iPSC lines during generation or maintenance process. Due to the nature of the stem cell-based products, they cannot be sterilized. The assessment of the microbiological sterility is of the highest importance and should be performed not only on the final product. This should include the mycoplasma, bacteriology and viral testing supplemented by endotoxins detection assay and should have a negative result. The genetic stability and fidelity of the iPSC lines should be evaluated by residual vector testing and karyotype. To eliminate the risk of potential cell transformation and the risk of malignancy development in patients, residual vector testing has to be ≤ 1 plasmid copy per 100 cells in seed and master cell banks and the karyotype should be normal on more than 20 metaphases. So far, techniques with high precision such as single nucleotide polymorphism (SNP) and whole genome analysis or other genetic markers are not required but could be performed for information. To give an appropriate dosage of cells, viability should be >60%. Calculation of doubling time and detection of cell debris are not required but could provide useful information. To manage the risk associated with the presence of non-desired or spontaneously differentiated cells, iPSCs have to be characterized by the expression of a minimum of two markers from the standard human pluripotent stem cells panel (positive for Oct4, TRA-1-60, TRA-1-81, SSEA-3, SSEA-4, Sox2, Nanog). A combination of one intracellular and one extracellular marker should be used and should be >70%. Finally, for the potency assay, reflecting the biological activity of the cells, embryoid body formation or directed differentiation of monolayer cultures to produce cell types representative of all three embryonic germ layers is mandatory. The teratoma formation in severe combined immune-deficient (SCiD) mouse injection assay is not mandatory for the iPSC due to a reproducibility problem, high cost and non-ethical procedure. Molecular pluripotency assays such as mRNA array- and RNA-Seq-based gene expression assays could be kept for information if they are performed molecular pluripotency assays such as mRNA array- and RNA-Seq-based gene expression assays could be for information but are not required. For the iPS-derived differentiated therapeutic products, the minimal criteria are mostly identical except for the phenotypical characterization, which should validate the absence of pluripotent stem cell markers, the expression of differentiation markers unique to the therapeutic product and assess 100% purity of the therapeutic cellular product without any contaminating other lineage cell types.

This consensus on CQA and minimum testing requirements for clinical-grade iPSC lines will evolve with the advances in scientific understanding and development in technology and best practices. The Global Alliance for iPSC Therapies (GAiT), which facilitates the development of general clinical-grade iPSC standards by community engagement and consensus building to support the global application of iPSC-derived cellular therapeutics, is in charge of the future evolution of the consensus on quality and safety standards required for a clinical-grade iPSC. Moreover, GAiT presents objectives to achieve consensus on donor selection and screening criteria and consent standards, which with future commercialization and global distribution also require ethical review.

5. Conclusion

It is quite remarkable that in just over 10 years, research using iPSC has led to several clinical studies, with many more applications expected to follow. In few years, the iPSC-based therapies induced a switch to a mass production of clinical-grade iPSC for the benefit of a large population at affordable costs, with the generation of clinical-grade iPSC banks, and with a stronger involvement of biopharmaceutical companies. This shift led to many efforts for the standardization of generation, maintenance and differentiation procedures, and for the establishment of quality and safety standards for the clinical-grade iPSC and their derivatives prior to transplantation to patients.

There are still a number of challenges that must be overcome for iPSCs to reach their full potential. The improvement of manufacturing procedures for a large-scale production would provide higher quality cells for clinical iPSC-based therapies. Quality and safety controls are also challenging. Predicting cancer risk based on sequence information is a formidable task, and failure to detect oncogenic mutations is not necessarily a warrantor of the non-tumorigenicity of iPSC-based products, suggesting that recommendations should still evolve with scientific advances.

Due to their large potential in regenerative medicine, such as the generation of complex 3D structures, tissues or organs, more challenges in differentiation protocols in 3D structures have to be overcome for the up-coming year, without compromising quality and safety of iPSCs.

Conflict of interest

The authors declare no conflict of interest.

Author details

Valérie Vanneaux
Department of Biotherapies, Saint-Louis Hospital, Paris, France

*Address all correspondence to: valerie.vanneaux@aphp.fr

References

[1] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;**126**:663-676. DOI: 10.1016/j.cell.2006.07.024

[2] Eguizabal C, Aran B, Chuva de Sousa Lopes SM, Geens M, Heindryckx B, Panula S, et al. Two decades of embryonic stem cells: A historical overview. Human Reproduction Open. 2019;**2019**(1):1-17. DOI: 10.1093/hropen/hoy024. eCollection 2019

[3] Mandai M, Watanabe A, Kurimoto Y, Hirami Y, Morinaga C, Daimon T, et al. Autologous induced stem-cell-derived retinal cells for macular degeneration. The New England Journal of Medicine. 2017;**376**:1038-1046. DOI: 10.1056/NEJMc1706274

[4] Cynata Therapeutics [Internet]. 2019. Available from: https://www.cynata.com/graftversushostdiseaseaftversushostdisease [Accessed: 30 June 2019]

[5] Fate Therapeutics [Internet]. 2019. Available from: https://fatetherapeutics.com/pipeline/immuno-oncology-candidates/ft500 [Accessed: 30 June 2019]

[6] Rossignol J, Crane AT, Fink KD, Dunbar GL. Will undifferentiated induced pluripotent stem cells ever have clinical utility? Journal of Stem Cell Research and Therapy. 2014;**4**:189

[7] Galat V, Galat Y, Perepitchka M, Jennings LJ, Iannaccone PM, Hendrix MJ. Transgene reactivation in induced pluripotent stem cell derivatives and reversion to pluripotency of induced pluripotent stem cell-derived mesenchymal stem cells. Stem Cells and Development. 2016;**25**(14):1060-1072. DOI: 10.1089/scd.2015.0366

[8] Nori S, Okada Y, Nishimura S, Sasaki T, Itakura G, Kobayashi Y, et al. Long-term safety issues of iPSC-based cell therapy in a spinal cord injury model: Oncogenic transformation with epithelial-mesenchymal transition. Stem Cell Reports. 2015;**4**:360-373. DOI: 10.1016/j.stemcr.2015.01.006

[9] Griscelli F, Desterke C, Feraud O, Divers D, Oudrhiri N, Tosca L, et al. Genomic landscape analyses of reprogrammed cells using integrative and non-integrative methods reveal variable cancer-associated alterations. Oncotarget. 2019;**10**(28):2693-2708. DOI: 10.18632/oncotarget.26857

[10] Feng C, Jia YD, Zhao XY. Pluripotency of induced pluripotent stem cells. Genomics, Proteomics & Bioinformatics. 2013;**11**(5):299-303. DOI: 10.1016/j.gpb.2013.08.003

[11] Melton C, Judson R, Blelloch R. Opposing microRNA families regulate self-renewal in mouse embryonic stem cells. Nature. 2010;**463**(7281):621-626. DOI: 10.1038/nature08725

[12] Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, et al. Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell. 2009;**4**:381-384. DOI: 10.1016/j.stem.2009.04.005

[13] Liang G, Zhang Y. Genetic and epigenetic variations in iPSCs: Potential causes and implications for application. Cell Stem Cell. 2013;**13**(2):149-159. DOI: 10.1016/j.stem.2013.07.001

[14] Kim K, Doi A, Wen B, Ng K, Zhao R, Cahan P, et al. Epigenetic memory in induced pluripotent stem cells. Nature. 2010;**467**:285-290. DOI: 10.1038/nature09342

[15] Polo JM, Liu S, Figueroa ME, Kulalert W, Eminli S, Tan KY, et al. Cell

type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. Nature Biotechnology. 2010;**28**:848-855. DOI: 10.1038/nbt.1667

[16] Ghosh Z, Wilson KD, Wu Y, Hu S, Quertermous T, Wu JC. Persistent donor cell gene expression among human induced pluripotent stem cells contributes to differences with human embryonic stem cells. PLoS ONE. 2010;**5**:e8975. DOI: 10.1371/journal.pone.0008975

[17] Boland MJ, Nazor KL, Loring JF. Epigenetic regulation of pluripotency and differentiation. Circulation Research. 2014;**115**:311-324. DOI: 10.1161/CIRCRESAHA.115.301517

[18] Utikal J, Maherali N, Kulalert W, Hochedlinger K. Sox2 is dispensable for the reprogramming of melanocytes and melanoma cells into induced pluripotent stem cells. Journal of Cell Science. 2009;**122**:3502-3510. DOI: 10.1242/jcs.054783

[19] Kim JB, Greber B, Arauzo-Bravo MJ, Meyer J, Park KI, Zaehres H, et al. Direct reprogramming of human neural stem cells by OCT4. Nature. 2009;**461**:649-643. DOI: 10.1038/nprot.2009.173

[20] Lo Sardo V, Ferguson W, Erikson GA, Topol EJ, Baldwin KK, Torkamani A. Influence of donor age on induced pluripotent stem cells. Nature Biotechnology. 2017;**35**(1):69-74. DOI: 10.1038/nbt.3749

[21] Musunuru K, Sheikh F, Gupta RM, Houser SR, Maher KO, Milan DJ, et al. Induced pluripotent stem cells for cardiovascular disease modeling and precision medicine: A scientific statement from the American Heart Association. Circulation: Genomic and Precision Medicine. 2018;**11**(1):1-30. DOI: 10.1161/HCG.0000000000000043

[22] Jeong HC, Cho SJ, Lee MO, Cha HJ. Technical approaches to induce selective cell death of pluripotent stem cells. Cellular and Molecular Life Sciences. 2017;**74**(14):2601-2611. DOI: 10.1007/s00018-017-2486-0

[23] Schuldiner M, Itskovitz-Eldor J, Benvenisty N. Selective ablation of human embryonic stem cells expressing a "suicide" gene. Stem Cells. 2003;**21**:257-265. DOI: 10.1634/stemcells.21-3-257

[24] Kotini AG, de Stanchina E, Themeli M, Sadelain M, Papapetrou EP. Escape mutations, ganciclovir resistance, and teratoma formation in human iPSCs expressing an HSVtk suicide gene. Molecular Therapy—Nucleic Acids. 2016;**5**:e284. DOI: 10.1038/mtna.2015.57

[25] Itakura G, Kawabata S, Ando M, Nishiyama Y, Sugai K, Ozaki M, et al. Fail-safe system against potential tumorigenicity after transplantation of iPSC derivatives. Stem Cell Reports. 2017;**8**:673-684. DOI: 10.1016/j.stemcr.2017.02.003

[26] Sadelain M, Papapetrou EP, Bushman FD. Safe harbours for the integration of new DNA in the human genome. Nature Reviews. Cancer. 2012;**12**:51-58. DOI: 10.1038/nrc3179

[27] Kinamura Y, Shofuda T, Higuchi Y, Nagamori I, Oda M, Nakamori M, et al. Human genomic safe harbors and the suicide gene-based safeguard system for iPSC-based cell therapy. Stem Cells Translational Medicine. 2019;**8**(7):627-638. DOI: 10.1002/sctm.18-0039

[28] Ben-David U, Biran A, Scaffidi P, Herold-Mende C, Boehringer M, Meshorer E, et al. Elimination of undifferentiated cancer cells by pluripotent stem cell inhibitors. Journal of Molecular Cell Biology. 2014;**6**:267-269. DOI: 10.1093/jmcb/mju012

[29] Lynch CL, Altun G, Tran HT, Garitaonandia I, Slavin I, Loring JF, et al. Specific lectin biomarkers for isolation of human pluripotent stem cells identified through array-based glycomic analysis. Cell Research. 2011;**21**:1551. DOI: 10.1038/cr.2011.148

[30] Miki K, Endo K, Takahashi S, Funakoshi S, Takei I, Katayama S, et al. Efficient detection and purification of cell populations using synthetic microRNA switches. Cell Stem Cell. 2015;**16**:699-711. DOI: 10.1016/j.stem.2015.04.005

[31] Parr CJC, Katayama S, Miki K, Kuang Y, Yoshida Y, Morizane A, et al. MicroRNA-302 switch to identify and eliminate undifferentiated human pluripotent stem cells. Scientific Reports. 2016;**6**:32532. DOI: 10.1038/srep32532

[32] Zhao T, Zhang ZN, Rong Z, Xu Y. Immunogenicity of induced pluripotent stem cells. Nature. 2011;474(7350):212-215. DOI: 10.1038/nature10135

[33] Zhao T, Zhang ZN, Westenskow PD, Todorova D, Hu Z, Lin T, et al. Humanized mice reveal differential immunogenicity of cells derived from autologous induced pluripotent stem cells. Cell Stem Cell. 2015;**17**(3):353-359. DOI: 10.1016/j.stem.2015.07.021

[34] Liu S, Yuan M, Hou K, Zhang L, Zheng X, Zhao SX, et al. Immune characterization of mesenchymal stem cells in human umbilical cord Wharton's jelly and derived cartilage cells. Cellular Immunology. 2012;**278**(1-2):35-44. DOI: 10.1016/j.cellimm.2012.06.010

[35] Nguyen D, Hägg DA, Forsman A, Ekholm J, Nimkingratana P, Brantsing C, et al. Cartilage tissue engineering by the 3D bioprinting of iPS cells in a nanocellulose/alginate Bioink. Scientific Reports. 2017;7(1):658. DOI: 10.1038/s41598-017-00690-y

[36] Westenskow PD, Bucher F, Bravo S, Kurihara T, Feitelberg D, Paris LP, et al. iPSC-derived retinal pigment epithelium allografts do not elicit detrimental effects in rats: A follow-up study. Stem Cells International. 2016;**2016**:8470263. DOI: 10.1155/2016/8470263

[37] Deuse T, Hu X, Gravina A, Wang D, Tediashvili G, De C, et al. Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. Nature Biotechnology. 2019;**37**:252-258. DOI: 10.1038/s41587-019-0016-3

[38] Roy KR, Smith JD, Vonesch SC, Lin G, Tu CS, Lederer AR, et al. Multiplexed precision genome editing with trackable genomic barcodes in yeast. Nature Biotechnology. 2018;**36**:512-520. DOI: 10.1038/nbt.4137

[39] Strohkendl I, Saifuddin FA, Rybarski JR, Finkelstein IJ, Russell R. Kinetic basis for DNA target specificity of CRISPR-Cas12a. Molecular Cell. 2018;**71**:816-824.e3. DOI: 10.1016/j.molcel.2018.06.043

[40] Dakhore S, Nayer B, Hasegawa K. Human pluripotent stem cell culture: Current status, challenges, and advancement. Stem Cells International. 2018;**2018**:7396905. DOI: 10.1155/2018/7396905

[41] Okita K. iPS cells for transplantation. Current Opinion in Organ Transplantation. 2011;**16**(1):96-100. DOI: 10.1097/MOT.0b013e32834252a2

[42] Taylor CJ, Peacock S, Chaudhry AN, Bradley JA, Bolton EM. Generating an iPSC bank for HLA-matched tissue transplantation based on known donor and recipient HLA types. Cell Stem Cell. 2012;**11**(2):147-152. DOI: 10.1016/j.stem.2012.07.014

[43] Tokunaga K, Nakatsuji N, Nakajima F. HLA-haplotype banking and iPS cells. Nature Biotechnology. 2008;**26**(7):739-740. DOI: 10.1038/nbt0708-739

[44] Gourraud P-A, Gilson L, Girard M, Peschanski M. The role of human leukocyte antigen matching in the development of multiethnic "haplobank" of induced pluripotent stem cell lines. Stem Cells. 2012;**30**(2):180-186. DOI: 10.1002/stem.772

[45] Gornalusse GG, Hirata RK, Funk SE. HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells. Nature Biotechnology. 2017;**35**:765-772. DOI: 10.1038/nbt.3860

[46] Sullivan S, Stacey GN, Akazawa C, Aoyama N, Baptista R, Bedford P, et al. Quality control guidelines for clinical-grade human induced pluripotent stem cell lines. Regenerative Medicine. 2018;**13**:859-866. DOI: 10.2217/rme-2018-0095