Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Induced pluripotent stem cells (iPSCs): Where are we and where are we heading?

Arshad Jamal*

Department of Biology, College of Science, University of Hail. Kingdom of Saudi Arabia

ARTICLE INFO

Review

Article history:
Received: June 04, 2023
Accepted: August 14, 2023
Published: November 15, 2023

Keywords:

Reprogramming, induced pluripotent cells (iPSCs), personalized medicine, Translational research

ABSTRACT

Induced Pluripotent Stem Cells (iPSCs) offer a unique therapeutic tool. The extent of the ongoing clinical studies substantiates the role of these cells in a highly promising area of personalized medicine. The employment of this technology in translational research is on the horizon. However, still, many questions remain unanswered. In this review, we discussed certain challenges and difficulties currently faced during the safe and stable clinical application of iPSCs. Furthermore, we describe the potential therapeutic scenarios employing iPSCs for the betterment of human health and the improvement of the health care sector.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.11.13

Introduction

The over-expression of the transcription factors Oct4, Sox2, Klf4 and c-Myc, for the reprogramming of iPSCs from mouse somatic cells, by Takahashi and Yamanaka coined the term "Yamanaka factors" (1). Following on, the aforementioned factors, and/or combinations of other similar factors were extensively used to reprogram numerous human and mouse body cells into iPSCs (2-5). iPSCs depict vast dedifferentiation potency and attain features like embryonic stem cells (ESCs). In fact, ESCs and iPSCs are structurally identical, and under *in-vitro* conditions, these cells are capable to give rise to cells of the three germ layers (ectoderm, endoderm and mesoderm) and develop almost all cells of adult organisms. Moreover, iPSCs are capable of producing living and potent animals (6-9). This unprecedented reprogramming approach developed great interest among the scientific, academic and medical community since iPSCs offer a promising source of pluripotent cells. Further, iPSCs are generated from somatic cells obtained in a most unharmful method, maintaining the discrete genetic settings, hence are autologous in nature and exhibiting minimal immune rejections risks (10). Unlike, blastocyst-derived human ESCs, the iPSCs are exempted from ethical concerns. The original reprogramming procedures are being streamlined to overcome several critical experimental issues, like the use of integrative vectors for the administration of the transcription factors. Besides Yamanaka factors, other reprogramming factors, microRNAs and/or small molecules and epigenetic regulators have appeared to cooperate or substitute these factors for reprogramming iPSCs. Reprogrammed cells hold great potential for high throughput screens for drug discovery, toxicity tests and *in-vitro* models for disease. Above all, reprogramming opens up the option of remedy by using the patients own cells (11).

Herein, we review the eminent challenges and difficulties currently faced during the safe and stable clinical application of iPSCs. Furthermore, we suggest potential applications and therapeutic scenarios employing iPSCs for the improvement of human well-being.

Challenges

The inception of iPSCs technology is conferred a landmark event in the remedial and therapeutic settings (12). The advantages associated with the application of iPSCs are notable and widely accepted, primarily due to their pluripotent capacity and their potential to create a patient-derived disease model (13). However, numerous distinguished barriers and drawbacks are hampering the use of promising cells in clinical research.

First, the generation and expansion and of iPSCs under laboratory settings, including all the necessary safety and pluripotency assessments, cost about 10-20,000 US dollars and also involves lengthy procedures (14). Likewise, the cost of clinical studies can reach up to 1 million dollars. Obviously, there is a serious need to find out a cost-effective solution to this hindrance, which would allow the iPSCs translation to the clinics.

One of the major concerns, inhibiting the application of iPSCs in clinics in related to genomic stability. It is a well-established fact that the reprogramming process leads to chromosomal aberrations and other types of mutations at significant frequencies (15). In this regard, the first clinical trial involving iPSCs was abandoned upon identification of DNA aberrations in patients' iPSCs, which were not present in the primary fibroblasts (16). The possible

* Corresponding author. Email: imarshadjamal@gmail.com

causes of the onset of DNA mutations in the iPSCs could be the reprogramming process, during subsequent passaging of these cells, or the preexisting mutations in the primary cells (17). Alarmingly, many of the mutations and aberrations could lead to tumorigenic potential. The safety concerns related to the use of iPSCs in clinics are of foremost importance; hence, requires strict programming and manufacturing conditions ensure the therapeutic potential of these cells.

iPSCs-derived immunogenicity in the host is yet another main obstacle concerning their application in translational research. The landmark announcement in 2011, that aberrant gene expression in some cells differentiated from iPSCs can evoke T-cell-dependent immune response in syngeneic mice brought about general doubt regarding the potential application of these cells in clinical settings (18). Following on, Zhao and coworkers studied the immunogenic response of several distinct iPSCs-derived cells in humanized mice and reported that the different derivatives triggered different immune responses (19). Therefore, the immune responses provoked by the iPSCs and their derivatives vary with the use of different cell lines. These immune responses could be the result of epigenetic alterations exhibited by the cell lines or genetic anomalies enticing the aberrant immunogenic product (20). A robust understanding of the immune responses generated by these cells and their derivatives could be a big leap forward toward greater safety and with minimal need for immunosuppressive treatments in the case of iPSCs transplants (21).

Prospects

Currently, time, cost (autologous transplants) and immunogenicity (allogeneic transplants) related to iPSCs technology remain the main obstacles keeping these cells away from clinics (22). Regarding the immunogenicity issue, the possible substitution of allogeneic transplants could sort out the problem (42,43) (Figure 1). However, concerns linked to immunogenicity remain prime downside. In this regard, many scientists are advocating the HLA-characterized iPSCs biobank set up (Table 1). Consequently, this would not only offer cost-effective technology itself but also reduce the immune rejection risk and enhance the possibility of bringing the iPSCs closer to clinics (23). Evidently, the HLA system is very polymorphic, therefore taking into consideration the loci HLA-A, HLA-B and HLA-DR are sufficient to diminish the rejection risk and the doses of immunosuppression needed. Assumingly, HLA- homozygous and blood group O donors dependent iPSCs biobank would streamline the donor-recipient matching (24). Hypothetically, 140 and 150 (selected donors) HLA-homozygous iPSCs lines are enough to match 90% and 93% of Japan and United Kingdom recipients, respectively (25, 26). It is vital to note that HLA- characterized iPSCs biobank intended for a remedial application requires more rigorous safety checks and efficient manufacturing protocols to guarantee the reliability of the likely therapeutics generated (27).

Further, critical aspects required to make these cells a likely translational product include the normalization of reprogramming protocols and the application of good manufacturing practices (28). The current iPSCs production restricts their application to laboratory settings, thus limiting the broader applicability. Therefore, it is imperative to regulate the protocols for a large-scale expansion of iPSCs, since cellular therapies would require a substantial amount of them to be attainable (29). Moreover, the tracking of the iPSCs-bound toxicity, tumorigenicity, also the safety of cells, is crucial for the therapeutics (30).

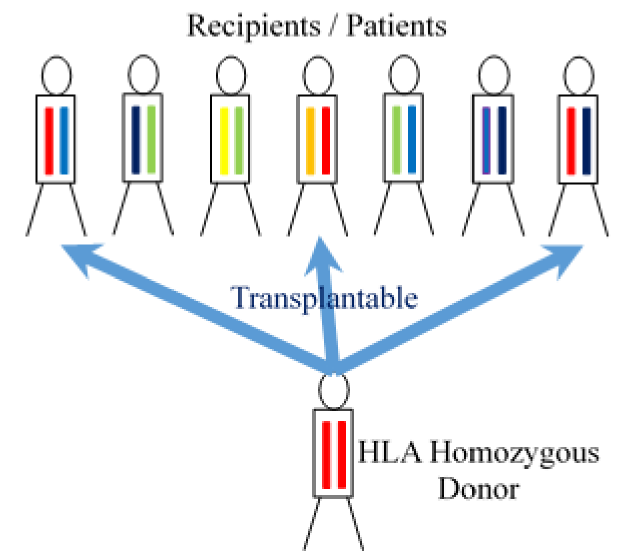

Figure 1. Schematic presentation of the cells from HLA-homozygous donors (red colored) can be used for cell therapies in recipients / patients who have at least one of the same HLA. In the illustration, the donor cells can be used in 3 of the 7 recipients / patients.

The current differentiation strategies do not lead to the iPSCs-derived, specific lineage of interest, and exhibit unwanted phenotypic heterogeneity and inadequate maturity with reduced efficiency rates (31, 32). The introduction of certain vital transcription factors via viral vectors abets non-specific incorporation within the genome, ergo compromising the safety of the iPSCs and their by-products (33). One more approach, focused on imitating the embryonic development in the presence or absence of several decisive compounds in the cell culture medium, aiming to check the regulation of specific relevant cellular pathways and setting apart the pluripotency of iPSCs (34). However, the shortcomings related to the existing approaches thwart to attain absolute maturity, particularly, in cardiomyocytes, and diseases where the affected phenotype is only expressed at the terminal stage (35). To sort these limitations new approaches are being explored. For instance, some groups are working on different small molecule cocktails to achieve a more mature differentiation state, the exploitation of the cellular niche, and the adaptation of 3D techniques (36, 37).

Conclusion

It is quite apparent that the therapeutic potential of iPSCs goes far beyond the basic research, their leap from bench to bedside is imminent. Cellular therapies illustrate the success of this groundbreaking research with the progress of numerous ongoing clinical trials (Table 2). For instance, in disease models such as age-related muscular atrophy and Parkinson's diseases, iPSCs demonstrated promising therapeutic ability. Over the years, iPSCs have evolved as a robust tool in the evolution of modern therapeutics (Figure 2), and can further be seen as a transitio-

Table 1. Brief information of iPSC repositories (38-41).

Name	Allies	Geographic Region	Products	Link
California Institute for Regenerative Medicine (CIRM)	Fujifilm Cellular Dynamics International (FCDI)	United States	40 diseases including 239 neurodevelopmental disorders, 131 liver disease, 442 heart disease, 65 neurodegenerative disease, 175 eyes disease, 191 lung disease, and 302 controls	https://www.cirm.ca.gov/researchers/ipsc-repository/about (accessed on 30 October 2022) https://www.fujifilmcdi.com/cirm-ipsc-products/ (accessed on 30 October 2022)
Center for iPS Cell Research and Application (CiRA)	ATCC, RIKEN, RUCDR	Japan	39 lines including 3 diseases: two neurodevelopmental diseases and a bone disorder	https://www.cira.kyoto-u.ac.jp/e /research/material_1.html (accessed on 30 October 2022)
European Bank for induced pluripotent Stem Cells (EBiSC)	HipSci	Europe	36 diseases, 895 iPSC lines including 359 normal control lines	https://ebisc.org/search (accessed on 6 June 2023)
Human Induced pluripotent Stem Cell Initiative (HipSci)	ECACC, EBiSC	United Kingdom	15 disease statuses, 339 disease lines, and 496 normal lines	https: //www.hipsci.org/lines/#/lines (accessed on 30 October 2022)
Institute of Physical and Chemical Research (RIKEN)		Japan	14 disease categories including 231 diseases, 753 patients, and 3110 iPSC lines; 718 health control lines	https://cell.brc.riken.jp/en/hps / patient_specific_ips (accessed on 30 October 2022)
Human Disease iPSC Consortium Resource Center (Taiwan Human Disease iPSC Consortium)	BCRC	Taiwan	10 normal lines, 74 disease lines of 23 diseases	http://ipsc.ibms.sinica.edu.tw/sc hedule.html (accessed on 30 October 2022)
WiCell Research Institute (WiCell)	N/A	United States	1377 iPSC lines including 308 disease lines of 40 disease types	https://www.wicell.org/home/stem-cells/catalog-of-stem-cell-line s/advanced-search.cmsx (accessed on 30 October 2022)

Table 2. Current iPSC-based clinical trials (as of 2021) (43).

Location	Company	Disease	Cell Type	Clinical Phase	Clinical Trial Identifier
Australia, United Kingdom	Cynata Therapeutics Limited	Graft vs. host disease	iPSC-derived mesenchymal stem cell	Phase 1	ClinicalTrials.gov: NCT02923375
United States	Fate Therapeutics	Cancer	iPSC-derived Natural Killer (NK) cell	Phase 1	ClinicalTrials.gov: NCT03841110
China	Beijing University of Chinese Medicine	Chronic heart failure	iPSC-derived cardiomyocytes	Phase 2/3	ClinicalTrials.gov: NCT03759405
	Help Therapeutics	Heart failure	iPSC-derived cardiomyocytes	Phase 1/2	ClinicalTrials.gov: NCT03763136
Japan	Kyoto University Hospital	Parkinson disease	iPSC-derived dopaminergic progenitors	Phase 1/2	ICTRP: JPRNUMIN000033564
	Osaka University, Cuorips Inc.	Myocardial ischemia	iPSC-derived cardiomyocytes sheet	Phase 1	ClinicalTrials.gov: NCT04696328

nal factor to develop other remedial products of interest as platelets. Nonetheless, the application of iPSCs in clinics are still an uphill battle. Addressing and understanding their prominent downsides such as immunogenicity, genetic instability, toxicity, and the increased cost of their production, together with the need to normalize the technical protocols and expedite the scaling up of this promising technology would validate its application in clinics. The key point is that advanced and novel techniques are being investigated to understand and interpret these problems,

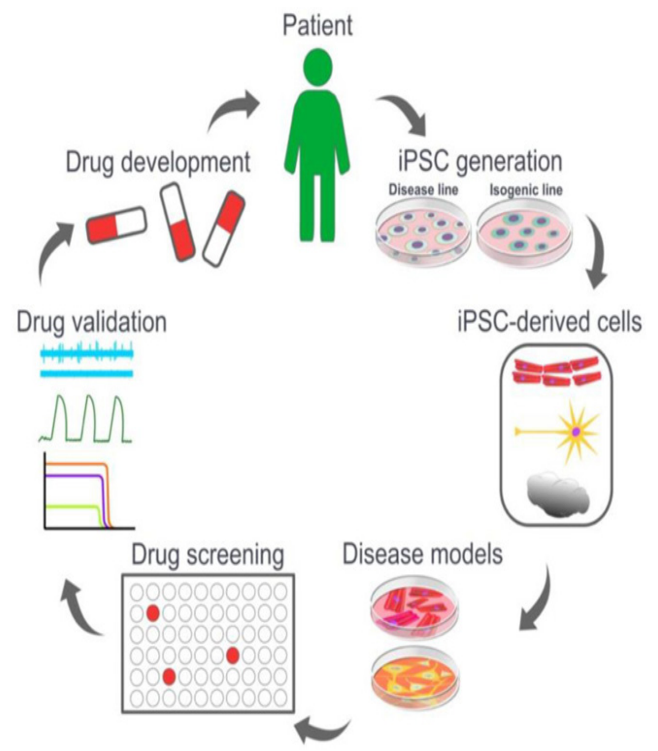

Figure 2. Disease modeling and drug screening using patient-specific induced pluripotent stem cells (44).

which will open up the way for the categorical application of this technology on a broader scale.

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 [PMID: 16904174 DOI:10.1016/j.cell.2006.07.024]
2. Yu J, Thomson JA. Pluripotent stem cell lines. Genes Dev 2008; 22: 1987-1997 [PMID: 18676805 DOI: 10.1101/gad.1689808]
3. Jaenisch R, Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. Cell 2008; 132: 567-582 [PMID: 18295576 DOI: 10.1016/j.cell.2008.01.015]
4. Lengerke C, Daley GQ. Disease models from pluripotent stem cells. Ann N Y Acad Sci 2009; 1176: 191-196 [PMID: 19796247 DOI: 10.1111/j.1749-6632.2009.04962.x]
5. Ho R, Chronis C, Plath K. Mechanistic insights into reprogramming to induced pluripotency. J Cell Physiol 2011; 226: 868-878 [PMID: 20945378 DOI: 10.1002/jcp.22450]
6. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature 2007; 448: 313-317 [PMID: 17554338 DOI: 10.1038/nature05934]
7. Maherali N, Sridharan R, Xie W, Utikal J, Eminli S, Arnold K, Stadtfeld M, Yachechko R, Tchieu J, Jaenisch R, Plath K, Hochedlinger K. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. Cell Stem Cell 2007; 1: 55-70 [PMID: 18371336 DOI: 10.1016/j.stem.2007.05.014]
8. Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, Bernstein BE, Jaenisch R. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature 2007; 448: 318-324 [PMID: 17554336 DOI: 10.1038/nature05944]
9. Blelloch R, Venere M, Yen J, Ramalho-Santos M. Generation of induced pluripotent stem cells in the absence of drug selection. Cell Stem Cell 2007; 1: 245-247 [PMID: 18371358 DOI: 10.1016/j.stem.2007.08.008]
10. Bellin M, Marchetto MC, Gage FH, Mummery CL. Induced pluripotent stem cells: the new patient? Nat Rev Mol Cell Biol 2012; 13: 713-726 [PMID: 23034453 DOI: 10.1038/nrm3448]
11. Kimbrel EA, Lanza R. Current status of pluripotent stem cells: moving the first therapies to the clinic. Nat Rev Drug Discov 2015; 14: 681-692 [PMID: 26391880 DOI: 10.1038/nrd4738]
12. Omole A.E., Fakoya A.O.J. Ten years of progress and promise of induced pluripotent stem cells: Historical origins, characteristics, mechanisms, limitations, and potential applications. PeerJ. 2018;6:e4370. doi: 10.7717/peerj.4370.
13. Karagiannis P., Nakauchi A., Yamanaka S. Bringing induced pluripotent stem cell technology to the bedside. JMA J. 2018;1:6–14.
14. Bravery C.A. Do human leukocyte antigen-typed cellular therapeutics based on induced pluripotent stem cells make commercial sense? Stem Cells Dev. 2015;24:1–10. doi: 10.1089/scd.2014.0136.
15. Turinetto V., Orlando L., Giachino C. Induced pluripotent stem cells: Advances in the quest for genetic stability during reprogramming process. Int. J. Mol. Sci. 2017;18:1952. doi: 10.3390/ijms18091952.
16. Attwood S., Edel M. iPS-cell technology and the problem of genetic instability—can it ever be safe for clinical use? J. Clin. Med. 2019;8:288. doi: 10.3390/jcm8030288.
17. Yoshihara M., Hayashizaki Y., Murakawa Y. Genomic instability of iPSCs: Challenges towards their clinical applications. Stem Cell Rev. Rep. 2017;13:7–16. doi: 10.1007/s12015-016-9680-6.
18. Zhao T., Zhang Z.-N., Rong Z., Xu Y. Immunogenicity of induced pluripotent stem cells. Nature. 2011;474:212–215. doi: 10.1038/nature10135.
19. Zhao T., Zhang Z., Westenskow P.D., Todorova D., Hu Z., Lin T., Rong Z., Kim J., He J., Wang M., et al. Humanized mice reveal differential immunogenicity of cells derived from autologous induced pluripotent stem cells. Cell Stem Cell. 2015;17:353–359. doi: 10.1016/j.stem.2015.07.021.
20. Liu X., Li W., Fu X., Xu Y. The immunogenicity and immune tolerance of pluripotent stem cell derivatives. Front. Immunol. 2017;8:645. doi: 10.3389/fimmu.2017.00645.
21. Chhabra A. Inherent immunogenicity or lack thereof of pluripotent stem cells: implications for cell replacement therapy. Front. Immunol. 2017;8:993. doi: 10.3389/fimmu.2017.00993.
22. Doss M.X., Sachinidis A. Current challenges of iPSC-based disease modeling and therapeutic implications. Cells. 2019;8:403. doi: 10.3390/cells8050403.
23. de Rham C., Villard J. Potential and limitation of HLA-based banking of human pluripotent stem cells for cell therapy. J. Immunol. Res. 2014;2014:518135. doi: 10.1155/2014/518135.
24. Nakajima F., Tokunaga K., Nakatsuji N. Human leukocyte antigen matching estimations in a hypothetical bank of human embryonic stem cell lines in the japanese population for use in cell transplantation therapy. Stem Cells. 2007;25:983–985. doi: 10.1634/stemcells.2006-0566.
25. Taylor C.J., Peacock S., Chaudhry A.N., Bradley J.A., Bolton E.M. Generating an iPSC bank for HLA-matched tissue transplantation based on known donor and recipient HLA types. Cell Stem Cell. 2012;11:147–152. doi: 10.1016/j.stem.2012.07.014.
26. Okita K., Matsumura Y., Sato Y., Okada A., Morizane A., Okamoto S., Hong H., Nakagawa M., Tanabe K., Tezuka K., et al. A more efficient method to generate integration-free human iPS cells. Nat. Methods. 2011;8:409–412. doi: 10.1038/nmeth.1591.
27. Huang C.Y., Liu C.L., Ting C.Y., Chiu Y.T., Cheng Y.C., Nicholson M.W., Hsieh P.C.H. Human iPSC banking: Barriers and opportunities. J. Biomed. Sci. 2019;26:87. doi: 10.1186/s12929-019-0578-x.

28. Blackford, S.J.I.; Ng, S.S.; Segal, J.M.; King, A.J.F.; Austin, A.L.; Kent, D.; Moore, J.; Sheldon, M.; Ilic, D.; Dhawan, A.; et al. Validation of current good manufacturing practice compliant human pluripotent stem cell-derived hepatocytes for cell-based therapy. Stem Cells Transl. Med. 2019, 8, 124–137.
29. Lavon, N.; Zimerman, M.; Itskovitz-Eldor, J. Scalable expansion of pluripotent stem cells. In Advances in Biochemical Engineering/Biotechnology; Spinger: Berlin, Germany, 2018; Volume 163, pp. 23–37.
30. Andrews, P.W.; Ben-David, U.; Benvenisty, N.; Coffey, P.; Eggan, K.; Knowles, B.B.; Nagy, A.; Pera, M.; Reubinoff, B.; Rugg-Gunn, P.J.; et al. Assessing the safety of human pluripotent stem cells and their derivatives for clinical applications. Stem Cell Rep. 2017, 9, 1–4.
31. Li, K.; Kong, Y.; Zhang, M.; Xie, F.; Liu, P.; Xu, S. Differentiation of pluripotent stem cells for regenerative medicine. Biochem. Biophys. Res. Commun. 2016, 471, 1–4.
32. Piga, D.; Salani, S.; Magri, F.; Brusa, R.; Mauri, E.; Comi, G.P.; Bresolin, N.; Corti, S. Human induced pluripotent stem cell models for the study and treatment of Duchenne and Becker muscular dystrophies. Ther. Adv. Neurol. Disord. 2019, 12, 1–28.
33. Maffioletti, S.M.; Gerli, M.F.M.; Ragazzi, M.; Dastidar, S.; Benedetti, S.; Loperfido, M.; Vandendriessche, T.; Chuah, M.K.; Tedesco, F.S. Efficient derivation and inducible differentiation of expandable skeletal myogenic cells from human ES and patient-specific iPS cells. Nat. Protoc. 2015, 10, 941–958.
34. Teotia, P.; Chopra, D.A.; Dravid, S.M.; Van Hook, M.J.; Qiu, F.; Morrison, J.; Rizzino, A.; Ahmad, I. Generation of functional human retinal ganglion cells with target specificity from pluripotent stem cells by chemically defined recapitulation of developmental mechanism. Stem Cells 2017, 35, 572–585.
35. Penney, J.; Ralvenius, W.T.; Tsai, L.H. Modeling Alzheimer's disease with iPSC-derived brain cells. Mol. Psychiatry 2019, 1–20.
36. Machiraju, P.; Greenway, S.C. Current methods for the maturation of induced pluripotent stem cellderived cardiomyocytes. World J. Stem Cells 2019, 11, 33–43.
37. Madrid M, Sumen C, Aivio S, Saklayen N. Autologous Induced Pluripotent Stem Cell-Based Cell Therapies: Promise, Progress, and Challenges. Curr Protoc. 2021 Mar;1(3):e88. doi: 10.1002/cpz1.88. PMID: 33725407.
38. Lee, J.-J.; Lin, C.-Y.; Chen, H.-C.; Hsieh, P.C.H.; Chiu, Y.-W.; Chang, J.-M. Opportunities and Challenges of Human IPSC Technology in Kidney Disease Research. Biomedicines 2022, 10, 3232. https://doi.org/10.3390/ biomedicines10123232
39. Azeez S, Jafar S, Aziziaram Z, Fang L, Mawlood A, Ercisli M. Insulin-producing cells from bone marrow stem cells versus injectable insulin for the treatment of rats with type I diabetes. Cell Mol Biomed Rep 2021; 1(1): 42-51. doi: 10.55705/cmbr.2021.138888.1006.
40. Fazeli F, Ahanjan M. The capacity of stem cells in treatment of diabetes. Cell Mol Biomed Rep 2022; 2(4): 230-244. doi: 10.55705/cmbr.2022.357066.1060.
41. Kim, J.Y., Nam, Y., Rim, Y.A. et al. Review of the Current Trends in Clinical Trials Involving Induced Pluripotent Stem Cells. Stem Cell Rev and Rep 18, 142–154 (2022). https://doi.org/10.1007/s12015-021-10262-3
42. Yamanaka S. Pluripotent stem cell-based cell therapy-promise and challenges. Cell Stem Cell. 2020;27:523–31. https://doi.org/10.1016/j.stem.2020.09.014.
43. Kusumoto D, Yuasa S, Fukuda K. Induced Pluripotent Stem Cell-Based Drug Screening by Use of Artificial Intelligence. *Pharmaceuticals*. 2022; 15(5):562. https://doi.org/10.3390/ph15050562
44. Xu Z, Yang J, Xin X, Liu C, Li L, Mei X and Li M (2023), Merits and challenges of iPSC-derived organoids for clinical applications. Front. Cell Dev. Biol. 11:1188905. doi: 10.3389/fcell.2023.1188905