EDITORIAL
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Advanced cellular technologies: biotechnology and regulatory challenges

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The average life expectancy of humans has been steadily increasing. However, it is undeniable that, in addition to living longer, one wants to live with quality. In this scenario, the prospect of using cells as tools has become an important and profitable focus of worldwide research.

In a recent study, Silva Junior and Ramalho[1] emphasized that in the next two decades, due to an aging population, diseases of the circulatory system will continue to account for most of the deaths, albeit with a tendency to decrease, followed by cancer and degenerative diseases such as Alzheimer's and dementia. These diseases have a high impact on the cost of both public and private healthcare systems. The authors also consider the trend of increasing causes of external death, concentrated in aggression and car accidents, with great impact on the population.

On the other hand, results from completed and ongoing clinical studies indicate an enormous therapeutic potential of stem cell therapy (SC) in the treatment of degenerative, autoimmune and genetic disorders[2].

The ethical and safety issues surrounding the use of human embryonic stem cells (hESC), induced stem cells (iPSC) and mesenchymal stem cell (MSC) based therapy have been widely discussed. Simple evidence of interest in these subjects can be found in the PubMed database (www.ncbi.nlm.nih.gov), in which, between the years 2008 and 2017, one can find a significant number of articles for the following keywords: *"human embryonic stem cell"* (hESC), *"human iPSC"* (hiPSC) and *"human mesenchymal stem cells"* (hMSC). A total of 13,515, 10,288 and 29,464 articles were found, respectively, in the search conducted on February 6, 2018. Interest in these fields of science increased precipitously for hiPSC and hMSC, but remained virtually steady in this period for hESC. Research studies on hMSC were the most numerous (Figure).

hESC are cells with normal karyotype, capable of dividing indefinitely and turning into any cell type both *in vitro* and *in vivo*[3]. They originate from the pluripotent internal cell mass of the pre-implantation embryos[4] and are identified by specific markers. However, it is questionable whether it is morally acceptable to seek new therapies to cure illnesses at the expense of destroying an early human embryo. This ethical dilemma is treated differently in many countries. In Italy, for example, any research with hESC is prohibited, while in the UK research is permitted, but its therapeutic or reproductive use is prohibited. In Brazil, Law 11.105, of March 24, 2005[5] permits the research and therapeutic use of hSEC and assigns to the National Health Surveillance Agency (Anvisa) its regulations (Decree 5,591, of November 22, 2005[6]). The proposal of Brazilian regulations and the legal possibility of registration and commercialization of advanced therapy products in Brazil will be further detailed in the articles by Garcia et al. and Parca et al., published in this issue, which also addresses the main risks involved in the production and delivery of advanced therapy products in the article by Silva Junior et al.

The plasticity of hESC stimulates countless clinical applications, but the control of their *in vivo* proliferation did not prove simple, leading to the development of teratomas,[7,8]. To overcome this important limitation, the complete differentiation in the desired cell type prior to injection seems promising and with potential for clinical application[9]. In the *ClinicalTrials.org* database, in a survey conducted on February 6, 2018, 34 studies were identified using hMSC, seven of which were completed. In Brazil, there is a study at the Federal University of São Paulo in the recruitment phase (Table).

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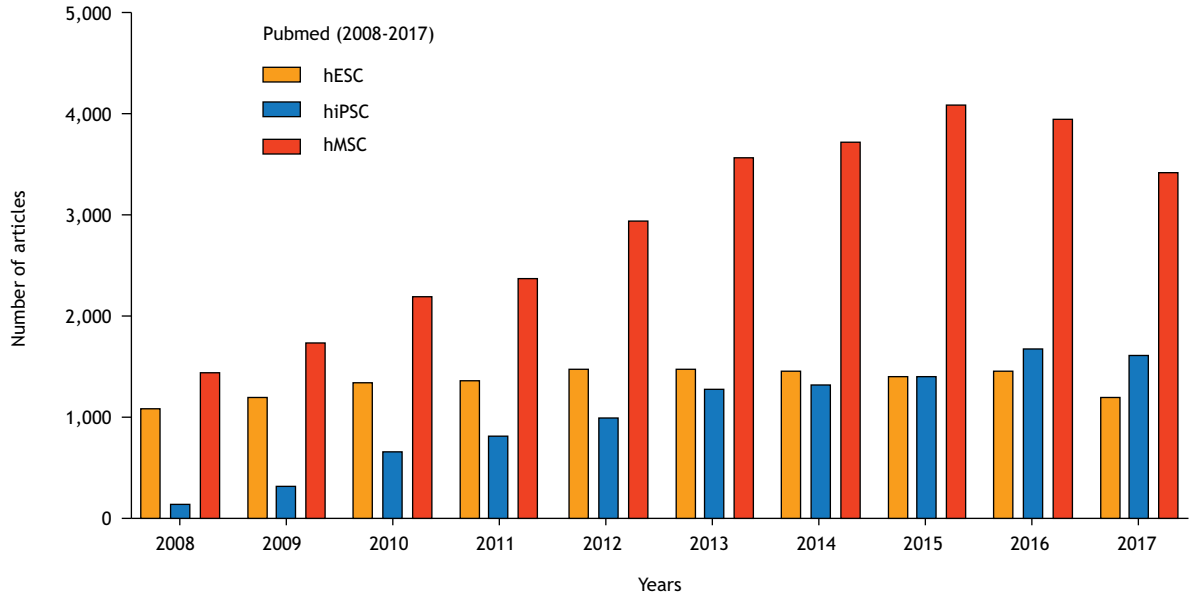

Figure. Number of articles published between 2008 and 2017 in the PubMed database for the following keywords: human embryonic stem cell (hESC), human iPSC (hiPSC) and human mesenchymal stem cells (hMSC), as per the subtitle.

iPSCs have emerged as an alternative to the use of embryos. They are obtained from somatic cells, but share several similarities with hESC (their plasticity, in particular), from the overexpression of some factors such as c-MYC[10]. IPSCs can be obtained from patients with specific diseases and enable the evaluation of drugs and the generation of *in vitro* models of human diseases. From the therapeutic point of view, there is no risk of immunological rejection. Similar to hESC, undifferentiated iPSCs, when implanted, may result in tumors due to uncontrolled proliferation and differentiation caused by genetic abnormalities. The therapeutic use of iPSCs was initiated in patients with macular degeneration[11]. In parallel, 21 clinical studies were conducted (www.clinicaltrials.gov, accessed Feb/6/2018) for the treatment of diabetes, neurological disorders, cardiac problems, among other diseases, including two studies that have already been completed (Table).

Progress in the clinical application of iPSCs requires detailed analysis of mutations present in somatic cells coupled with the use of rigid standard operating procedures for the routine verification of genetic abnormalities prior to clinical use[12]. In this issue, Biagi et al. discusses the potential of cardiomyocytes obtained from iPSC for the discovery of new drugs, with great potential impact in reducing the number of animals used in in vivo tests, in addition to greater predictive power.

Mesenchymal stem cells, similar to fibroblasts, although with elongated nuclei and condensed chromatin, are often isolated from bone marrow, adipose tissue, umbilical cord blood and dental pulp[13,14]. They are multipotent cells that have the capacity for self renewal. The International Society of Cell Therapy (ISCT – International Society for Cellular Therapy) established the minimum criteria for the uniform characterization of MSC, such as plastic adherence, differentiation potential in

Table. Clinical studies registered in the ClinicalTrials.org database according to their progress for human embryonic stem cells (hESC), human iPSC (hiPSC) and human mesenchymal stem cells (hMSC).

Situation on February 6, 2018	hESC	hiPSC	hMSC
Not recruiting yet	1	3	31
Recruiting	12	8	57
Recruiting through invitation	1	3	4
Active, not recruiting	4	2	28
Suspended	1	0	3
Finished	1	1	4
Complete	7	2	60
Withdrawn	1	0	7
Unknown[1]	6	2	50
Subtotal	**34**	**21**	**244**

[1] The study has passed its completion date and its situation has not been verified for over two years.

osteogenic, chondrogenic and adipogenic lineage, expression of CD105, CD73, CD90 and absence of CD45 hematopoietic markers, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR[15].

MSCs have been known since the early studies from the 1970s[16]. In the last few decades, many basic and clinical studies have investigated the therapeutic use of MSC as in the treatment of osteoporosis[17], tendon repair[18], kidney disease[19], liver[20] and myocardial infarction[21]. The results show that MSCs from bone marrow and adipose tissue, both allogeneic and autologous, are safe, simple and effective for the treatment of autoimmune diseases and chronic inflammatory diseases, such as Type I diabetes mellitus, discussed in this issue by Leal-Lopes et al. Concern about the use of MSC is due to the risk of undesired and potential differentiation to suppress the antitumor immune response and

promote angiogenesis that may contribute to tumor growth and metastasis. Calcification of the infarcted myocardium occurred after the use of MSC of the unfractionated bone marrow[22]. More than two hundred clinical studies are registered on the ClinicalTrials.org database (www.clinicaltrials.org, on February 6, 2018), of which two are in the recruitment phase in Brazil: one evaluating the MSC for advanced glaucoma treatment (University of São Paulo, School of Medicine) and another in transplant patients with severe resistance to corticosteroid treatment (Hospital das Clínicas de Porto Alegre, Center for Cellular Technology).

The use of human cells, adult or embryonic, transcends clinical applications. Complex biotechnological processes have been developed and associated, for example microfluidics, resulting in technologies capable of allowing new strategies for toxicological studies such as the *organ-on-a-chip*[23], as well as associated to nanoparticles as reported in this issue by Jasmin and Borojevic. There is great expectation that new models of three-dimensional culture, associated or not with microfluidics, can promote significant reduction of tests in animal and, simultaneously, increase the predictability of the tests[24,25,26], as it has been occurring with the models of equivalent skin presented in our themed issue by De Vecchi et al. The challenges related to three-dimensional cell culture are discussed in detail by Cavalheiro et al., as well as the regulatory prospects for the use of stem cells and iPSC in alternative methods to the use of animals, described in this issue by Biagi et al.

It is fundamental to emphasize that the use of advanced therapy products as well as cellular technology for alternative tests depends on the strict quality control of the inputs and processes. In the present issue, this aspect is addressed by Carias et al., who emphasize the tests previously mentioned in the scientific literature or in health norms, applied to the evaluation of the quality of human primary cells, that can be inserted in the routine of the Centers of Cellular Processing.

Also in this issue, Folgueras-Flatschart et al. draw attention to the major contaminants of cell cultures as well as the incorrect determination of cell identity. Also, the need for replacement of fetal bovine serum by the human equivalent, discussed in the articles by Meneses et al. and Takamori et al., including as source the platelet-rich plasma described by Teixeira et al. We also have in this issue an article by da Silva et al. which describes the use of cellular technology for the development of toxicological tests applied to the quality control of injectable products in Brazil.

In this context, in addition to the application of advanced cellular technologies to the therapy of various diseases, the disruptive potency of hESC, iPSC and MSC in the field of Toxicology is undeniable.

The increasing ability to mimic *in vitro* tissues, combined with the development of analytical and imaging tools, has enabled the development of *in vitro* tests that can reduce the use of animals and, in some cases, even replace them for some specific toxicological conditions. The European Union Reform Laboratory for alternatives to animal Testing (EURL ECVAM) developed the DB-ALM, Database Service on Alternative Methods to Animal Experimentation). According to information obtained from the website (https://ecvam-dbalm.jrc.ec.europa.eu/ - accessed on Feb/2/2018), DB-ALM is a public and factual database service that provides reviewed information on the development and applications of advanced and alternative methods for animal experimentation in Biomedical Sciences and Toxicology, both in research and for regulatory purposes.

The current version of DB-ALM covers the following Data Set:

1. **Topic summaries:** Thematic review of data sheets in executive summary form on alternative methods, available in DB-ALM for an entire topic area (e.g.: Percutaneous Absorption, Eye Irritation).

2. **Method descriptions:** two levels of detail: i) abstracts of methods covering the scientific principle, the needs addressed, the main applications and the current status of the development, validation or acceptance of the method; ii) protocols with detailed technical instructions to allow the transfer of a method to a laboratory.

3. **Project descriptions and studies:** method evaluations, including integrated European Union (EU) projects and formal validation studies selected as summary records, referenced with related data sectors.

4. **Compounds and test results:** lists of substances (more than 3,000) and individual investigations carried out using methods included in DB-ALM.

5. **People and institutions:** Information on persons and institutions active in the field of alternative methods is provided on the basis of voluntary participation.

6. **Bibliography:** All references analyzed for compilation of data sheets.

For example, in the cell culture subject, it is possible to find a protocol on the cultivation of stem cells (*Method Summary 165 - Differentiation of induced-pluripotent stem cells into post-mitotic neurons and glial cells - mixed culture*), which discloses all stages of human stem cell differentiation induced in neural precursor cells and in mixed cultures of postmitotic neurons and glial cells. With respect to local toxicity, the EpiOcular™ ocular irritation tests (*Method Summary 164*) and SkinEthic Human Skin Epithelium Model™ are noteworthy. For dermal sensitization analysis, the KeratinoSens™ method (*Method Summary 155 - OECD TG n. 442D*) enables us to differentiate between chemical compounds that sensitize the skin from those that don't.

What can be concretely observed is that progress in the field of Cellular Technology, Bioinformatics (dealing with *big data* of these sciences), and recent discoveries concerning Adverse Outcome Pathways have transformed the Toxicology in a more predictive science and Regulatory Toxicology in a subarea that increasingly seeks to elucidate the mechanisms behind xenobiotic-induced adverse events[27,28]. Today, multidisciplinary approaches, based on knowledge of physical, chemical and biological processes,

integrate in vitro, in silico, and toxic methods capable of identifying toxic effects of toxic compounds. What has been seen in practice is the use of these methods in Integrated Testing Strategies, together with experimental data generated by alternative (non-animal) tests, such as in vitro tests and traceability (*High Throughput Contend Screening*), contributing to analyzes with greater predictive power and applicability to humans[29].

Thus, it remains clear that cellular technologies are presented as promising therapeutic tools and as models for alternative toxicological tests, but for legal use and marketing, the establishment of standard operating protocols for handling and the demonstration of safety and quality are still under construction.

Committed to contributing to regulatory and innovation progress, the Brazilian Industrial Development Agency (ABDI), in partnership with Anvisa, promoted, in Brasília, on May 8 and 9, 2017, the International Seminar on Advanced Therapy Products and New Technologies using human cells.

The focus of the seminar was to discuss regulations that allow the advancement of knowledge-frontier technologies in the health area, such as gene and cell therapies, tissue bioengineering, and its benefits for people with chronic degenerative diseases.

Specialists, researchers and technicians of Anvisa, the Ministries of Health (MS), Science, Technology, Innovation and Communication (MCTIC), Industry, Foreign Trade and Services (MDIC), Attorney Generals (AGU) , Brazilian universities, Inmetro, Fiocruz and ABDI, as well as representatives of national and international companies and startups participated in the event, proving that the country is effectively moving towards innovation and technological application in this field.

Over the course of the event, it was agreed that it would be relevant to discuss some of the topics in the form of scientific articles that addressed the different perspectives addressed in the seminar. Thus, in this thematic issue of Visa em Debate, we consolidate the joint effort of authors, editors and journal staff. Over the next few pages, you will find several approaches to using cells as tools for both disease therapy and chemical hazard assessment. The opportunities and challenges are immense, but the determination to deepen knowledge and the possible application for people's well-being are equally spectacular.

We wish you a good reading!

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Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.

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