Universidade de Lisboa

Faculdade de Farmácia

The role of Mesenchymal Stem Cells in the development of ATMPs

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Monografia orientada pela Professora Doutora Joana Paiva Gomes Miranda, Professora Auxiliar, FFUL.

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Resumo

Os *Advanced Therapy Medicinal Products* (ATMPs) tornaram-se uma realidade com a evolução da pesquisa científica e tecnologia, que levaram à evolução terapêutica sentida nos últimos anos. Estas terapêuticas compartilham as funcionalidades gerais de um medicamento - funções farmacológicas, imunológicas ou metabólicas - e, para além disto, apresentam um perfil biológico e atuam como uma importante opção terapêutica para doenças para as quais não existem alternativas. Estas características biológicas acarretam preocupações extra em termos de regulamentação, acesso ao mercado e a nível ético, que são exploradas neste trabalho.

As células estaminais mesenquimais (MSCs) são células multipotentes capazes de se diferenciar em células da linhagem mesodérmica, mantendo a sua própria existência por auto-renovação, possuindo também diversas funções imunomoduladoras e um secretoma rico em fatores tróficos, entre outras. O conjunto destas características constitui a oportunidade perfeita para o desenvolvimento de ATMPs derivados de MSCs, direcionados a várias áreas terapêuticas e aplicações, tema este que tem sido um dos focos da comunidade científica.

O perfil imunomodulador que as MSCs apresentam, em particular, é uma "mina de ouro" no que toca ao controlo das disfunções do Sistema Imunitário e o seu *modus operandi* é uma fonte de esperança na procura de melhores tratamentos para doenças autoimunes, que prejudicam a qualidade de vida dos pacientes. Doenças como a Doença do Enxerto versus Hospedeiro, Osteoartrite, Artrite Reumatoide, Esclerose Múltipla, Lúpus Eritematoso Sistémico, Doença Inflamatória Intestinal e Diabetes Tipo 1 têm agora a possibilidade de obter mais alternativas terapêuticas e os medicamentos com MSCs já aprovados comprovam este facto, juntamente com o grande número de ensaios clínicos com estas indicações.

No entanto, apesar destas terapêuticas apresentarem um grande potencial e benefícios para o paciente, a fase de desenvolvimento terapêutico não tem sido fácil e têm surgido desafios significativos no caminho para um maior progresso. Este trabalho destaca os principais mecanismos da terapêutica com MSCs em cada doença autoimune referida, a fim de identificar suas vantagens e detetar possíveis pontos de melhoria no desenvolvimento da terapia de MSC.

Palavras-chave: terapia avançada, células mesenquimais estaminais, imunomodulação, autoimune

Abstract

Advanced Therapy Medicinal Products (ATMPs) became a reality with the development of scientific research and technology, that made the recent years' therapeutic evolution possible. These therapies share the general functionalities of common medicines - pharmacological, immunological or metabolic functions - and, in addition, present a biological profile and act as an important therapeutic option for diseases without other alternatives. These biological characteristics carry extra burdens regulation-wise, in market access and ethically, that are explored in this work.

Mesenchymal Stem Cells are multipotent and capable of differentiating into cells from the mesodermal lineage whilst maintaining their own existence by self-renewing, also carrying several immunomodulatory functions and a secretome with abundant trophic factors, among others. These characteristics make up the perfect opportunity for the development of MSC ATMPs, directed to various therapeutic fields and applications, a subject that has been a focus of the scientific community.

The immunomodulatory profile MSCs present, in particular, is a "golden mine" regarding the idea of controlling dysfunctions of the Immune System, and their modus operandi is a source of hope for the achievement of better treatment for autoimmune diseases that taunt patients' quality of life. Diseases such as Graft Versus Host Disease, Osteoarthritis, Rheumatoid Arthritis, Multiple Sclerosis, Systemic Lupus Erythematosus, Inflammatory Bowel Diseases and Type 1 Diabetes can now gain further therapeutic alternatives and the already approved MSC medicines verify this fact, along with the vast number of clinical trials with these indications.

However, even though these therapies may present great potential and benefits for the patient, the development phase hasn't been easy and significant challenges have arisen against higher progress. This work underlines the major mechanisms of MSC therapy in each referred autoimmune disease, in order to pinpoint their advantages and detect possible improvement points in MSC therapy development.

Keywords: advanced therapy, mesenchymal stem cells, immunomodulation, autoimmune

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List of Abbreviations

ATMP	Advanced Therapy Medicinal Product
DNA	Deoxyribonucleic Acid
EMA	European Medicines Agency
CHMP	Committee for Medicinal Products for Human Use
GMP	Good Manufacturing Practices
GCP	Good Clinical Practices
UPI	Unique Patient Identifier
GLP	Good Laboratory Practices
EU	European Union
EC	European Commission
CAT	Committee for Advanced Therapies
ESC	Embryonic Stem Cells
MSC	Mesenchymal Stem or Stromal Cells
ASC	Adult Stem Cells
αSMA	α Smooth Muscle Actin
BM	Bone Marrow
BM-MSC	Bone Marrow-derived Mesenchymal Stem Cells
AD-MSC	Adipose Tissue-derived Mesenchymal Stem Cells
UC-MSC	Umbilical Cord-derived Mesenchymal Stem Cells
HLA	Human Leukocyte Antigens
ISCT	International Society for Cell Therapy
TF	Transcription Factor
GNDF	Glia cell-Derived Neurotrophic Factor
VEGF	Vascular Endothelial Growth Factor

MHC	Major Histocompatibility Complex
IS	Immunological System
IL	Interleukin
IFN	Interferon
DC	Dendritic Cells
NK	Natural Killer
FBS	Foetal Bovine Serum
GVHD	Graft Versus Host Disease
aGVHD	Acute Graft Versus Host Disease
cGVHD	Chronic Graft Versus Host Disease
Th1	T helper lymphocytes 1
Th2	T helper lymphocytes 2
OA	Osteoarthritis
RA	Rheumatoid Arthritis
MS	Multiple Sclerosis
EAE	Experimental Autoimmune Encephalomyelitis
SLE	Systemic Lupus Erythematosus
RNA	Ribonucleic Acid
ANA	Antinuclear Antibodies
SLEDAI	Systemic Lupus Erythematosus Disease Activity Index
UC	Ulcerative Colitis
CD	Crohn’s Disease
TNF	Tumour Necrosis Factor
T1D	Type 1 Diabetes
AMI	Acute Myocardial Infarction

USA	United States of America
NA	Not Applicable
IV	Intravenous
IA	Intra-Arterial
IM	Intramuscular
BBB	Blood-Brain Barrier

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1 Advanced Therapy Medicinal Products

1.1 Overview of ATMPs

According to the European Medicines Agency, an Advanced Therapy Medicinal Product (hereafter "ATMP") is a medicine for human use that is based on genes, cells or tissues. Gene-based medicines make use of recombinant genes, carrying DNA from different sources, for a medicinal outcome - therapeutic, prophylactic or diagnostic result. Cell-based therapy medicines are based on cells whose biological characteristics have been altered, in order to be used for a different purpose rather than their normal functions in the human body. The outcome of these medicines has, too, to be therapeutic, prophylactic or diagnostic. Tissue-based medicines are created through tissue engineering, which makes them capable of regenerating, repairing or replacing other human tissue that, most likely, is damaged. Even though these are the main types of Advanced Therapy Medicinal Products, it's possible to create a combined ATMP which must contemplate the combination of one of the aforementioned types of ATMPs and one or more medical devices (Figure 1) (1).

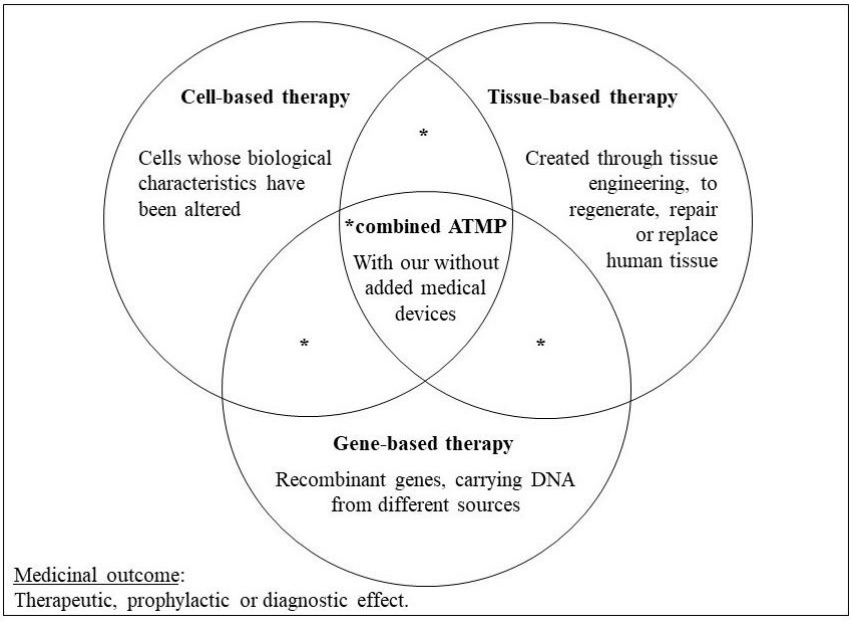

Figure 1 - Diagram representation of Advanced Therapy Medicinal Products.

ATMPs have become an important and needed evolution of medicine, especially in cases of exhaustion and failure of other treatment options or in cases of rare diseases with scarce options of treatment, acting as new and specific opportunities. These therapies have acted as a paradigm shift when it comes to the therapeutic reality of life-threatening diseases

and have opened new horizons to clinical research, with a special importance when it comes to genetic diseases (2).

This type of medicines require special attention from the European Medicines Agency (EMA), due to their biological profile and characteristics. These cannot be approved nationally by each country, having to go through a single central marketing authorisation process, with their safety and efficacy closely monitored pre and post authorization. These are only a small part of the specific details ATMPs demand when it comes to regulation and social and economic acceptance, aspects that will be addressed hereafter (1).

1.2 General Aspects of ATMP Regulation

The first European Regulation regarding Advanced Therapy Medicinal Products was released in November 2007, contemplating the first concerns and specific rules relating to ATMPs in subjects such as the authorization process, supervision and pharmacovigilance, that is, the issues under the responsibility of the EMA. This Regulation presented the first official definition of ATMP, which is still used for its characterization, stating that it must regulate any medicine that falls under such definition (3). With this document, the EMA established the creation of a Committee for Advanced Therapies within the agency, with members from the Committee for Medicinal Products for Human Use (CHMP), members who represent clinicians and representatives of patients' associations, all of which must be chosen for their qualifications and knowledge in the field. The Committee's functions are mostly based on the development of opinions and advice concerning the quality, safety and efficacy of ATMPs, providing help fitting drugs in development under the ATMP definition via the submission of a specific form, requesting and delivering the opinion of the Executive Director of the Agency or the Commission on any appropriate subject or, finally, assisting scientifically or advising on any suitable subject on which the knowledge of its members can be helpful (3). This being said, the Committee acts as a facilitator towards the development and approval of new ATMPs, making the information and processes flow easier and enabling quicker communication with the EMA, in general. Other aspects, such as the Good Manufacturing Practices (GMP) and Good Clinical Practices (GCP) concerning the Clinical Trials were left for later development by the European Commission (EC) (3).

In terms of packaging, ATMPs shall contain specific information in the outer packaging or in the immediate packaging in case of an outer packaging not existing (3). In ATMPs for autologous use, there must be a "for autologous use only" mention, along with the unique

patient identifier (UPI) - a medical identification number, individual to each patient, on which all their medical information is stored. The general requirements regarding the well-made packaging of medicines, according to the current legislation, and any special warning that may be necessary for the exact medicinal product, must also be ensured (3). In the post-authorization period, the market authorization holder shall guarantee the close follow-up of efficacy, security and adverse reactions concerning the product, presenting the measures previously foreseen upon the submission of the marketing authorization application. These may be presented as a risk management system if the need of further accompaniment is verified (3). Finally, another aspect of great importance is the traceability of the product and its starting and raw materials, since the majority of these components come into contact with living cells or tissues that the ATMP may contain, possibly of human origin. These substances shall be traceable from their sourcing, through their lifespan in manufacturing, packaging, storage, transport, delivery to the place of use by the patient, and the administration itself (3).

Since the publication of this document, the EMA has continuously analysed the effectiveness of the regulation behind the ATMPs and found some grey areas and subjects that should be addressed and, at the time, were not. Specifically, the EMA found that the regulation should make the process between the scientific discoveries and patient access to these innovative medicinal products easier and quicker. Thus, the EC and the EMA have developed an Action Plan in order to improve the ATMP regulation domain and reduce its uncertainties, with the main objectives of facilitating their development and patient's access to novel therapies (4).

Through this action plan, the EMA has already developed guidelines on various topics. In 2017 Guidelines on GMP were released, which emphasized the need to carry out a rigorous quality control on the components used in these products, specifically those that are animal-derived, due to the intrinsic variability that they entail and the high risk of transmissibility of infectious diseases. The differences in stability of these products in comparison to other types of therapeutics must also be taken into consideration as to keep the product effective, since the complex nature of the cells or tissues that constitute them and their special needs regarding manufacturing and storage (5). These GMP are risk-based, varying with the type of biological material in the product and the risks they presuppose, always presenting patient's safety as the main objective (6). Still in 2017, an adaption of the Good Laboratory Practices (GLP) was released, acknowledging the possibility of the ATMPs not following these general principles in specific products if accompanied by a proper justification and complementary

documentation, due to their specific biological characteristics (7). In 2018, the EMA released an update on the evaluation process of ATMPs, regarding marketing authorisation applications. As previously defined, the ATMP marketing authorisations are approved in a centralised process, led by the Committee for Advanced Therapies who compose the first opinion and then deliver the process to the CHMP who declare the final opinion and the possibility, or not, of an authorisation by the Commission. With this document, these processes were clarified and the developers were given more time to answer the Committees' questions (8).

Some of the subjects still in development are: a revision of the safety and efficacy follow up of ATMPs, with the intention to identify risks in earlier stages rather than in the post-authorisation phase and the mapping of more suitable post-authorization studies on these medicines, based on more recent knowledge (9); improving the Commission's capacity to provide scientific support to ATMPs developers; the publication of guidelines that define the requirements for ATMPs to enter the clinical trial phase and the establishment of their Good Clinical Practices, aiming to reach equality in clinical trials across the EU (4). This selection of targets, part of an ambitious and extensive but crucial action plan, is helping the EMA lead developers to an augmented and straightforward research and patients to a safer and clearer usage of Advanced Therapy Medicinal Products.

A particular challenge in the development of such novel and complex therapies is their economic expense and the impact they entail on companies. To alleviate this impact and have more developers enter the production process of ATMPs, the EMA has put into practice some incentives following the advisory services already available, such as the CAT. Firstly, an ATMP can acquire the Orphan Medication designation if it is intended for the diagnosis, prevention or treatment of a life-threatening or chronically debilitating disease, that affects no more than 5 in 10 000 people in the EU, that carries a significant benefit for its patients and that isn´t economically viable without any incentive. With this designation, the company obtains a 10-year marketing exclusivity once the product enters the market and the EMA provides scientific support during the product-development phase along with the possibility of fee reductions (10). Furthermore, the EMA has developed the PRIME pathway, which serves as a Priority Medicines scheme to support the development of medicines that target a still unmet need of the EU population (2). Through this pathway, the assessment of these medicines' applications is prioritised and accelerated by the EMA, along with their contribution with scientific advice (11). This tool helps new therapies reach the patients who

need them earlier, in order to minimise the effects of previously untreated diseases. Moreover, ATMPs not yet approved can be used in EU member states' hospitals under a Hospital Exemption. This requires the authorization of the national competent authorities and the update of their own legislation to accommodate such law and its guidelines (12).

The rapid evolution of the European regulation concerning ATMPs in the last decade has not only facilitated their production and the involvement of additional, and also smaller, companies in the research and manufacturing of life changing advanced therapies, but also made them widely available and a safer choice for patients with the added benefit of targeting a bigger number of previously untreatable diseases, representing a new step towards a better quality of life.

1.3 Concerns regarding the use of ATMPs

Despite the vast effort the EMA put into the continuous development of ATMP's regulation and the growing rigour and control towards this type of medicinal products, in 2020 the EMA felt the need to warn the population about the use of unproven cell-based therapies (13). The document emitted by the Agency pointed out the promotion of these uncontrolled therapies by individuals, companies and hospitals and highlighted their characteristics and the subsequent importance of well-designed clinical trials as part of their development, along with the CAT's close monitoring before and after they enter the market. This doubtful usage of cell-based therapies tends to increase the population's insecurities towards these medicines and difficult their involvement with clinical trials or even their receptivity towards them.

Ethical concerns are another pronounced source of divergences regarding ATMPs, including worries about the procedures used in genetic research, the storage of genetic materials and who has access to them, mainly focusing on the possibility of this information being used for any aspect other than the specific medicinal product (2). Thus, the general population shows apprehension in factors such as data protection and misuse of this information, confidentiality and also the modification of DNA in gene therapy, since these medicines are so profoundly different from the traditional therapies (14). These concerns grow substantially bigger when it comes to paediatric research, hence why the EMA stated that all ATMPs ought to follow the agreed paediatric investigation plan and present all

clinical trials' results as it denotes (15). This highlights a further need which is the education of the population and clarification regarding the new ATMPs.

Apart from the most common concerns, ATMPs carry extra burdens: in what comes to autologous products, there is not a clear conclusion on which part owns the product: the patient or the developer and, in general, there are still some difficulties in establishing a determined risk/benefit ratio in these novel therapies (16). Religious motives also affect the popular acceptance of ATMPs, since some of the genetic procedures may go against the devotee's beliefs (14). Overall, the patient must always be informed of the vast characteristics of the medicine product and/or clinical trial and their outcomes, may these be positive or negative, and the health professionals shall work against patient's disinformation and possible therapeutic misconception, on which the patient believes they are being given an approved medicine instead of being a part of clinical research (17).

From another point of view, an ATMP commercialization can be prohibited by a specific country's government even before it has the chance to be targeted to a specific patient, since each government is able to ban particular therapies that don't align with their ethical views or cause any specific ethical concern to their population (18).

The economic side of not only the development, but also the utilisation of ATMPs also faces some obstacles - as touched on before, the development of these novel therapies ends up being a costly process and perhaps impossible without the incentives the EMA put in place for the companies that venture into this side of therapeutics. However, the development phase isn't the end of this product's lifetime and, for a successful implementation on the patient's treatments, proper access to them, even at an economic level, is crucial. Due to the high price point of production, ATMPs become expensive medicinal products to the patient and this represents a great barrier for the accessibility to needed medicines (2). Since these therapeutic products offer benefits that the patient deeply needs and can't be offered by any other drug, with a lifetime impact, the reimbursement of therapeutic costs by the Healthcare System is the only viable solution. Various economic measures have been put in place to calculate viable reimbursements and to help decide which medicines benefit from said reimbursement or not, along with, if that reimbursement alone is capable of making such medicine affordable to the patient (19). Nevertheless, it is decisive that the ATMP in question presents benefits that are significantly superior to other alternatives and proportional to its price increase in order to receive the suitable reimbursement and become a viable and positive replacement of failed therapeutics (19). Even though the ATMP's marketing authorisation

submission and approval is a central EMA process, the reimbursement calculation and its implementation are national measures taken on by the government of each EU member state. Health service infrastructures shall too be adapted to this innovation and its requirements, and this adjustment also depends on the country's economic possibilities. Therefore, there can be significant disparities on these therapies' pricing points and accessibility throughout the EU and, therefore, on each patient's access to the medication (2).

At the end of the day, the regulation of Advanced Therapy Medicinal Products turns out not to be the only concern regarding these therapies, and the marketing authorisation application by the developer is only the start of a demanding market access journey. Subjects such as ethics and the economic position of each patient or country end up being a considerable obstacle on the way to the great advantage such innovative medicinal products deliver. Table 1 summarizes the pros and cons of ATMPs.

Advanced Therapy Medicinal Products	
Pros	A novel therapeutic option in exhaustion and failure of other treatments, and an important new hope in rare diseases treatment
	Possibility of acquiring the Orphan Medication designation and benefits
	Assessment prioritised and accelerated by the EMA through the PRIME pathway
	Possible utilisation at EU member states' hospitals under a Hospital Exemption
Cons	Need for extra quality and safety control, adverse reactions supervision and product traceability
	Possible extra packaging requirements and special warnings
	High economic expense, either at the production phase or in market access and patient accessibility
	Health service infrastructures' needed adaptation to these therapies
	Ethical and religious concerns

Table 1 - The pros and cons of Advanced Therapy Medicinal Products.

2 MSCs (Mesenchymal Stem Cells)

2.1 Different types of MSCs, isolation techniques and sites, identification criteria

The human body presents a selection of cells with multipotent differentiation abilities, capable of evolving into specialised cell types, namely stem cells (20,21). Stem cells are usually divided in two different types: embryonic stem cells (ESCs) and adult stem cells, whose characteristics such as isolation sites and differentiation capacities diverge. Embryonic Stem Cells' differentiation and proliferation is not restricted to a specific lineage, since these cells can differentiate into the three primordial germ layers (20). Despite their attractive proliferation profile and theoretical capacity to differentiate into any cell type, ESCs are not the safer option when it comes to therapy development with stem cells due to the possible teratoma formation (20). Adult Stem Cells differentiate into mesodermal lineage cells, part of the vascular and lymphatic systems: osteocytes, adipocytes and chondrocytes, and acquire the recommended designation Multipotent Mesenchymal Stromal or Stem Cells, hereafter referred to as MSCs (20,22). Figure 2 demonstrates the differentiation profile of pluripotent and multipotent cells.

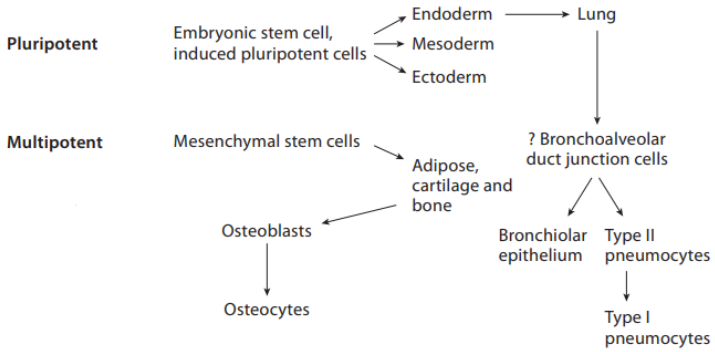

Figure 2 – Description of Stem Cells potency and differentiation. [Adapted from (23)]

The Mesenchymal Stem Cells were discovered over 50 years ago, by Alexander Friedenstein and his team, in 1976 (24). There are various sources of Mesenchymal Stem Cells throughout the human organism, which vary in concentration, accessibility and, consequently, isolation procedures, their efficiency and comfortability for the patient. These sources are the bone marrow, adipose tissue and birth-associated tissues, which present similarities: all of them are connective tissues and exhibit perivascular niches - specific environments around vessels where MSCs reside in the human body - something that is

known due to the expression of α smooth muscle actin (αSMA) on all the tissue types MSCs were obtained from (25,26).

The bone marrow (BM) was the first source of MSCs found and has, since then, become the most well-known source for obtaining these cells. The isolation of MSCs from the BM is an invasive method that relies on anaesthesia in order to collect bone marrow from donors, either from the iliac crest of the pelvic bone or from the femoral head during orthopaedic surgery. These isolation techniques carry risk of infection, which makes them only suitable to use in clinical or preclinical circumstances. Their isolation from this source is possible by density gradient centrifugation, finishing with the assembly of the mononuclear cells portion. Then, these cells are seeded in culture dishes to adhere and expand (Figure 3) (26).

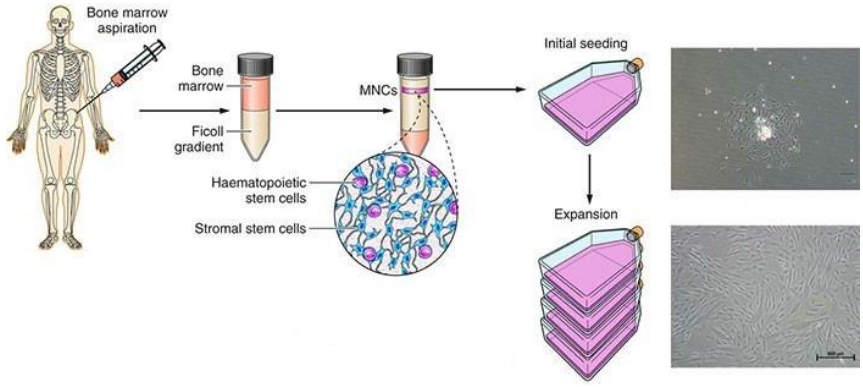

Figure 3 - Illustration of the isolation and expansion of BM-MSC. [Adapted from (27)]

The adipose tissue is also considered an easily accessible source of MSCs. Their collection is done by suction or excision of fat tissue, subcutaneous or visceral, from various areas, such as the abdomen, inguinal, kidney, femoral, gluteal or brachium areas (26). Due to the extraction of these cells being much less invasive than the isolation of BM-MSCs and the fact that their abundance in the adipose tissue is naturally high, this option is becoming more frequent. Following the extraction step, these tissues are digested by collagenases and go through centrifugation, where the vascular stroma fraction is set apart - this fraction contains, apart from the ASCs, hematopoietic and endothelial cells, among others (28). The final step is the adhesion and expansion of ASCs in the adequate culture plate and medium, just as the BM-MSCs isolation method (Figure 4) (26,28).

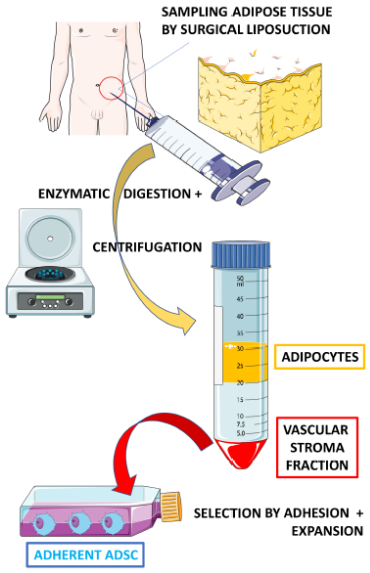

Figure 4 - Isolation process of AD-MSCs. (28)

Another source of MSCs is the human umbilical cord and other neonatal tissues. The umbilical cord possesses conjunctive tissue, the Wharton's jelly and vasculature, and the UC-MSCs can be obtained from the whole umbilical cord, from the Wharton's jelly alone or from the umbilical cord blood - these bear differences in MSC abundance, and the umbilical cord blood tends to have a lower yield of cells (26,29). The isolation and expansion method regarding these cells varies with their compartment of origin, however it is similar to the methods used with BM-MSCs and AD-MSCs: enzymatic digestion, cell filtration or density gradient dissociation (29). The birth-associated tissues end up having a low efficiency MSC isolation, which makes some researchers question it. Despite that, the UC-MSCs carry advantages: this extraction doesn't cause any pain or inconvenience to the donor, the cell's self-renewal properties are faster due to their fetal nature, the Human Leukocyte Antigens matching with the donor is not a problem since the cells are autologous, to name a few.

In addition to these sources, MSCs have also been found and extracted from other tissues like dental pulp, gingival tissue, ligaments, the synovial membrane and peripheral blood, however these are not used in MSC therapy (26).

A crucial factor concerning MSCs' isolation is the age of the donor, since it affects the cell's performance. MSCs obtained from older donors have a lower proliferation rate, tend to suffer oxidative damages and younger donors offer cells that age slower in culture (30). This is due to molecular changes associated with age, such as DNA methylation or histone acetylation. Other disparities found between MSCs can be originated by different intrinsic and extrinsic signals and effects the cells were submitted to, before extraction from the donor (31).

One of the major problems in what comes to therapeutic research with MSCs is the identification of these cells. It is very important that all researchers follow the same criteria for identifying this type of cells so there aren't discrepancies in which cells are being used to develop medicines and how they're being characterised. Facing this issue, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cell Therapy (ISCT) developed a proposal of minimal identifying criteria for human MSCs intended for research, in 2006. From this paper on, the scientific community defined MSCs based on the following characteristics (Table 2) (22):

- Adherence to plastic;
- Specific surface antigen expression;
- Multipotent differentiation potential.

Each characteristic alone doesn't identify an MSC, but the combined use of the three is what identifies these cells the best.

Criteria	Specifications
Adherence to plastic	When in the adequate culture medium, MSCs must be adherent to plastic.
Specific surface antigen expression	A minimum of 95% of the MSC population must express, as surface antigens: CD73, CD90 and CD105. These must not express CD11b, CD14, CD19, CD34, CD35, CD45, CD79a or HLA class II.
Multipotent differentiation potential	These cells must be able to differentiate into the mesoderm lineage: osteoblasts, adipocytes and chondroblasts.

Table 2 - Specifications for each characteristic for identification of MSCs, based on the ISCT minimal identification criteria from 2006. (22)

New knowledge about the MSCs brought the need to update these criteria and develop novel specifications, even though some researchers still don't meet this minimum (32). ISCT updated the criteria in 2019, with some recommendations (33):

- Researchers should include in their data the tissue from which the cells were extracted;
- The term "stem" should only be used if the cells effectively show evidence of stemness - that is, self-renewal and the specific differentiation properties. If not, they shall be named Mesenchymal Stromal Cells and not Mesenchymal Stem Cells. The abbreviation MSC can still be used, upon explanation of the specific term that's being used: "stem" or "stromal";
- Clinical research data with these cells must include functional assays that present the therapeutic mechanism of action of the cells in question.

Therefore, the most important update to these criteria is the abandonment of the term "stem" to characterise MSCs and the adoption of the term "stromal". The term "stromal" describes connective tissue cells, which is accurate in what comes to MSCs (21).

Regulatory-wise, MSCs used therapeutically are considered to be ATMPs by the EMA and can be somatic-cell therapy medicines if their biological characteristics are altered so they're used to diagnose, prevent or even cure a disease, or tissue-engineered medicines if they're altered to repair, replace or regenerate human tissue (1).

2.2 Characteristics and factors that offer therapeutic potential

Since their discovery, MSCs have become part of the most studied therapeutic products, cell-based, either by the industry or scholarly, due to their specific properties and actions in vivo that offer them therapeutic potential (32).

MSCs CHARACTERIZATION

Mesenchymal Stem Cells have a natural proliferation function, also characterised by the capacity to differentiate into mesodermal lineage cells (31). This characteristic is proven in vitro, and it has been shown that the presence and quantity of O_2 in the culture medium affects their proliferation by regulating the transcription factor hypoxia-inducible factor 1: a TF that manages the expression of cycle progression controlling genes. A lower O2 concentration replicates the human body environment and therefore increases MSC proliferation (26). Time is also a crucial factor in what comes to MSC proliferation, due to cell ageing. Just as the

human organism's regenerative capabilities decrease with age, so do stromal cells' and they enter a senescence stage (26). This age evolution is promoted by high cell replication - between 20 and 50 times - overwhelming stimuli, metabolic stress or even failed attempts to repair DNA damage, which can be seen as a defence mechanism (26). The cells then abandon their usual fibroblast-like shape and become irregular, flatter, bigger and less replicative, until the replication stops altogether but the metabolism and general phenotype are maintained - reflecting the whole human body's natural ageing process (31,34). MSC's ageing is a complex point whose cause is most likely the loss of telomere length, since there are differences in the cell's morphological constitution and telomerase activity (34). Apart from this, there are other factors that can cause or affect the cell's ageing such as the selected MSC expansion culture mediums or the cell's tissue of origin and donor age (26,34). After some time in senescence, cell cycle arrest is inevitable. This stage of MSC life is still being studied in order to understand its significance in the cell's functions and therapy options (26).

HOMING ABILITY

When Mesenchymal Stem Cells enter the human body though a transfer, it can result in the housing in a non-specific tissue, the homing in a niche that is similar to the one of their origin and the migration to a damaged location, and there is still not much information on which one is going to happen in which circumstances, as well as if the cells are going to be well received or eliminated from the organism (26). Certain elements, such as adhesion molecules and receptors take part in the migration of MSCs and homing in their destination (30). To reach their final target, the cells need to make use of endothelial cells, attach themselves and migrate to the tissue of choice, and the detailed mechanisms of how they travel through the tissues and choose their homing place is not yet clarified (35). However, it is supposed that the way migration and homing of MSCs work is in all aspects similar to the leukocytes' chemotactic attributes, since these cells have shown migration towards the diseased site in response to inflammatory mediators, but with different adhesion molecules such as selectins and integrins (35,36). It is suggested that the diseased tissues express specific molecules that promote MSCs migration (30). The delivery method is crucial to this issue's importance: direct applications into the damaged tissue don't require the migration attributes, unlike systemic applications. Whether the systemic applications are intra-arterial or intravenous is also something to have in consideration due to points like the lung first-pass effect in the intravenous route or the various risks associated with the intra-arterial option (26).

MSCs SECRETOME

Another useful MSC characteristic is the capacity to produce trophic factors that stimulate nearby cells (37). The substances these cells secrete, just as their phenotype, are thought to be defined by their interactions with the environment where they reside - the niche (37,38). These interactions are provided by the injured tissue and, with this, the damaged tissues are able to control what MSCs produce and, consequently, use them for their benefit (38). For example, MSC therapy in degenerative diseases such as acute kidney injury or liver cirrhosis may be beneficial due to this capacity: the MSCs secrete growth factors (e. g. hepatocyte growth factor) that act via a paracrine activity and improve the organ's functions (26). Neurotrophic factors are also some of the MSCs' most secreted, namely the glia cell-derived neurotrophic factor (GNDF), vascular endothelial growth factor (VEGF), nerve growth factor and the ciliary neurotrophic factor, which are very beneficial in neurodegenerative diseases for regeneration (39).

Finally, these cells interact with the immunological system (IS), modulating its activity. The IS is blind to the MSCs, since they don't express MHC class II on their cell surface (HLA-class II), neither the costimulatory molecules CD40, CD80, CD83, CD86 or CD154, which is a big privilege in MSC therapy, offering the possibility to use foreign MSCs in another human body (allogeneic use) without rejection or need for immunosuppression - they are hypoimmunogenic (26,30). However, there is the need to monitor closely the expression of HLA-class I molecules, considering that these may foster the expression of HLA-class II under specific stimuli and, consequently, the activation of T lymphocytes (26). Nevertheless, inflammation leads to the activation of T lymphocytes by the MSCs due to the upregulation of HLA-class II receptors. Although, since the MSCs don't secrete costimulatory molecules, this activation is suboptimal and ends up causing T cell anergy (loss of response by the T lymphocytes) and a reduction of cytotoxicity, thus uprising the immunosuppression (30,40). This described process only happens in vivo and never in in vitro experiencing, which leads researchers to conclude there could be a "licensing signal" amidst inflammation factors, an important aspect that might influence MSC therapeutic (26). Moreover, the interaction of MSCs with T lymphocytes doesn't end here, and these cells aren't the only ones with whom MSCs interact - something that is favourable to their therapeutic profile (20). These interactivities are done either directly (cell-cell) or indirectly (by means of secreted substances) (30). Regarding T lymphocytes, studies designate that, more than influencing their deactivation, MSCs also promote the development of regulatory T cells, downgrading

the immune response (20). T helper lymphocytes are also affected, since Mesenchymal Stem Cells alter the interleukin-4 (IL-4) and interferon-g (IFN-g) concentrations (30).

Dendritic cells have, as some of their purposes in the immune system, the stimulation of T lymphocytes and antigen presentation. The MSCs counteract these cells by affecting not only their core functions but also their maturation, differentiation of DC14+ into DCs and by fomenting the creation of tolerogenic dendritic cells, which have immunosuppressive properties (20,30,41). Decreasing the antigen presentation abilities of dendritic cells is a very positive point in what comes to organ transplantation, since it can reduce rejection (20). Antibody production is part of B lymphocytes' functions along with adaptive immunity, and untouched MSCs can't directly affect these cells. However, MSCs treated with interferon-γ are capable of inhibiting their proliferation in vitro. In vivo, intact MSCs suppress the overproduction of antibodies by B lymphocytes when that's the case, affect their chemotaxis by downregulating certain receptors' expression and, by influencing T cells and DCs, end up influencing B lymphocytes too (20,42).

The human organism's innate immunity is mostly assured by Natural Killer (NK) cells, which stop the proliferation of tumours and infections (43). The MSCs, directly or indirectly through soluble factors, are able to alter NK's phenotype and suppress their actions towards targets that express HLA-I molecules. Still, NK cells recognize MSCs as cytotoxic and fitting to lysis due to their expression of NK activation ligands. After treatment with IFN-γ, MSCs become resistant to NK by expressing more MHC class I antigens (20).

MSC-DERIVED EXTRACELLULAR VESICLES

Recently, MSC derived extracellular vesicles have been studied and developed, in order to acquire the same benefits as with MSC therapy, but without the burdens that cell therapies involve (44). These vesicles originate from fractions of MSC's cytosol, and they supposedly maintain the cell's therapeutic value, such as their trophic function, growth factors secretion and inflammation downregulation (30,37). Trials with these vessels have proven their effectiveness, mostly in tissue regeneration (45–47). Still, some concerns around this new discovery have been brought up regarding the persisting lack of knowledge on MSC characterization and how it is exponentiated when we're talking about an MSC-derived product, raising the need to further investigate the origin cells before investing on derived products' further research and testing (32).

All of this being said, MSCs portray important immunomodulating characteristics that reveal high potential for therapeutic success, particularly in immune disorders, mostly immune rejection or autoimmunity, and in regenerative therapy (30). Although, these cells' behavioural differences in in vitro/in vivo research and the remaining lack of knowledge on some of these cells' features are certain to create some difficulties in these therapies' development (26).

2.3 Cultures and growing conditions

The culture conditions and mediums to grow Mesenchymal Stem Cells are a topic of high relevance and especially studied, since it may guide the cell's characteristics. To work towards standardisation and possible replication of therapies, following rightly the Good Manufacturing Practices, these cultures must be well controlled and documented.

Serums like the Foetal Bovine Serum (FBS) or Serum AB shouldn't be used, since their composition isn't well described and can affect the cell's special undifferentiated condition or their future behaviour *in vivo* (26). Plus, synthetic products should be discarded and human derived products prioritised, free of strange substances to the human body (48). The culture's environment must be hypoxic, since we've already noted that lower O2 concentration mimics the cells' natural domain and promotes their growth (26). These details have been pinpointed in low scale culturing of MSCs, and future high scale cultures need to be studied further, since their attributes change.

The Mesenchymal Stem Cells' differentiation can be generated in vitro or prepared before the in vivo injection, through the manipulation of cell signalling (26). Firstly, an adipocyte-like cell can be obtained through stimulation with substances like dexamethasone, indomethacin or isobutylmethylxanthine. Then, the cell develops a lipid vacuole, typical of an adipocyte, and accumulates specific substances: lipoprotein lipase and fatty acid-binding protein. The Wnt/β-catenin signalling is indispensable for this differentiation (34). Chondrogenesis can be started by culturing in transforming growth factor-β1, transforming growth factor-β3, insulin-like growth factor or fibroblast growth factor 2. These cells then acquire the chondroblast morphology and aspect, and start secreting proteoglycan and collagen type II, when regulated by determined pathways (49). The development into an osteoblast is achieved by stimuli with bone morphogenetic protein 2, β-glycerophosphate, ascorbic acid or vitamin D3. This process depends mostly on the runt transcription factor 2,

accompanied by particular transcription factors. As an osteoblast, the presence of alkaline phosphatase L and calcium becomes more prominent (50).

MSCs' age is an all-around important factor, as it has been detailed before, and that is once again proved, in the differentiation phase. After a high quantity of passages, the cells have shown restrictions in differentiation: it becomes strict to a certain type of cell, instead of the usual whole mesodermal lineage (34).

3 Autoimmune Diseases MSC Therapy

Therapy with Mesenchymal Stem Cells has been widely studied in the last few years, and positive results have shown up in different diseases' studies, along with doubts and other not so good aspects. It's important to note that none of the therapies with MSCs mentioned registered significant adverse effects.

3.1 Graft Versus Host Disease

Graft Versus Host Disease (GVHD) can present itself as a chronic or acute disease. It is characterised by an after-transplant immunologic reaction, usually only associated with allogeneic hematopoietic stem cell transplant and no other types of transplantation (51). In this pathology the organism develops an exacerbated inflammation response against the foreign lymphocytes, that can start either around the first 100 days after the transplant, acting as an acute disease (aGVHD), or after this time, being labelled chronic GVHD (cGVHD) (52). These two presentations of the disease differ not only in the timeframe of appearance but also on the pathophysiology: aGVHD can be distinguished by the response of T helper lymphocytes 1 (Th1) and cGVHD can be typified by the reaction of T helper lymphocytes 2 (Th2) (53,54). Consequently, cGVHD follows the usual autoimmune diseases' profile, while aGVHD's behaviour differs from the norm.

As with most autoimmune diseases, GVHD is usually controlled with corticosteroid and immunosuppressant therapy. However, the response to this therapeutic profile is well below what's desired (55). With the failure of this medication, the disease evolves to what can be, at its worst, a tumour recurrence with high chance of mortality (55,56).

To circumvent these complications, and due to the immunomodulation attributes of MSCs especially regarding lymphocytes, a first experience of MSC therapy in GVHD took place with a paediatric patient carrying a form of severe unresponsive GVHD. After the infusion with BM-MSCs from his mother, a haploidentical donor, the immunosuppression was drastic and the patient's clinical condition improved significantly (57). Beyond immunosuppression, MSC's stimulation of regulatory T cells helps conserve the transplanted graft's anti-leukaemia action, that's overpowered by the usual immunosuppressant therapy, this way increasing the tumour repression. Although this result was surprisingly positive, this level of benefits wasn't coherent on all trials, which reveals the need to further research this

disease's pathophysiology along with MSC's characteristics and benefits in this situation (56). Still, all trials presented advantages when compared to placebo.

3.2 Osteoarthritis and Rheumatoid Arthritis

Osteoarthritis (OA) and Rheumatoid Arthritis (RA) are two articular diseases, different in their pathophysiology. Osteoarthritis consists in cartilage destruction leading to defects in the bone, in which case the chondrocytes can't maintain the usual environment needed for healthy articulations. This pathology can be erosive or nonerosive, the first galloping quicker, and both deriving from unknown mechanical and chemical alterations (58). On the other hand, Rheumatoid Arthritis is an established autoimmune disorder, in which there is inflammation in the synovial tissue that evolves to synovial outgrowths, also called villi - projections made of cells (synovial lining cells, lymphocytes and macrophages) and blood vessels. The synovial tissue thickens, called pannus, secretes enzymes that tear the cartilage and produces less fluid, reducing cartilage lubrication and leading to damage (58).

MSCs are a promising therapeutic option for OA by acting in two fronts: their immunomodulation characteristic reduces immune cartilage attack and their differentiation capacity into chondrocytes is crucial to help regenerate the already diseased cartilage (56). This therapy has been trialled in animal models and acted as supposed, leading to cartilage regeneration and amelioration of symptoms (59).

RA's therapy options are more developed than OA's, with antirheumatic drugs already in the market. However, MSCs can still bring benefits to these patient's quality of life by improving the therapeutic outcome of the antirheumatic drugs, which is still not at the desired level (60). Since RA is an autoimmune disease, MSC's immunomodulatory function is useful in lowering inflammation, inflammatory factors and fibroblast-like synoviocytes - a specialised cell, part of the pannus, that's an important part of RA's pathogenesis (56,60).

Particularly, these diseases' pathogenesis is far from being fully clarified, which makes their differentiation tougher and, consequently, therapeutic development harder to adapt and evolve.

3.3 Multiple Sclerosis

Multiple Sclerosis (MS) is an autoimmune disease, a consequence of autologous T lymphocytes' attack to the white matter of the central nervous system, demyelinating the nerve fibres (61). This demyelination leads to losses in nerve conduction, axonal loss and

consequential cognitive decline in the patient - affects the vision, motor dexterity, the memory and other neurological aspects (62,63).

The pathogenesis of MS is based in the cytokines secreted by T helper 1 lymphocytes, which secrete IL-17 and IL-5 cytokines that contribute to the disease's pathogenicity in a way that still remains unclear (61). Thus, MSCs can be beneficial in a similar way to the aforementioned diseases: these cells interact with the immune system repressing T helper lymphocytes and increasing the proliferation of T regulatory cells. Even though this type of therapy is helpful and important, studies have concluded that the MS patient's T lymphocytes pathologically secrete higher quantities of IL-2 cytokine, so the developed medicine should be adapted to this detail (64).

Therapy with MSCs has been proven beneficial by trials with EAE - experimental autoimmune encephalomyelitis - the animal model of Multiple Sclerosis. Adding to the immunomodulatory suppression of T helper cells and stimulation of T reg cells, MSCs have fostered the remyelination by secreting trophic factors and improved optic function and general disabilities via myelin repair (60,65). Fairly recent studies with EAE mice have also led to the observation that the use of the intramuscular route of administration with MSCs for the treatment of MS is not as beneficial as the intravenous, intracerebral or intraperitoneal routes, even though it is the safest (60,66).

3.4 Systemic Lupus Erythematosus

Systemic Lupus Erythematosus (SLE) is a chronic disease with an autoimmune profile, where mostly B lymphocytes but also other immune cells attack the organism's RNA-binding proteins, phospholipids, cell nucleus and their own DNA (67,68). This happens due to the production of Antinuclear Antibodies (ANA) along with the suitable environment for the disease's development, since producing these antibodies is usual in the population but it's not the only factor for developing SLE (69). This pathology is more common in females in their reproductive age, which suggests that endocrine, sex and other genetic factors might be preponderant (69).

MSCs can offer an alternative to the usual corticosteroid and immunosuppressive therapies, that frequently fail to bring patients to long term disease remission. These cells are able to suppress autoreactive B and T lymphocytes and also Dendritic Cells, which also play a significant role in this disease, in addition to down regulating inflammation (70).

The SLEDAI score, Systemic Lupus Erythematosus Disease Activity Index, has been used as a tool to measure MSC therapy effectiveness, with positive results. Most of the studies published have witnessed disease remission, with lower levels of proteinuria (very common in SLE due to renal damage), antibodies and inflammation cytokines, along with reduction of organ dysfunction as a result of growth factors secretion by MSCs (70,71). However, these therapies didn't save every patient from disease relapse and a novel increase of autoantibodies, which can possibly be explained by the diverging disease severities, by complications and different treatment characteristics, which uncovers the need for standardisation and further research, once again (72,73).

3.5 Inflammatory Bowel Disease

Inflammatory Bowel Diseases are globally emerging chronic digestive tract pathologies that can be either Ulcerative Colitis (UC) or Crohn's Disease (CD) (74). These diseases consist in idiopathic inflammation caused by immune responses to intestinal normal microbiota or even certain types of food, resulting in the immune cells attacking the intestine - autoimmune disease (28,56). The main difference between UC and CD is the localization of the inflammation flare-ups: while, in Ulcerative Colitis, the inflammation occurs in a part of the digestive tract (usually in the colon), in Crohn's Disease this inflammation is spread through the whole digestive tract (75). UC's pattern of inflammation is more permanent whilst Crohn's Disease can be intermittent, and UC tends to form ulcers quicker.

The more serious factor of these diseases is fistulisation, especially complex fistulas located in the perianal and anal zones, which occurs in 40 to 50% of Crohn's Disease patients (28). These require drainage and therapies such as immunosuppressants. Antibiotics have been tested but with disappointing results and adverse reactions. In the worst cases, there might be the need to surgically place a seton and continue its drainage for months, coupled with therapeutic such as anti-TNF-α, and this alternative still doesn't help the clinical condition of 25% of patients (28).

The low effectiveness of the available therapeutic options for this condition opens the door to MSC therapy. Just as in the previous autoimmune diseases, it's MSCs' immunomodulatory attributes that offer hope in this pathology: MSCs will upregulate T helper lymphocytes, that are usually in deficit in CD, along with suppressing T lymphocytes, dendritic cells and macrophages that take part in the disease's chronic inflammation condition (76). Studies have shown that the treatment with MSCs in Crohn's Disease has led to clinical

remission and also closing of the wounds in more than 50% of the cases, without excluding the need to surgery, which augments the patient's living quality (28). Some other researchers have come to the conclusion that the coating of MSCs with antibodies against addressin and vascular cell adhesion molecules leads to a better delivery to the inflammation site in this disease, as well as found that some modifications to the cells enhance their immunosuppression capacity, both of whose shall be optimised and standardised in order to obtain a potentiated and reproducible medicine (28,56).

3.6 Type 1 Diabetes

Type 1 Diabetes (T1D) is a metabolic disease in which the patient suffers from hyperglycaemia, due to pancreatic β cell damage - insulin secreting cells. With suboptimal insulin secretion and concentration, the glycaemic homeostasis is not assured (77). This disease is classified as autoimmune due to the role of T lymphocytes in the destruction of pancreatic β cells, although its pathophysiology is not fully clarified yet (78).

Therapeutic options in regard to T1D are centred around the replenishment of the needed insulin concentration levels, by insulin administration. However, these therapeutics can cause serious side effects like hypoglycaemic episodes that can lead to mortality (79). Moreover, these therapeutics don't target the disease's genesis but only the insulin scarcity. Therefore, therapeutic with MSCs can be a coadjutant, since these cells can differentiate into cells with pancreatic β cells phenotype and also act as a protection against the destruction of the remaining cells by T lymphocytes through immunomodulation (20,79). Plus, MSCs can foment β cells regeneration by upregulating growth factors, hence amplifying healthy cells quantity and, consequently, insulin production (77).

Research and trials with diabetic mice and human diabetic patients have not only shown that the transplantation of differentiated MSC cells into insulin-producing cells successfully reduced hyperglycaemia and originated functional pancreatic islets but also proven the pancreatic β cell protection and regeneration capacities of MSCs (20,60,80–83).

4 Approved/To Be Approved Therapeutics

4.1 Approved Medication

The first Advanced Therapy Medicinal Product with MSCs approved by the EMA was Alofisel, approved in 2018 with the Orphan Medication designation (84). Before this and from this time on, more therapies have shown successful results in clinical trials and obtained approval all over the world, making their way into patient's treatment options. Apart from the EU, countries like South Korea, India, Japan and New Zealand have conducted successful clinical trials that ended up in ATMP approval (Table 3).

Approved Advanced Therapy Medicinal Products with MSCs

Country of Approval	Name	Clinical Indication	MSC Source	Date of Approval	Marketing Authorization Holder
EU	Alofisel	Crohn's Disease (complex fistulas)	Adipose Tissue	2018	Takeda Pharma A/S
India	Stempeucel	Critical Limb Ischemia (due to Buerger's Disease or Atherosclerotic Peripheral Arterial Disease)	Bone Marrow	2016	Stempeutics Research Private Ltd.
Japan	Temcell HS	Acute Graft Versus Host Disease (aGVHD)	Bone Marrow	2015	JCR Pharmaceuticals Co.
Japan	Stemirac	Traumatic Spinal Cord Injury	Bone Marrow	2018	Nipro Corporation
Canada	Prochymal	Acute Graft Versus Host Disease (aGVHD)	Bone Marrow	2012	Osiris Therapeutics, Inc.
New Zealand					Mesoblast Ltd. (since 2013)

South Korea	Queencell	Subcutaneous tissue defect	Adipose Tissue	2010	Anterogen Co.
South Korea	Cellgram	Acute Myocardial Infarction (AMI)	Bone Marrow	2011	Pharmicell Co., Ltd.
South Korea	Cupistem	Crohn's Disease (complex fistulas)	Adipose Tissue	2012	Anterogen Co.
South Korea	Cartistem	Osteoarthritis, knee articular cartilage defects	Umbilical Cord	2012	MEDIPOST Co., Ltd.
South Korea	Neuronata-R	Amyotrophic Lateral Sclerosis	Bone Marrow	2014	CORESTEM Inc.

Table 3 - Approved MSC-based therapeutics. (32,84,85)

Through the analysis of the approved therapeutics, we can conclude that there have been 10 approved therapies, all of them approved post 2010, which leads us to the conclusion that the recent developments in MSC knowledge have been fruitful and this type of medicines is now making a difference in the therapeutic treatment of severe diseases. Bone Marrow is the most common MSC source, which is predictable due to the higher understanding of this source. South Korea is the country with more MSC therapies approved, by far, and with more than one marketing authorisation holder. In the USA (under the FDA), the process has been slower and less fruitful: there have been difficulties replicating pre-clinical studies' results in clinical trials and, therefore, there still isn't an approved medicinal product containing MSCs in the USA (32). Furthermore, the following diseases, of the previously mentioned, already have a MSC therapy alternative in the market: Acute Graft Versus Host Disease, Crohn's Disease, Osteoarthritis (knee defects).

4.2 Clinical Trials

Apart from the approved medicinal products above stated, there is still an extensive number of ongoing clinical trials, yearning to replicate the positive preclinical results in a clinical perspective. The general unfiltered number of clinical trials with MSC therapies, that are or have been active, reach more than one thousand entries (86). However, some of these clinical trials have either already been finished, withdrawn or their status is unknown.

In order to achieve a better perspective on how the development of the (possibly promising) therapies for the aforementioned and discussed diseases is at the moment, a representative summary of the terminated or completed clinical trials, with results, for these indications is presented (Table 4).

Disease	Study Title	Cell Source	Closing Year	Phase	Results	Efficacy Outcome
Graft Versus Host Disease	Treatment of Refractory Acute Graft-Versus-Host Disease by Sequential Infusion of Allogenic Mesenchymal Stem Cell	Allogenic Bone Marrow	2019	1/2	No adverse events related. 5 complete responses*, 2 partial responses**, and 1 patient did not respond***.	Positive
Osteoarthritis and Rheumatoid Arthritis	Treatment of Knee Osteoarthritis With Autologous Mesenchymal Stem Cells	Autologous Bone Marrow	2015	1/2	No adverse events related. Enhancement of cartilage quality and pain relief (87). Maintenance of clinical efficacy 2 years after (88).	Positive
	Treatment of Knee Osteoarthritis	Allogeneic Bone Marrow	2014	1/2	No adverse events related. Efficacy	Positive

	With Allogenic Mesenchymal Stem Cells				superior to hyaluronic acid. Enhancement of cartilage quality (89).	
	Phase 1/2a Clinical Trial to Assess the Safety of HB--adMSCs for the Treatment of Rheumatoid Arthritis	Autologous Adipose Tissue	2022	1/2	No adverse events related. Safety and efficacy were verified (90).	Positive
Multiple Sclerosis	Phase I-II Clinical Trial With Autologous Bone Marrow Derived Mesenchymal Stem Cells for the Therapy of Multiple Sclerosis	Autologous Bone Marrow	2016	1/2	NA	NA
	Stem Cells in Rapidly Evolving Active Multiple Sclerosis	Autologous Bone Marrow	2019	1/2	NA	NA
Systemic Lupus Erythematosus	Pilot Trial of Mesenchymal	Allogeneic Umbilical	2019	1	NA	NA

	Stem Cells for Systemic Lupus Erythematosus	Cord				
	Treatment of Systemic Lupus Erythematosus With Pooled Allogenic Mesenchymal Stem Cells	Allogeneic Olfactory Mucosa	2021	1/2	NA	NA
Inflammatory Bowel Disease	Umbilical Cord Mesenchymal Stem Cell Treatment for Crohn's Disease	Umbilical Cord	2017	1/2	NA	NA
	Stem Cell Coated Fistula Plug in Patients With Crohn's RVF	Autologous NA	2020	1	NA	NA
Type 1 Diabetes	Mesenchymal Stem Cells to Intervene in the Development of Type 1 Diabetes: a Blinded Randomized Study	Autologous NA	2020	2	NA	NA
	Mesenchymal Stem Cells in Patients With	Allogeneic Adipose Tissue	2021	NA	NA	NA

	Type 1 Diabetes Mellitus					

Table 4 – Completed/terminated clinical trials' representative summary.

**Resolution of acute GVHD in all involved organs; **Organ improvement of at least 1 stage without worsening in any other organ system; ***MR or stable disease or worsening disease; NA - not available/not applicable.*

By analysing the completed/terminated clinical trials available in the chosen database, it is possible to deduce that there are a vast number of clinical trials taking place in recent years, however not many of these studies have their results published. From the available literature, the results of these trials are positive with no significant adverse reactions, which is an optimistic paradigm.

Finished clinical trials on Osteoarthritis are very focused on the knee injuries and Inflammatory Bowel Disease related finished clinical trials focus mostly on complex fistulas, indications that already have a therapeutic option in the market. This leads to the need for the completion of more expansive research concerning these diseases.

Finally, approved therapeutics are used in clinical trials for applications that are different from the approved clinical indication, which is common in more ordinary therapeutics and might lead these therapies to a more flexible use.

5 Challenges in MSC Therapy

5.1 Lack of studies on the basic characteristics of MSCs and standardisation in therapy development

The development of novel MSC-based therapies has been a challenge for researchers, not only economically but also due to all the unknown factors surrounding these cells. MSC's phenotype is still not fully understood and, therefore, neither is the cell's impact on their own environment and the environments they may be delivered into (91). As mentioned before, the majority of studies have shown that the therapy with these cells hasn't created significant adverse effects, however there may still be other effects and long-term consequences that are not uncovered. Particularly, BM-MSCs have become the most well-known type of MSCs, however AD-MSCs and others may be lacking additional research (91). Further scientific knowledge about MSCs and their characteristics in all their possible sources, expansion and applications is essential to ensure controlled and reproducible therapeutic development and will reduce the divergent therapeutic results currently seen in clinical trials (32).

To help direct and standardise ongoing and future research, the criteria to identify and select these cells is crucial. The criteria published by the ISCT was recently updated, as previously touched on, however there are still a lot of doubts and incongruencies regarding this subject: these cells are taken as a whole and some species and origin differences are not considered, however these may affect factors such as surface markers and secreted molecules - something that also should be further researched and standardised, for future reference and to avoid false negatives or positives while identifying these cells (32).

Furthermore, MSCs are a valuable kind of cells not only for their different immunomodulatory and trophic capacities but also for their differentiation attributes, and these attributes ought to be warranted and confirmed during these cells' manipulation, especially after cell expansion and culturing, making sure that these processes didn't damage the cells or affect their properties (92). Thus, adequate assays must be defined in order to analyse these cell characteristics and make sure that the cells being used are in perfect conditions and also that the culturing mediums, time and conditions are adequate (32,93). Moreover, the name used in this cell's characterization must always represent the cell's capacity to differentiate and this capacity must be proven, since it has been already concluded that not all MSCs show true stemness, in comparison to multipotency (91).

This leads to another MSC research problem, that resides in the difference that has been stated between how these cells act in vitro or in vivo. Therefore, it's important that these differences are studied and also the reasoning behind them so that, in the future, these problems can be avoided and in vitro research fully represents what happens in vivo and mimics the latter environment (21,94,95).

Finally, general compliance of MSC research with good practises either in a production level or in a clinical setting are crucial, especially due to the ATMP profile of these therapies and the consequent extra specific requirements they entail in order to achieve the highest quality but also safety, and reach every patient who could benefit from them (96).

5.2 Tumorigenesis

MSC's differentiation and replication profile shows some of the same characteristics as tumours, mostly due to quick cell expansion and to the genetic alterations these cells are able to easily present. The reports in tumorigenesis regarding MSCs are dubious and studies have shown that these cells can either be a part in repressing tumours or active tumorigenic agents. Moreover, some researchers have even reported alterations in MSC's phenotype, mostly in extreme cell expansion, which may lead to cancer on a long-term basis (97).

This subject is an ongoing discussion and research focus, since the origin and timing of chromosomal abnormalities in these cells have still not been clarified. The possible abnormalities can start at the very beginning of the cell's lifetime - in the donor's organism - and, therefore, be affected by the donor's predisposition to tumorigenic factors and genetic data (97). However, these early-stage abnormalities are very rare and, therefore, the anti-tumorigenic controls should be focused on other steps of development (98).

Manufacturing can be a point of tumorigenicity of MSCs, either due to the mediums used in culture, to stimuli during the expansion to acquire certain characteristics or to extreme cell duplication. Culturing of these cells leads to alterations in the cell cycle, such as lowering DNA's ability to self-repair, which makes abnormalities more likely (20). Moreover, the highest proliferative rate induced on the cells, obtained with the use of growth factors, the more common are these abnormalities and less auto DNA repair is done, making this the most dangerous step in MSC managing (99). Thus, it's important to analyse these factors and proceed with a culture method that minimises the risk of abnormalities and, consequently, tumorigenesis, possibly even with the analyses of the cell's karyotype and exclusion of any altered cell (97).

In the end, these cells can acquire tumorigenicity in the host's body, after infusion. This process is influenced by MSC's highly variable phenotype and can also be potentiated by different and unpredictable stimuli in the destiny tissue, as well as the host's level of immunosuppression (97,100).

With this in mind, tumorigenesis in MSCs is not frequent, however its consequences are not to be underestimated, so preventive processes and routine analyses shall be set in place during therapy development and after infusion, in order to minimise this occurrence.

5.3 Other therapy challenges

Apart from the two aforementioned challenges about MSC therapy, other details constitute important challenges in the production of this type of therapies.

DELIVERY METHODS

The method of delivery of MSCs is one of the most important factors in MSC therapy, since the efficacy of therapies depend on it, that has been highly researched and that still entails some doubts. The two most used methods are either systemic delivery or local injection, and systemic delivery can be done either through intravenous (IV) or intra-arterial (IA) administration. Topical administration can be used when the skin is injured and intramuscular (IM) route is also acceptable when beneficial. Systemic delivery methods are beneficial due to the advantages of being in the blood circulation: close interactions with the immune system and rapid distribution (44).

The IA route is not the most used, mostly due to the possibility of occlusions in microvessels and cerebral cell cloths, that may cause a cerebral infarct (30,44). However, this delivery system can be the most effective in certain situations, such as acute myocardial infarction when the cells are targeted to the heart, and can be securely used with the appropriate caution in cell dosage and size, as well as the velocity of injection (101).

On the other hand, IV delivery of MSCs is the most used method of delivery, since it is an easier path and carries less risks. Nevertheless, intravenous injection leads to the entrapment of cells, mostly in the lung, and consequential reduction of the quantity of cells that reach the desired location, which leads to less efficacy, need for dosage adjustment and less impact of MSC produced soluble factors on the organism (44,102). Embolisms can also happen in this route of administration, however they are less likely to happen than in IA (30).

Furthermore, MSC's homing capacity offers them the ability to migrate towards the injured tissue after injection (103).

Local injection of MSCs is an option especially in a specific tissue regeneration or when the MSC secretome is needed in a specific location. This method causes less cell losses during migration and is beneficial when MSCs are needed in the nervous system (since they are not in the bloodstream and, therefore, don't need to pass through the blood-brain barrier (BBB)) and also in cardiac diseases. As a downside, this route of administration can cause tissue formation in problematic zones and there are less benefits from immunomodulatory and secretome characteristics of MSCs (30,44,104).

CELL LIFE SPAN

Beyond the route of administration of MSCs, the number and time between administrations also has to be defined and it depends, apart from the method of delivery chosen, from the in vivo MSC life span.

Some studies have shown that MSC's life span in vivo, after delivery, tends to be short. The therapeutic effect of MSCs in these patients wore off quickly, with half-lives around 24 hours (105,106). Additionally, MSCs have demonstrated loss of differentiation capacities due to cell ageing, with later senescence and cell arrest, as was concluded before. All these factors lead to the conclusion that MSC administration doesn't have a long-term profile and, consequently, repeated administrations will be needed to achieve a prolonged effect. Moreover, frequent injections of MSC may cause an immune response to these cells from the immune system, with the consequent secretion of antibodies, something that is undesired and may cause complications (30).

This implies that a healthy pharmacokinetic equilibrium between the route of administration of an MSC therapy and the dosage and posology has to be found, similarly to conventional therapies, with the added need of further cell life-span research and to minimise adverse effects linked to the chosen method of administration.

6 Conclusions

Advanced Therapy Medicinal Products, in their diverse definition, are a big part of the future of therapies and represent a step ahead in the pharmaceutical field. With scientific evolution acting towards diagnosis, more complex and diversified pathologies are documented and, therefore, the need for more robust therapies arises, with a special emphasis on biological therapies as the key for novel therapies. The biological character of ATMPs demands exact and critical regulation, to assure the safety of the patient and quality of the approved products, which is harder to achieve in biological-sourced products. EMA's continuous efforts to support manufacturers have a positive impact in the increasing quantity of companies in the field and a higher number of developing ATMP therapies, ultimately resulting in more therapeutic options for patients in need. Subjects such as economy, ethics and religion also perform a barrier in these therapeutics' development, but stand farther from the drug monitoring agencies spectrum, which leaves these subjects in the hands of governors and the general population, never discarding the importance of these structures being adequately informed by trustworthy scientific entities.

Part of the development of ATMPs is highly related to the use of Mesenchymal Stem Cells and their role in therapeutic progress, due to the promising profile of these cells: being biologically sourced and not secreting HLA-class II molecules, these are a known substance to the body and don't cause an immune response; their innate attributes of interaction with the IS cells and modulating their activity, trophic factors secretion and migration and homing, make them a weapon towards immunologically mediated diseases - safe if rightfully used. Still, to safely and effectively use these cells to perform pharmaceutical treatment, it is crucial that most of the doubts around their characteristics are clarified, that the research around these cells is expanded towards all cell sources and types, and that the terms of their clinical usage are standardised - such as their identification criteria and assays, culturing conditions and presented data in clinical trials.

The utilisation of MSCs in Autoimmune Diseases therapy is justified by the immunomodulatory capacities of these cells, since these diseases are originated by a defect in the Immune System, causing its cells to attack the body's own organs. MSCs' capacity to downregulate inflammation and to modulate and decrease the action of cells such as T helper lymphocytes whilst promoting T regulatory lymphocytes is the most valuable tool in the therapeutics of diseases such as GVHD, OA and RA, MS, CD and T1D. The downregulation

of Dendritic Cells and B lymphocytes is especially important in SLE and the regeneration of tissue and secretion of trophic factors are key in the treatment of OA and MS, respectively. The already approved medication in this field follows this pattern of action, with approved medications for GVHD, OA and CD, but leaving behind treatment alternatives for RA, MS, SLE and T1D. The similar form of attack by the IS that is seen in most of these diseases makes researchers believe that already approved therapies for one of these might be effective in a different disease, something that is already taking part in clinical trials, that most likely might achieve success and, consequently, increase the clinical indications of these drugs in a near future.

Nevertheless, taking a step back in research development of therapies with MSCs and reverting to the study of basic characteristics of these cells, whilst exploring their already known features and enlightening persistent doubts would, without question, be a positive step and a helping hand towards a better use of these valuable elements and diminish a significant part of the recognized challenges of MSC therapy.

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