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DEVELOPING CELL THERAPIES AS DRUG PRODUCTS

Running title: Cell therapies at bedside

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ABSTRACT

In the last 20 years, the global regulatory frameworks for drug assessment have been managing the challenges posed by using cellular products as new therapeutic tools. Currently, they are defined as “Advanced Therapy Medicinal Products”, comprising a large group of cellular types that either alone or in combination with gene and tissue engineering technology have the potential to change the natural course of still lethal or highly invalidating diseases, including cancers, opportunistic infections and chronic inflammatory conditions. Globally, more than 50 cell-based products have obtained market authorization. This overview describes the advantages and unsolved challenges on developing cells as innovative therapeutic vehicles. The main cell therapy players and the legal framework are discussed, starting from chimeric antigen receptor (CAR) T-cells for leukemias and solid tumors, dealing then with lymphocytes as potent anti-microbiological tools and then focusing on mesenchymal stem/stromal cells whose role is between regenerative medicine, immunology and anti-tumour therapy.

KEY WORDS: Chronic inflammatory diseases • Clinical trials • Haematological Malignancies • Immune tolerance • Opportunistic Infections • Mesenchymal stem/stromal cells • Solid Tumours

INTRODUCTION

The burden of chronic diseases, including cancers, opportunistic infections and immune-mediated disorders (Global Burden of Disease Study 2013 Collaborators, 2015), together with the advent of systems medicine (Wang R-S., et al., 2015), have prompted the scientists to search for therapeutic strategies alternative to chemical agents. Hence, the idea of harnessing the power of cells to treat a number of invalidating conditions has progressed through clinical trials from theory to novel treatment strategies. The general belief is that restoration of function is better accomplished by cells performing the appropriate therapeutic duty than by any chemical compound. This has paved the way for the development of the so-called “Advanced Therapy Medicinal Products” (ATMPs) (Hanna et al., 2016), a new class of agents classified as follows: 1) “somatic-cell therapy medicinal products” containing cells that either have been manipulated to change their biological characteristics or that are not intended to be used for the same essential functions in the body; they can be used to cure, diagnose or prevent diseases; 2) “gene therapy medicinal products” containing genes that lead to a therapeutic, prophylactic or diagnostic effect; they work by inserting recombinant genes into the body, usually to treat genetic disorders, cancer or degenerative diseases; 3) “tissue-engineered medicinal products” containing cells or tissues that have been modified, so that they can be used to repair, regenerate or replace human tissues (Commission Directive 2009/120/EC). This review discusses those cell therapies that have become a bedside reality, i.e., chimeric antigen receptor (CAR) T-cells, pathogen-specific T-cells and mesenchymal stem/stromal cells (MSCs) with a view on the regulatory issues that oversee this matter in Europe (Figure 1).

THE LEGAL FRAMEWORK IN EUROPE

ATMPs are governed by the Directive 2001/83/EC and the Regulation 2004/726/EC, amended by Regulation 2007/1394/EC, which set specific rules concerning their centralised marketing

authorisation, supervision and pharmacovigilance (Detela G. & Lodge A, 2019). ATMPs are considered as industrially-produced drugs, with the sole exclusion of those products falling under the “Hospital Exemption” rule (Regulation 2007/1394/EC) that are issued on a national basis (Tatjana et al., 2017). Therefore, ATMPs must be produced according to Good Manufacturing Practice (GMP) (Eudralex: https://ec.europa.eu/health/documents/eudralex/vol-4_en) and in compliance with Investigational Medicinal Products or Marketing Authorization specifications in sites authorized by National Competent Authorities. Moreover, the Regulation 2007/1394/EC refers to the Directive 2004/23/EC on donation, procurement and testing of human cells and tissues, and to the Directive 2002/98/EC on human blood and blood components, so that any use of human cells has to be in compliance with the quality requirements therein described. The “Committee for Advanced Therapies” within the European Medicines Agency (EMA) provides expertise to evaluate ATMPs (<https://www.ema.europa.eu/en/committees/committee-advanced-therapies-cat>). In the United States, the Food and Drug Administration (FDA) “Center for Biologics Evaluation and Research” regulates cellular therapy products, gene therapy products, and medical devices. European and United States regulations are only partially harmonised. To improve harmonisation, on September 17th, 2004, the EMA and FDA agreed to undertake a program to provide parallel scientific advice (Center for Biologics Evaluation and Research SOPP 8001.6).

Hospital Exemption

Hospital Exemption applies to those ATMPs not intended to be marketed and “prepared on a non-routine basis according to specific quality standards, and used within the same Member State in a hospital under the exclusive professional responsibility of a medical practitioner, in order to comply with an individual medical prescription for a custom-made product for an individual patient”. This rule enables patients to receive ATMPs when no authorised medicinal

product is available. However, the application of the Hospital Exemption has been matter of debate between Industry and Academia, since countries have differently interpreted the definition of “non-routine basis” (Tatjana et al., 2017).

Good Clinical Practice

Clinical trials with ATMPs are governed by the European Regulation 2014/536/EC. ATMPs pose specific challenges to clinical trial design, since their manufacturing and *in vivo* persistence require the implementation of particular logistical arrangements. It is recognised that in some cases, it could be difficult to produce robust pre-clinical data due to the absence of specific animal models. In this scenario, clinical studies are needed to be performed with the highest attention to the safety issues (EMA/CAT/852602/2018). The article 4 of Regulation 2007/1394/EC mandated the European Council to define guidelines on Good Clinical Practice for ATMPs that were finally adopted in October, 2019 (https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-10/atmp_guidelines_en.pdf).

Production

GMP rules for ATMPs were adopted in November 2017 (https://ec.europa.eu/health/sites/health/files/files/eudralex/vol4/2017_11_22_guidelines_gmp_for_atmps.pdf). This is the key document to understand how a GMP facility should be organized in terms of personnel, pharmaceutical quality system, production facilities, quality control and product release criteria. The personnel play a crucial role in ATMP manufacturing. Each manufacturing site must have at least one Qualified Person, whose main responsibility is to certify that each batch has been produced and controlled in accordance with the requirements of the marketing or clinical trial authorisation. In addition, a person responsible for the production ensures that manufacturing is performed in accordance with the specifications and that all the validations are done regularly, while a person responsible for quality control ensures for all the quality procedures. Quality defects and process deviations must be identified, the

causes investigated, and corrective and/or preventive actions should be applied. Importantly, adequate measures must be in place to ensure the full traceability of the ATMP after release. GMP for ATMPs gives particular relevance to the "risk-based approach". The legislator agrees that ATMPs are complex products and often their behaviour is not totally known, especially in the early stages of development. Thus, application of a risk-based approach may allow the release of a product that cannot undergo full testing prior to distribution, because it requires immediate administration in a non-cryopreserved form. In this case, an adequate control strategy could be developed by testing product intermediates or by performing in-process controls. Since ATMPs cannot be terminally sterilized, their manufacture requires an appropriate level of environmental cleanliness to minimise the risks of particulate or microbial contamination. According to GMP, ATMPs must be handled in a class A environment (under laminar flow) within a class B area, and there should be evidence that the production area classification, according to the International Standard Organization 14644-1 2015 (see Supplementary Table), is maintained during the whole manufacturing period. Therefore, production areas should be ventilated with air systems able to control particle contamination, temperature, humidity and differential pressure between rooms. In addition, the quality of starting and raw materials is a key factor in the production of ATMPs. Indeed, the use of xenogeneic cells/tissues poses additional risks of transmitting known and unknown pathogens, thus the selection of donor animals must be strictly controlled, selecting animals bred in pathogen-free conditions. The use of antimicrobials may be employed to reduce the bioburden, but antibiotics should be removed as soon as possible to ensure that they do not interfere with the sterility testing. Moreover, particular attention must be paid to the transmissible Spongiform Encephalopathy. Thus, compliance with the latest version of the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products (EMA/410/01 rev.3) is required.

ATMPs are also characterized by biological variability and by peculiar, often poorly characterised, pharmacodynamics and pharmacokinetics. In this scenario, the production process must be validated. Validation process comprises four steps: qualification of personnel and equipment; description of the validation strategy; performance of the validation experiments and collection of the results. Validation of aseptic processing is the simulation of the manufacturing process by using a microbiological nutrient growth medium to test whether the procedures are adequate to prevent contaminations during production and the report is a key document of the entire validation process. Validation of the analytical methods is performed according to the International Conference on Harmonization Guideline Q2 (R1) (EMA CPMP/ICH/381/95) and is intended to demonstrate that procedures and testing are suitable for the intended use.

CHIMERIC ANTIGEN RECEPTOR (CAR) T-CELLS FOR HAEMATOLOGICAL MALIGNANCIES

Having dissected the regulatory framework and basic elements of ATMP manufacturing, here we deal with one of the most advanced, promising and complex ATMP, namely the engineering of T lymphocytes through the introduction of a gene sequence carried by a viral vector coding for a protein, known as CAR. This molecule is expressed on T-cell surface and is able to redirect their specificity towards a certain target by combining the antigen-specific recognizing capacity of the ScFV of a monoclonal antibody with the T-cell activation and killing machinery (Figure 1). CAR T-cells differ according to the type and number of co-stimulatory domains employed and the role played by this component of the CAR is crucial for granting the T-cell activation, expansion and persistence. In particular, second-generation CAR T-cells include one co-stimulatory domain, while third-generation CAR T-cells include two co-stimulatory domains.

CD19

(<https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=2764>)

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directed CAR T-cell therapies for B-cell malignancies are currently the most advanced T-cell therapies tested in clinical trials and have demonstrated unprecedented efficacy. Indeed, preliminary reports showed high rates of remissions in patients affected by relapsed, highly refractory B-cell precursor acute lymphoblastic leukemia (BCP-ALL) who had previously been considered incurable (Grupp et al., 2013; Maude et al., 2015). Several trials, as summarized in Table 1, showed dramatic responses also in patients with other relapsed/refractory (r/r) B-cell malignancies, including B-cell Non-Hodgkin lymphoma (B-NHL) and chronic lymphoid leukemia (CLL) (Neelapu et al., 2017; Porter et al., 2011).

B-cell precursor acute lymphoblastic leukemia

To date, three large studies have been conducted on BCP-ALL by using CAR constructs different in terms of costimulatory domains and viral platform, and all showed an antitumour efficacy exceeding expectations, with complete remission (CR) rates ranging from 70% up to 90% (Davila et al., 2014; Maude et al., 2014; Lee D.W., et al., 2015). Briefly, Lee et al. reported the outcomes of a phase I trial on 21 children and young adults with BCP-ALL or B-NHL treated with second-generation (CD28 was the co-stimulatory molecule employed) CAR T-cells (Lee D.W., et al., 2015). The CR rate in BCP-ALL reached 70%; 10 patients subsequently underwent allogeneic haematopoietic stem cell transplantation (HSCT) and remained disease-free. The group of Davila initially published the results of 16 adults with r/r BCP-ALL treated with CD28-z-CAR T-cells, showing a CR rate of 88%; 44% of the patients subsequently underwent allogeneic HSCT and did not experience relapse (Davila et al., 2014). Finally, Maude et al. reported the results obtained with the administration of CAR T-cells generated using a different second-generation construct, containing the 4.1BB costimulatory molecule (Kymriah™) in children and young adults with BCP-ALL (Maude et al., 2014). Thirty subjects younger than 24 years were treated, and a CR rate of 90% was reported. Notably, a long persistence of the infused CAR-T cells was shown, since genetically-modified T-cells were

detectable up to two years after infusion. Moreover, 19 out of 27 responding patients maintained CR for 2-24 months without further therapy. These extraordinary results were confirmed in an international study (Maude et al., 2018), thus leading to the recent approval by both FDA and EMA of Kymriah™ for the treatment of children and young adults with r/r BCP-ALL. However, peculiar toxicities have been described, the most relevant being cytokine release syndrome and neurotoxicity. Cytokine release syndrome is a non-antigen specific reaction that occurs because of high-level activation of immune cells, leading to the massive release of cytokines. It was reported to occur more frequently and with greater severity in patients with higher leukemia burden (Maude et al., 2014 and 2018; Lee et al., 2015). Neurotoxicity is characterized by a wide spectrum of clinical signs, ranging from headache to severe encephalopathy, usually it is self-limiting, although fatal cases were reported (Maude et al., 2014 and 2018; Lee et al., 2015). Albeit the remarkable responses obtained, a substantial proportion of patient relapses and CD19^{dim} or negative recurrence is the main cause of treatment failure (Sotillo et al., 2015). In order to reduce the risk of immunological escape, bispecific CAR T-cells simultaneously targeting different surface antigens are currently being tested. In particular, since an innovative, CD22 (<https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=2786>) - directed CAR T-cell approach has been evaluated in a phase I clinical trial on 21 children and adults with BCP-ALL (including patients with CD19^{dim} or negative B-ALL), showing 73% CR, the CD19-CD22 bispecific construct represents a promising dual approach (Fry et al., 2018). The results of the first 6 adults affected by r/r BCP-ALL treated with CD19/CD22 CAR T-cells have recently been reported (Dai et al., 2020). Despite obtaining CR in all the treated patients, 50% of them relapsed 3-10 months after treatment. Further studies are required to shed light on the actual risk of antigen escape in patients treated with bispecific constructs.

Non-Hodgkin lymphoma and chronic lymphoid leukemia

In 2015, Kochenderfer et al. reported the results of CAR T-cell treatment of 11 B-NHL and 4 CLL patients (Kochenderfer et al., 2015). Four of the 7 evaluable patients with aggressive B-NHL achieved CR and 2 a partial response, whereas 3/4 CLL patients achieved CR. Parallely, Schuster et al. reported the results of 24 B-NHL patients treated with Kymriah™ showing an overall response rate of 68% (Schuster et al. 2015). These results were further investigated by the same group in a single center, phase IIa trial and, subsequently, by an international phase II trial showing response rates of 50% and 52%, respectively (Schuster et al., 2017 and 2019). In parallel, Neelapu et al. reported the multicenter experience with anti-CD19 Axicabtagene ciloleucel (Yescarta™), a second-generation CAR construct, for the treatment of r/r B-NHL (Neelapu et al., 2017), where 101 patients received the treatment, including 77 with diffuse large B-cell lymphoma and 24 with primary mediastinal B-cell lymphoma or transformed follicular lymphoma. The overall response rate was 82%, with a 54% CR rate. The outstanding responses obtained by Kymriah™ and Yescarta™ in patients affected by B-NHL also led to the recent approval by the FDA and EMA of these therapies in patients with r/r large B-cell lymphoma (Cuende et al., 2018).

Other haematological malignancies: current and future perspective

Beside BCP-ALL, CLL and B-NHL, further haematological malignancies, namely multiple myeloma (MM), T-cell ALL and acute myeloid leukemia (AML) still represent unmet needs requiring innovative approaches for r/r patients. For patients with r/r MM, different CAR T-cell approaches targeting several antigens have been explored, including: a) CD19 with encouraging preliminary results using second-generation 4.1BB-z CAR T-cells (Garfall et al., 2015); b) the κ immunoglobulin subtype with modest results (4/7 patients achieving stable disease) (Ramos et al., 2016); c) CD138, with 3/5 patients reaching stable disease (Guo B., et al., 2016). Interestingly, another antigen has been identified as putative target, the B-cell maturation antigen, a receptor of the tumour necrosis factor (TNF) superfamily upregulated

during B-cell differentiation into plasmablasts. A phase I study was conducted on 33 patients with r/r MM, exploring the safety and the efficacy of a B-cell maturation antigen (<https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=1889>) - directed, second-generation (4.1BB-z) CAR construct (Raje et al., 2019). Besides developing common side effects, namely cytokine release syndrome (76%) and neurotoxicity (42%), a remarkable response rate was reported, with 85% responding patients, including 42% of patients achieving CR. Unfortunately, 6/15 patients who achieved CR subsequently relapsed, and the median progression-free survival was 11.8 months. Taken together, these results revealed, for the first time, an encouraging anti-tumour activity of CAR T-cells targeting B-cell maturation antigen in MM.

The development of CAR T-cell approaches for T-ALL is hampered by several major obstacles, including the risk of T-cell blasts transduction and fratricide occurrence. Fratricide is the potential reciprocal killing of CAR T-cells that can recognize the target antigen not only on the cell surface of leukemia blasts but also of engineered T-cells.

In order to overcome the risk of blasts contamination in the drug product, third-party, off-the-shelf products have been exploited. To develop allogeneic CAR products avoiding the risk of graft-versus-host-disease (GvHD), two main approaches have been investigated for B-cell malignancies: the use of either gene-edited T-cells with a knock-out of the T-cell receptor (TCR) α -chain, or different cell platforms lacking allogeneic reactivity, such as natural killer (NK)-cells. Gene-edited, CD19-directed CAR T-cells with abrogation of native TCR have been tested in adult and paediatric patients with r/r B-cell malignancies showing encouraging results, although less promising than the autologous products (Graham et al., 2019). CAR NK-cells targeting CD19 have shown extraordinary early results in both preclinical (Quintarelli et al., 2020) and clinical studies (Liu E., et al., 2020), encouraging further clinical exploration. On the other hand, the risk of production failure related to fratricide can be reduced through

targeting of antigens more expressed on T-ALL blasts than on normal T cells, such as CD38 (<https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=2766>) (Naik J., et al., 2019), or those temporarily downregulated after T-cell activation, such as CD5 (Mamonkin et al., 2015). Gene-editing can also be exploited as strategy to circumvent fratricide, by knocking-out the target antigen on the normal lymphocytes before their transduction with a CAR construct (Gomes-Silva et al., 2017).

Similarly to T-ALL, the development of immunotherapeutic approaches for AML is hindered by the difficulty of identifying a suitable, tumour-specific and universal, target antigen. This type of target antigen would avoid the risk of killing normal haematopoietic progenitors by CAR T-cells. To date, few CAR constructs have been investigated in clinical trials, with two main antigens currently under exploration, namely CD33 (<https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=2601>) and CD123. Results of CD33-directed CAR T-cells are controversial, with minor benefit observed and the risk of severe on-target, off-tumour toxicity related to the expression of the molecule on healthy haematopoietic stem cells and on hepatocytes (Wang Q.S., et al, 2015). A strategy to circumvent this hurdle is represented by the use of transiently expressed CD33-CAR molecules, although in the preclinical setting this strategy obtained only transient efficacy (Kenderian et al., 2015). CD123 is overexpressed on AML blasts and has low expression on normal haematopoietic stem cells, qualifying as promising target. Preliminary evidence of the use of CD123 (<https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=1705>) - directed CAR T-cells for treatment of patients with AML showed promising anti-leukemic activity without myelosuppressive effects (Budde et al., 2017). Further data are needed to thoroughly evaluate the efficacy and safety of these novel approaches.

As for haematological malignancies, treatment with genetically-modified somatic cells has been implemented as new therapeutic strategies for several solid tumours (Figure 1) characterized by poor prognosis (Guo F. & Cui J. 2020), including high-risk neuroblastoma, metastatic Ewing's sarcoma, glioblastoma and pancreatic ductal adenocarcinoma. These gene therapy approaches are based on: (a) MSCs as a vehicle for delivering TNF Related Apoptosis

either alone or in combination with chemotherapy agents (Spano et al., 2019) that will be discussed in the paragraph dedicated to MSCs; and (b) lymphocytes engineered to express a CAR directed towards the disialoganglioside GD2 (Prapa et al., 2015).

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risk neuroblastoma patients, alone or in combination with checkpoint inhibitors, demonstrating safety and an early efficacy that deserve further investigations (Heczey et al., 2017). Similarly, CAR T-cells have been infused in patients affected by glioblastoma targeting a variety of antigens, such as IL-13R α 2 (<https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=1701>), epidermal growth factor receptor-vIII, and human epidermal growth factor receptor-2 suggesting important insights regarding safety and efficacy (Akhavan et al., 2019). In particular, multiple, sequential intratumoral and intraventricular infusion of autologous CAR T-cells targeting IL-13R α 2 were reported to be well tolerated and to induce a transient CR in a patient with recurrent multifocal glioblastoma (Brown et al., 2016). These data are particularly relevant, considering the possible off-target risks associated with the occurrence of a strong inflammation, upon CAR T-cell activation, that might induce intracranial hypertension, potentially lethal. In this particular setting, factors related to the selective identification of tumour-specific targets, the delivery route, the cell dosage and the preparative regimen represent key factors for a successful clinical translation against glioblastoma. Overall, these data indicate that genetically modified cytotoxic T-cells seem an attractive therapeutic possibility for solid tumours, thanks to the CAR-T specific recognition and cytotoxicity that can possibly circumvent the tumour-mediated immunological silencing. However, this goal is not easily achieved and, while the use of CAR T-cells for haematological malignancies have already obtained market authorization (Cuende et al., 2018), the way for a robust clinical translation of CAR T-cells in solid tumours is less straightforward. There are many reasons at the basis of this suboptimal clinical efficacy, and some are still not completely understood (D'Aloia et al., 2018). One crucial issue pertains the choice of the target antigen that may also induce undesired off-target side effects emerging late in the pre-clinical and clinical development (Lamers et al., 2013). In addition, it has been reported that *ex vivo* engineered

cells may express immunogenic vector-encoded epitopes that can be recognized and attacked by the patient immune system, compromising the persistence of CAR T-cells (Lamers et al., 2011). Other relevant issues may be related to the CAR T-cell doses, frequencies and route of administration since efficient homing and trafficking of CAR T-cells toward tumours and their complex microenvironment are not completely under control and may require additional tools to more precisely direct CAR-T (Zhao et al., 2020). In addition, CAR T-cells may find a hostile, immune-suppressive microenvironment that could exert an inhibitory effect on activated CAR T-cells, leading to treatment failure; in this case, novel combinatory strategies, including the use of checkpoint inhibitors, could counteract this mechanism, improving CAR T-cell antitumour efficacy (Heczey et al., 2017). Finally, the possible occurrence of antigen escape may be detrimental in the long-term control of the diseases, as already reported with the use of monoclonal antibodies (Jackson & Brentjens, 2015). As for haematologic malignancies (Fry et al., 2018; Dai et al., 2020), to face this issue, bispecific CAR T-cells have been developed with the goal of minimizing this risk (Hegde et al., 2013). Despite the hurdles just discussed, there are currently (September 2020) more than 500 clinical trials on CAR T-cells, with about 80 studies on solid tumours (<https://www.clinicaltrials.gov/>), so that implementation of their use in clinical practice is eagerly expected in the near future.

PATHOGEN-DIRECTED ADOPTIVE T-CELL THERAPY

In an era of intensive immunosuppression post-transplantation and expanding indications for novel immunotherapeutic agents, the number of patients at risk for developing potentially life-threatening conditions caused by opportunistic pathogens, including viruses and fungi, is rapidly increasing (Fernández-Ruiz & Aguado, 2018). Moreover, as experienced with the recent Coronavirus disease-19 pandemic, there is a significant threat posed by emerging pathogens. Despite a significant effort to develop new drugs, specific agents may not always

be available and, when present, prophylactic and preemptive pharmacotherapy is limited by toxicity and, to some extent, by emergence of resistance. More importantly, pharmacological treatment may fail to restore pathogen-specific immunity, which is the central requirement to achieve a long-lasting control of infection (Hakki et al., 2003). Given the correlation between the absence of T-cell immunity and viral disease, adoptive cell therapy is a rational and attractive alternative to pharmacologic one. Transfer of T-cells specific for viral or fungal antigens has been explored as a treatment strategy to prevent or clear infection with minimal toxicity (Figure 1), while enhancing immune surveillance through the emergence of pathogen-specific immune responses.

The first attempts at treating infection-related disease by restoring immune responses were conducted in the setting of HSCT, and were based on the use of unmanipulated lymphocytes from seropositive bone marrow donors (Papadopoulos et al., 1994; Hromas et al., 1994). The approach was successful, but the presence of alloreactive T-cells caused the development of GvHD, thus limiting its applicability. An elegant advancement in this strategy was the transduction of donor lymphocytes with a retroviral construct containing suicide genes, to induce susceptibility to drug-mediated lysis in case of GvHD development (Zhou et al., 2014). An earlier evolution was the replacement of unmanipulated lymphocytes, which contain a limited number of pathogen-specific T-cells, with cellular products enriched in antigen-specific T-lymphocytes. Pioneering studies conducted in the nineties proved that virus-specific cytotoxic T-cell clones or lines reactivated from the peripheral blood of HSCT donors could be successfully administered as prophylaxis/treatment against human Cytomegalovirus (hCMV) disease or EBV-positive post-transplant lymphoproliferative disease (PTLD) in patients given a T-cell depleted, human leucocyte antigen (HLA)-unrelated HSCT (Walter et al., 1995; Rooney et al., 1995). Since then, *ex-vivo* expanded pathogen-specific T-cells have been employed after T-cell depleted haploidentical HSCT to prevent EBV PTLD, and cure

hCMV disease and invasive Aspergillosis as well, with good efficacy and without the development of GvHD (Einsele et al., 2002; Perruccio et al., 2005; Comoli et al., 2007). These studies demonstrated that a high degree of mismatch between the lymphocyte donor and recipient did not represent a hurdle to *ex-vivo* expanded adoptive T-cell therapy for infections. Overall, the rate of response for these early studies and others (Doubrovina et al., 2012) was in the range of 90% for prophylaxis/preemptive therapy and 70-80% in the case of treatment for pathogen-associated disease, with minimal toxicity due to the development of GvHD (1-10%). However, these studies relied on rather complex and time-consuming methods for *ex-vivo* expansion of T-cells after stimulation with antigen-presenting cells either infected or loaded with pathogen particles. When peptides derived from immunodominant viral antigens became available, more rapid and GMP-compliant methodologies to obtain pathogen-targeted T-cells were investigated. Thus, *in vitro* generation of infectious agent-specific T-cells was achieved using HLA class I/peptide multimer cell sorting (Cobbold et al., 2005) or interferon- γ secretion capture assays (Moosmann et al., 2010; Peggs et al., 2011; Feucht et al., 2015). While the former approach allows selection of CD8⁺ antigen-specific T-cells, the latter option offers the advantage of enriching for either CD8⁺ or CD4⁺ T-cells, both necessary for long-lasting immune surveillance, without the need for HLA restriction. This is crucial for some pathogens, such as adenoviruses, that are mainly controlled by CD4⁺ cytotoxic T cells. The greater and more rapid availability of T-cells for therapy was counter-balanced by a 10-15% loss in response rate in the case of overt EBV, hCMV, or adenovirus disease, and a slight increase in the rate of GvHD post-adoptive therapy. The main obstacle to this approach, however, is the inapplicability to pathogen-naïve cell donors.

To overcome this hurdle and, in general, to hasten the procurement of large numbers of T-cells to treat pathogen-related disease, a different strategy, based on the use of partially matched, third party-derived, banked virus-specific T-cells was proposed (Haque et al., 2002). These

cells were first employed to treat EBV PTLD after solid organ transplantation, but were soon employed as “off-the-shelf” immediately available immunotherapy products also in the setting of cord blood and bone marrow transplantation (Barker J.N., et al., 2010; Tzannou et al., 2017). Nowadays, these products can be allocated based on their viral epitope specificity and HLA restriction element. Due to the partial match with the recipient, these “off-the-shelf” cellular products do not persist long term, albeit they have been demonstrated to induce long-lasting clinical responses in HSCT recipients (Withers et al., 2017), possibly through bystander stimulation of endogenous immunity, without significantly increasing the risk of untoward immune-mediated effects. Moreover, as clinical experience underlined the additive impact of multiple infections on patient outcome, focus was directed to the possibility of targeting multiple pathogens with a single cellular product (Leen et al., 2006). Results of early proof-of-principle studies, demonstrating feasibility and preliminary efficacy of multivirus-specific T-cells as prophylaxis or treatment of viral infections, were confirmed in almost 200 patients treated with HSCT donor (Gerdemann et al., 2013; Papadopoulou et al., 2014) or banked third-party-derived (Naik S., et al., 2016; Tzannou et al., 2017) products. Responses exceeded 50% even with third-party T-cells, with a rate of GvHD, mostly grade I, ranging from 0 to 20%, and only a few adverse events. The majority of data reported to date derive from phase I or II clinical studies that employed cellular products obtained with different methodologies, conducted in different clinical contexts with heterogeneous cohorts. Thence, optimal dosing and administration schedules are difficult to determine, and will likely depend on the different products and settings. In general, even when doses as low as $1 \times 10^4/\text{kg}$ were administered, clinical response was achieved. However, efficacy partly depends on *in vivo* expansion, and memory cells have a greater potential than terminally differentiated T-cells (Scheinberg et al., 2009); thus, selection of memory subsets may improve the quality of the product. Treatment failure has been largely associated with lack of recognition of target antigens by T-cells, either

due to the absence of relevant epitopes presented during stimulation on the endogenous pathogen strain or, in the case of mismatch between cell donor and recipient, to presentation of immunodominant responses by non-shared HLA antigens (Doubrovina et al., 2012).

Although most of the clinical data have been obtained in HSCT recipients, the use of pathogen-specific T-cells has been translated to other settings, including solid organ transplantation (Comoli et al., 2002) and primary immune deficiencies (Naik S., et al., 2016). In these cases, virus-specific T-cells were obtained either from the patient, or from HLA-partially matched family or third-party donors. The majority of patients were treated for EBV PTLD, and clinical results did not substantially differ from those observed after HSCT, although, in solid organ transplanted patients, *in vivo* expansion of transferred T-cells was partly inhibited by immunosuppressive therapy administered with the goal of preventing the rejection of the transplanted organ. Consequently, the rate of response was lower than in HSCT (Merlo et al., 2010). To overcome this hurdle, pathogen-specific T-cells have been genetically engineered to acquire resistance to calcineurin inhibitors (i.e., tacrolimus and cyclosporine) (Brewin et al., 2009), or to steroids (Basar et al., 2020). Currently, GMP-compliant procedures are available for the most common pathogens, but manufacturing conditions remain a limitation for many centers. Challenges for the immediate future include expanding accessibility to pathogen-specific cellular products using third-party banked T-cells, and the broadened application to other infections, such as adenoviruses, polyomaviruses JC and BK, respiratory viruses, and fungal species.

MESENCHYMAL STEM/STROMAL CELLS AS ADOPTIVE CELL IMMUNOMODULATORY THERAPY

MSCs are spindle-shaped cells endowed with self-renewal and multilineage differentiation capacities, first identified by Friedenstein and co-workers in 1976 (Friedenstein, 1976) in adult

bone marrow, where they substantially contribute to the creation of the haematopoietic stem cell *niche* by providing both the structural support and growth factors needed for development and differentiation of the lympho-haematopoietic system (Charbord, 2010). Subsequently, MSCs were isolated from a number of additional tissues, including adipose tissue, dental pulp, placenta and umbilical cord (Avanzini et al. 2009; Hass et al., 2011), and even generated from induced pluripotent stem cells (Lian et al., 2010). Because of the lack of a specific marker, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cell & Gene Therapy suggested the following three minimal criteria for MSC identification: i) plastic-adherence under standard culture conditions; ii) expression of CD105, CD73 and CD90, and lack of CD45, CD34, CD14 or CD11b or CD19 and HLA-DR surface molecules; iii) *in vitro* differentiation ability into osteoblasts, adipocytes and chondroblasts (Dominici et al., 2006). Their plasticity coupled with excellent expansion potential *ex vivo*, lack or low expression of both HLA class I/II and costimulatory molecules (Le Blanc et al., 2003) that allows their use across HLA barriers without preventive immunoablative treatment (Sundin et al., 2007), as well as the absence of ethical controversies, makes MSCs very attractive for cell-based therapy. In addition, thanks to the ability to interact with virtually any cell population involved in the inflammatory cascade, MSCs are particularly suitable for the use in immune-mediated disorders (Regmi et al., 2019) (Figure 1).

Immunological properties

MSCs prime naïve immune cells towards a tolerogenic profile by creating an appropriate microenvironment, called '*quasi-niche*' (Prockop D. J., et al., 2010), through the secretion of an array of bioactive molecules and vesicles (Figure 2), other than by cell-to-cell contact (Spees et al., 2016). T-cells were the first immunologic cell type shown to be influenced by MSCs. Co-culture of T-cells with MSCs results in the generation of a population of regulatory T-cells expressing CD4, CD25 and the master molecule of immune tolerance, namely the transcription

factor forkhead box factor (FoxP3) (Prevosto et al., 2007). Moreover, MSCs operate a rebalancing of the T-helper 1/2 ratio towards the T-helper 2 profile (Duffy et al., 2011), and potentially inhibit the proliferation of activated T lymphocytes (Aggarwal & Pittenger, 2005) mainly through the induction of cell cycle arrest at the G₀-G₁ phase (Glennie et al., 2005). MSCs also inhibit the cytolytic activity of both CD8⁺ and NK-cells via the non-classical HLA class I molecule, HLA-G (Selmani et al., 2008), and by down-regulating the expression of the NKp30, NKp44 and NKG2D receptors (Sotiropoulou et al., 2006; Spaggiari et al., 2006). In this regard, although MSCs have been shown to be susceptible to recognition and lysis by IL-2-activated NK-cells (Spaggiari et al., 2006), they may resist this attack when stimulated by interferon- γ (Krampera et al., 2006). Differently from T-cells, only scanty information is available on the effects of MSCs on B-cells, with some discrepancies between studies. Similarly to the effect on T-cells, MSCs may block also B-cells in the G₀/G₁ phases, thus interfering with immunoglobulin production (Corcione et al., 2006). However, contrasting evidence was obtained in *in vitro* studies showing a stimulatory effect of MSCs on immunoglobulin production (Rasmusson et al., 2007; Traggiai et al., 2008), whereas an inhibitory action on alloantibody production was shown in another one (Comoli et al., 2008). The different experimental conditions applied do not allow drawing firm conclusion. Noteworthy, there is evidence that MSCs regulate B-cell function through favouring the expansion of a regulatory subset that, by producing considerable amounts of IL-10, induces inhibition of immune response (Franquesa et al., 2015). Besides the action on lymphocytes/NK-cell populations, the interaction of MSCs with other immune cells, such as dendritic cells and monocytes/macrophages, significantly contribute to their potent and long-lasting effects. In details, MSCs are able to affect both the phenotype and function of dendritic cells, by inhibiting the differentiation of CD34⁺ precursors and CD14⁺ monocytes (Nauta et al., 2006), and by interfering with the activity of mature dendritic cells (Ramasamy et al., 2007).

The exposure of mature dendritic cells to MSCs resulted in a shift towards a less mature phenotype characterized by a decreased expression of HLA class II, CD80, CD86, CD40, and CD83 molecules, increased endocytic activity, and reduced production of IL-12 (Jiang et al., 2005). Therefore, the cells were severely impaired in their ability to stimulate proliferation of allogeneic T-cells. This abortive maturation was associated with the expression of a regulatory profile, characterized by the secretion of IL-10, which seems fundamental to delivery the immunosuppressive effect (Zhang B., et al., 2009). As far as the monocyte/macrophages is concerned, MSCs induce the functional skewing of monocytes into M2-like macrophages that secrete IL-10 and, in turn, suppress T-cell proliferation (François et al., 2012) and inhibit NK activation (Chiossone et al., 2016). Moreover, these MSC-educated M2-like cells are able to induce T-helper 2 polarisation of naïve CD4⁺ T-cells (Selleri et al., 2013), thus amplifying the immunomodulatory effect of MSCs. Finally, MSCs have been described to modulate macrophage polarization via a novel mechanism termed 'efferocytosis'. Briefly, the therapeutic activity of MSCs seems to depend on the ability of activated cytotoxic T- and NK-cells to induce their apoptosis and on the subsequent phagocytosis of apoptotic bodies by CD11⁺ cells which, in turn, induce the immunomodulatory effects mainly through the production of the enzyme 2,3 indoleamine-dioxygenase (Galleu et al., 2017). In addition, the efficiency of cytotoxic cells to induce MSC apoptosis has been shown to be a valuable predictive marker of therapeutic efficacy of MSCs, at least in the setting of GvHD (Galleu et al., 2017). At this point, it should be underlined that following intravenous infusion, MSCs accumulate in the lungs and become undetectable within few hours, with only a little amount of cells reaching the target organ (Leibacher & Hendschler, 2016). Consequently, the therapeutic potential of MSCs is guaranteed through either a direct (cell-to-cell contact, secretion of soluble factors and extracellular vesicles) or indirect (efferocytosis) action with the result of modulating the recipient's immune system.

Clinical applications

MSCs were first utilized in clinical trials in the mid-nineties, when they were administered to breast cancer patients undergoing autologous HSCT, in an attempt to accelerate haematopoietic recovery (Koç et al., 2000). This led to their use in the allogeneic setting where the subsequent evidence of a trend toward lower incidence and severity of GvHD solicited further studies (Frassoni et al., 2002). Acute GvHD is still the major cause of morbidity and mortality in patients receiving allogeneic HSCT and it is caused by clonal expansion of end-organ targeting T-cells that lead to an exaggerated inflammatory cascade, called “cytokine storm”. The use of MSCs in GvHD has developed more rapidly than in any other immune-mediated disease, thanks to a case report of a paediatric patient with severe steroid-refractory acute GvHD, which was successfully treated with haploidentical bone marrow-MSCs obtained from the mother (Le Blanc et al., 2004). In light of this seminal case report, several clinical trials applying autologous, haploidentical, or unmatched MSCs have been conducted in Europe and in the United States (Chen et al., 2015). Among the published trials, two large-scale multicenter phase II studies showed exciting results (Le Blanc et al., 2008; Ball et al., 2013), although not fully confirmed by a subsequent phase III trial where patients underwent treatment with an industrial product (Prochymal®, Osiris Therapeutics Inc.; Columbia, MD, USA) (Kurtzberg et al., 2014). The discrepancy can be explained in view of the difference between the MSC manufacturing in terms of culture conditions, passage number, cryopreservation, other than in patient selection (children *versus* adult) and type of transplantation (bone marrow, peripheral blood, or cord blood) (Rizk et al., 2016). It is likely that after many passages in culture (as is the case of prolonged manufacturing of MSCs), epigenetic reprogramming occurred, thus contributing to the loss of therapeutic efficacy (Moll et al., 2014). This has led a German group to use MSC batches obtained from pooled bone marrow mononuclear cells displaying high and identical potency *in vitro* to treat 69 patients (51 children and 18 adults) suffering from treatment-refractory grade

II (4%), III (36%) or IV (59%) acute GvHD (Bader et al., 2018). The day 28 overall response rate was striking (83%) with a median follow-up of 8.1 months. One advantage of MSC immunotherapy as compared with other therapies affecting lymphocyte number and function is the capacity of MSCs to interfere with GvHD pathophysiology, while better preserving the graft-versus-leukemia and graft-versus-infection effects (Auletta et al., 2015).

Systemic lupus erythematosus (SLE) is characterized by hyperactive B-cells producing auto-antibodies against RNA-binding proteins, phospholipids, and double-stranded DNA, which can lead to multiorgan dysfunction. Following early case reports, three phase II trials were carried out in China evaluating the safety and efficacy of MSCs in refractory SLE (Li et al., 2013; Gu et al., 2014; Wang D., et al., 2014). Remarkably, systemic administration of allogeneic MSCs improved cytopenia and reduced SLE disease activity in 35 patients with a mean follow-up of 21 months (Li et al., 2013). Clinical remission was mirrored by increased regulatory T-cell and decreased T-helper 17 subsets. A subsequent phase II study was an open-label trial conducted on 81 patients with active and refractory lupus nephritis who underwent one intravenous infusion of allogeneic MSCs (Gu et al., 2014). In total, 60.5% patients achieved disease remission during the 12-month follow-up, with a marked improvement of both renal function and disease activity index. In addition, the doses of concomitant steroid and immunosuppressive drugs could be tapered. Finally, a multicenter clinical trial was undertaken to assess the safety and efficacy of two intravenous infusions of umbilical cord-MSCs in 40 patients with refractory SLE (Wang D., et al., 2014). The overall survival rate was 92.5% and the MSC infusions were well tolerated, with no treatment-related adverse events. Thirteen (32.5%) patients achieved clinical response, while 11 (27.5%) partial response during 12 months of follow-up. Disease activity scores critically improved, mostly those related to lupus nephritis. It is also worth noting that serum antinuclear and anti-double-stranded DNA antibodies decreased after MSC treatment.

Rheumatoid arthritis is characterized by the expansion of synovial fibroblasts, aberrant leukocyte infiltration, and secretion of cytokines and proteases within the joints leading to cartilage destruction and bone erosion. Because of conflicting experimental data showing amelioration (Augello et al., 2007), no benefit (Djouad et al., 2005) or even worsening (Sullivan et al., 2012) of the disease in the well-established animal model, the collagen-induced arthritis model, clinical trials have lagged behind those of other autoimmune diseases. Currently, a few proof-of-concept studies utilizing MSCs for treatment-refractory RA have demonstrated satisfactory safety profile, and several are underway (Lopez-Santalla et al., 2020).

MSC adoptive cell immunotherapy represents a new frontier also for treating neurological diseases. The most commonly investigated conditions are multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS). MS is a chronic T-helper 1/17-mediated demyelinating disease that affects young adults and leads to progressive and irreversible damage of the central nervous system. ALS is a neurodegenerative disease that selectively affects motor neurons, thereby leading to bulbar, respiratory, and limb weakness. So far, MSCs from different sources and given either intravenously or intrathecally have been applied for treatment of these disorders with promising results, although the need to optimize the administration route, the dosage/s, and the eligibility criteria do not allow drawing firm conclusion (Abati et al., 2019). Interestingly, an increase of the proportion of regulatory T-cells and a reduction of activated dendritic cells and lymphocytes became evident, thus confirming the *in vivo* immunomodulatory effects of MSCs. By contrast, the possibility of regeneration via the transdifferentiation of MSCs into neuronal or glial cells, although theoretically possible, has yet to be proven.

As far as Crohn's disease is concerned, i.e., a segmental chronic inflammatory enteropathy, a number of open-label phase I-II studies were carried out testing the use of either autologous or

allogeneic systemic infusions of bone marrow- and placenta-derived MSCs (Ciccocioppo et al., 2018). These studies showed that this therapeutic approach is feasible and safe, as well as significantly effective, since disease remission was achieved in half the patients with a follow-up ranging from 6 weeks to 24 months despite only refractory cases were enrolled. However, the discrepancy between clinical outcomes and mostly the lack of endoscopic data may have resulted in an overestimation of the success rate. This prompted the Gastrointestinal Committee of the International Society for Cell & Gene Therapy to establish a Consensus in an effort to design informative and consistent clinical trials for the intravenous use of MSCs in this condition (Ciccocioppo et al., 2019a). Clearer and more unambiguous results were obtained when using MSC local injections for fistulising refractory Crohn's disease. This new treatment option gave excellent results in terms of both safety and efficacy (Ciccocioppo et al., 2019b). Notably, the results of the first phase III multicentre trial, where 212 patients were enrolled and randomly assigned to receive a single local injection of an industrial preparation of allogeneic adipose tissue derived-MSCs (Darvadstrocel, formerly Cx601®) or placebo, showed that MSCs performed better than placebo to achieve combined remission (51.5% versus 35.6% at week 24) within a shorter period of time (Panés et al., 2016). Following this evidence, Darvadstrocel (Alofisel®, Takeda) has received a positive opinion from the Committee for Medicinal Products for Human Use of the EMA. Moreover, fistula healing was maintained in most cases (56.3%) at one year (Panés et al., 2018), although further studies showed that the proportion of patients relapsing upon a longer follow-up increased over time (Ciccocioppo et al., 2015). Importantly, no safety concerns were reported, thus confirming the tolerability of the treatment, while, again, a sustained increase of regulatory T-cells was invariably observed in those cases where it was investigated (Ciccocioppo et al., 2019b).

Moving on to type 1 diabetes, upon strong experimental evidence of prevention of autoimmune attack against β -cells in both the streptozotocin (Tang et al., 2014) and non-obese (Lee R.H.,

et al., 2006) mouse models, as well as protection against the transfer of diabetes by T-cells isolated from treated mice (Madec et al., 2009), systemic infusions of autologous MSCs were applied in new-onset type 1 diabetes patients and proved successful in blocking disease progression and preserve β -cell function (Carlsson et al., 2015).

A 'cytokine storm' has been recognized as the main cause of respiratory failure and mortality in Coronavirus disease-19 (Vabret et al., 2020). Among the wide array of therapeutic options tested, MSCs has received much attention considering the growing number of trials registered in the database www.ClinicalTrials.gov. The rationale for MSC use in Coronavirus disease-19 patients lies on the promising data obtained in both animal model (McIntyre et al., 2016) and human (Matthay et al., 2019) acute respiratory distress syndrome, as well as on *in vitro* studies showing the ability of MSCs to shift the lung microenvironment towards an anti-inflammatory and anti-fibrotic pattern mostly through the production of keratinocyte growth factor and polarization of resident macrophages into regulatory ones (Lee J.W., et al., 2013). To date, two phase I studies have been published on the use of one (Leng et al., 2020) or three (Meng et al., 2020) intravenous infusions of MSCs in Coronavirus disease-19 showing the feasibility, safety and efficacy of this therapeutic strategy in rescuing patients with moderate to severe pneumonia.

Moving to application in solid tumours, MSCs can be also genetically modified to produce the cytotoxic molecule known as TRAIL, capable of inducing tumour cell apoptosis without promoting significant toxicity in healthy tissues, as reported instead with the systemic administration of TRAIL (Dianat-Moghadam et al., 2020). The first report of the clinical use of genetically-modified autologous bone marrow-derived MSCs suggested that the approach is feasible, safe and well tolerated (von Einem et al., 2017). Thanks to the possibility of consistently isolating and modifying MSCs from human adipose tissue by minimally invasive surgical procedures (Foppiani et al., 2019), adipose tissue-derived MSCs engineered to produce

TRAIL variants were tested in a variety of preclinical models, obtaining evidence of a specific cell-tumour pro-apoptotic activity without toxicity (Spano et al., 2019; Grisendi et al., 2010). Based on these findings, this strategy is being moved in the clinic in a phase I/II trial (expected to open enrollment in mid 2021) aimed at assessing safety and early efficacy for the treatment of locally advanced pancreatic ductal adenocarcinoma in combination with chemotherapy as first-line treatment.

CONCLUSIONS

As documented by the abundance of clinical data discussed in this review article, the last two decades have yielded significant progress in the application of cell-based products as a new therapeutics. The use of ATMPs perfectly fits with the need of precision medicine and systems pharmacology of the modern era. However, an extraordinary effort towards elimination of barriers between scientists, physicians, regulatory agencies and industry professionals should be undertaken to guarantee the realization of their potential. Surely, crucial points remain open, including the cost analysis and the reimbursement assessment, mostly because the traditional business model does not apply to ATMP manufacturing. Importantly, we should avoid the medical tourism of patients suffering from incurable diseases who search for last resort treatment, and may be attracted by unapproved cell therapies. Conversely, a greater awareness and support of patients and their families need to be built-up. Therefore, more than a simple novelty, the advent of cellular therapies represents an epochal cultural change where also the establishment of progressive politics and specific task forces will help this revolution and may represent a tremendous boost for both scientific and industrial development.

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CONFLICT OF INTEREST DISCLOSURE: R.C. is member of the Advisory Board Takeda Italia on Alofisel®. M.D. has patents in the field of cell and gene therapy and is co-founder of Rigenerand srl, a University start-up company developing gene therapy approaches for cancer. MD is also member of the Board of Directors of Rigenerand srl. M.D. roles are managed by

University of Modena & Reggio Emilia in accordance with internal regulations and conflict of interest policies.

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ABBREVIATIONS. ALS: amyotrophic lateral sclerosis; AML: acute myeloid leukemia; ATMP: advanced therapy medicinal product; BCP-ALL: B-cell precursor acute lymphoblastic leukemia; B-NHL: B-cell non-Hodgkin lymphoma; CAR: chimeric antigen receptor; CLL: chronic lymphoid leukemia; CR: complete remission; EBV: Epstein-Barr virus; EMA: European Medicines Agency; FDA: Food and Drug Administration; GMP: good manufacturing practice; GvHD: graft-*versus*-host disease; hCMV: human Cytomegalovirus; HLA: human leucocyte antigen; HSCT: haematopoietic stem cell transplantation; IL: interleukin; MM: multiple myeloma; MS: multiple sclerosis; MSC: mesenchymal stem/stromal cell; NK: natural killer; PTLN: post-transplant lymphoproliferative disease; r/r: relapsed/refractory; SLE: systemic lupus erythematosus; T-ALL: T-cell acute lymphoblastic leukemia; TNF: tumour necrosis factor; TRAIL: tumour necrosis factor-related apoptosis inducing ligand.

LIST OF HYPERLINKS:

CD19: <https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=2764>

CD22: <https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=2786>

B-cell maturation antigen:

<https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=1889>

CD33: <https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=2601>

CD38: <https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=2766>

TRAIL:

<https://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=5065>

CD123:

<https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=1705>

Interleukin-13

receptor

$\alpha 2$:

<https://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=1701>

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Table 1. Summary of the most relevant clinical trials on chimeric antigen receptor (CAR) T-cells for B-cell precursor acute lymphoblastic leukemia (BCP-ALL) and B-cell Non-Hodgkin lymphoma (B-NHL).

CAR construct	Disease	NCT number	Response	Toxicities	Reference
CD28-z- CD19 CAR T	Resistant/refractory ALL in children and young adults	NCT01593696	CR: 70% 48% consolidation with HSCT T cells persistence: up to 68 days	Fever, Hypokalaemia, Neutropenia, CRS (76% of the patients, 28% severe)	Lee DW 2015
CD28-z- CD19 CAR T	Resistant/refractory ALL in adults	NCT01044069	CR: 88% 44% consolidation with HSCT T cells persistence: 2-3 months	CRS	Davila ML 2014
4.1BB-z- CD19 CAR	Resistant/refractory	NCT01626495 NCT	CR: 81%-90% T cells persistence:	CRS (100% in NCT01626495,	Maude SL 2014

T	ALL in children and adults	02435849	up to 2 years	with 27% severe CRS; 77% in NCT 02435849) Neurotoxicity	Maude SL 2018
CD28-z-CD19 CAR T	Resistant/refractory ALL in adults	NCT01044069	CR: 83%	CRS (severe in 26%) Neurological toxicities (severe in 42%) Infections	Park JH 2018
CD28-z-CD19 CAR T	Refractory Aggressive B-NHL	NCT02348216	ORR: 82% CR: 54% OS at 18 mo: 52%	CRS, Neurotoxicity	Neelapu SS 2017
4.1BB-z-CD22 CAR T	Relapsed/refractory ALL	NCT02315612	CR: 73%	CRS, cytopenia	Fry TJ 2018
4.1BB-z-	Resistant/refractory	NCT03185494	CR:100%;	CRS (100%; all	Dai H

CD19/CD22 CAR T	fractory ALL in adults		50% relapse (1 CD19neg/CD22dim)	grade 1-2 No neurotoxicity	2020
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Abbreviations. CR: Clinical Response; CRS: Cytokine Release Syndrome; HSCT: Haematopoietic Stem Cell Transplantation; ORR: Overall Response Rate.

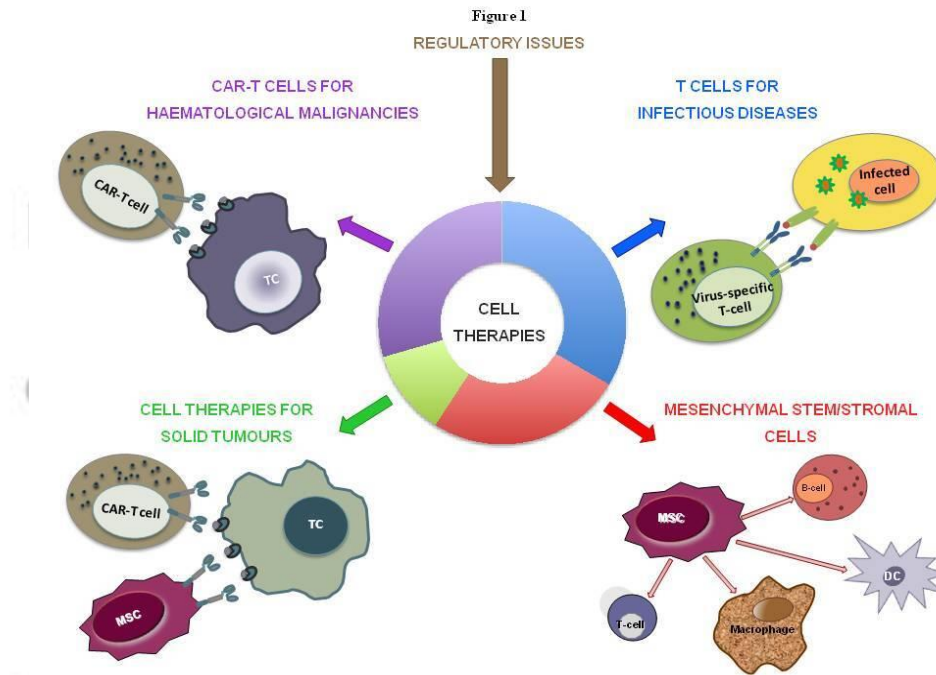


Figure 1. Schematic representation of the main topics discussed in the review. Cell therapies, mainly based on the use of CAR T/NK-cells for haematological malignancies and solid tumors expressing tumour-associated antigens, pathogen-specific T-cells engaging infected cells through the cognate interaction of their T-cell receptor with the MHC-pathogen antigen complex to treat opportunistic infections, and mesenchymal stem/stromal cells interacting with any immune cell population for the use in immune-mediated conditions and, upon *ad hoc* engineering, also in solid tumours, are discussed in this review, together with the regulatory issues governing their production, commercialisation and use in Europe. Abbreviations: CAR: chimeric antigen receptor; DC: dendritic cell; HMC: haematologic malignant cell; MHC: major histocompatibility complex; MSC: mesenchymal stem/stromal cell; NK: natural killer; STC: solid tumour cell.

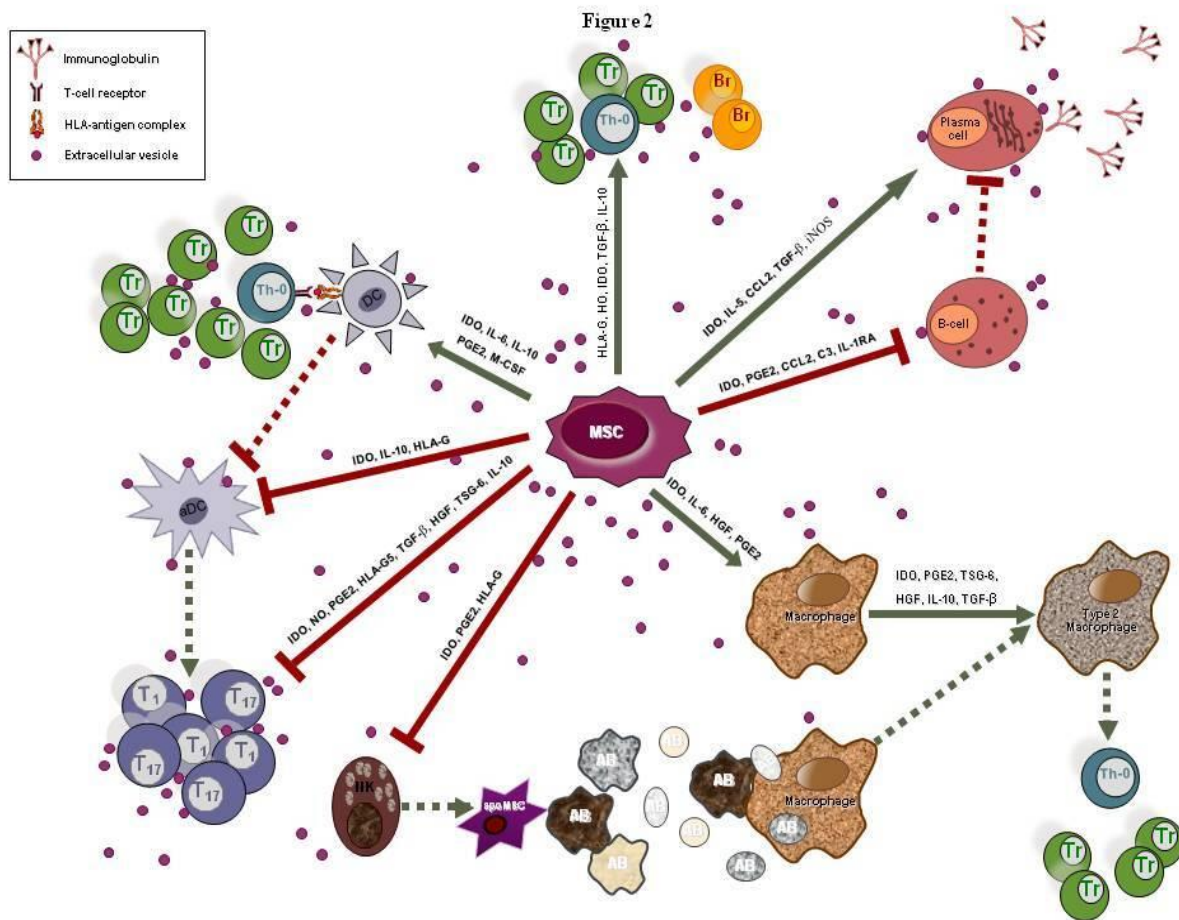


Figure 2. Main immunomodulatory mechanisms of mesenchymal stem/stromal cells. MSCs possess broad immunomodulatory properties that are displayed on any immune cell population through either cell-cell contact (Fas/FasLigand cognate interaction, Programmed Death1 system; notch pathway; Toll like receptors) or through the production of an array of bioactive molecules (as detailed in proximity of the lines) and extracellular vesicles that function as cargo of soluble mediators, including nucleic acids, mitochondria, growth factors, cytokines, lipids. The end result is the creation of a microenvironment – called ‘*quasi-niche*’ – that stimulate a phenotypic switch of the target cells. Green lines indicate permissive effects, red lines indicate inhibitory effects, dot lines indicate indirect actions of MSCs. In addition, engulfment of apoptotic bodies by macrophages, upon killing of MSCs from activated NK- and T-cells, results in the switch of macrophages towards a tolerogenic profile. Taken together, these events explain why the duration of the therapeutic effects of MSCs exceeds their survival in the host.

Abbreviations: aDC: activated dendritic cell; AB: apoptotic bodies; apoMSC: apoptotic mesenchymal stem/stromal cell; Br: regulatory B-cell; C3: complement; CCL: chemokine ligand; DC: dendritic cell; HGF: hepatocyte growth factor; HLA: histocompatibility locus antigen; HO: haemoxygenase; IL: interleukin; IL-1RA: interleukin-1 receptor antagonist; IDO: indoleamine 2,3-dioxygenase; iNOS: inducible nitric oxide synthase; MSC: mesenchymal stem/stromal cell; NK: natural killer cell; PGE2: prostaglandin E2; TGF: transforming growth factor; Th0: T-helper 0; T1: T-helper 1; T17: T-helper 17; Tr: regulatory T-cell; TSG: tumour necrosis factor-stimulated gene.