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IM - REVIEW

Regenerative medicine: the red planet for clinicians

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Abstract
Regenerative medicine represents the forefront of health sciences and holds promises for the treatment and, possibly, the cure of a number of challenging conditions. It relies on the use of stem cells, tissue engineering, and gene therapy alone or in different combinations. The goal is to deliver cells, tissues, or organs to repair, regenerate, or replace the damaged ones. Among stem-cell populations, both haematopoietic and mesenchymal stem cells have been employed in the treatment of refractory chronic inflammatory diseases with promising results. However, only mesenchymal stem cells seem advantageous as both systemic and local injections may be performed without the need for immune ablation. Recently, also induced pluripotent stem cells have been exploited for therapeutic purposes given their tremendous potential to be an unlimited source of any tissue-specific cells. Moreover, through the development of technologies that make organ fabrication possible using cells and supporting scaffolding materials, regenerative medicine promises to enable organ-on-demand, whereby patients will receive organs in a timely fashion without the risk of rejection. Finally, gene therapy is emerging as a successful strategy not only in monogenic diseases, but also in multifactorial conditions. Several of these approaches have recently received approval for commercialization, thus opening a new therapeutic era. This is why both General Practitioners and Internists should be aware of these great advancements.

Keywords Gene therapy · Regenerative medicine · Stem cells · Tissue engineering

Abbreviations

AAV	Adeno-associated viral
ADA	Adenosine deaminase deficiency
AdV	Adenoviral
CAR	Chimeric antigen receptor
ECM	Extracellular matrix
ESC	Embryonic stem cells
HSC	Haematopoietic stem cells
iPSC	Induced pluripotent stem cells
LV	Lentiviral vector
MSC	Mesenchymal stem cells
RM	Regenerative medicine
RV	Gammaretroviral vector
SCID	Severe combined immune-deficiency
SMA	Spinal muscular atrophy

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Introduction

During the course of a century, we have witnessed a radical change of the disease spectrum, moving from acute and infective to chronic and degenerative illnesses. We are expected, therefore, to change our therapeutic approach from rescuing to supporting patients in the long-term while reverting the aging processes. This has led to the advent of regenerative medicine (RM), a multidisciplinary field of health sciences (Fig. 1) that aims at repairing, regenerating, or replacing dysfunctioning cells, tissues or organs using allogeneic or autologous cells, supporting scaffolding biomaterials, gene modifications, and molecular cues, alone or in combination.

However, as recently emphasized, the boundaries of RM remain unclear "mainly due to the lack of a universally accepted definition, the lack of clarity of its potential modalities of application and the unjustified and misleading

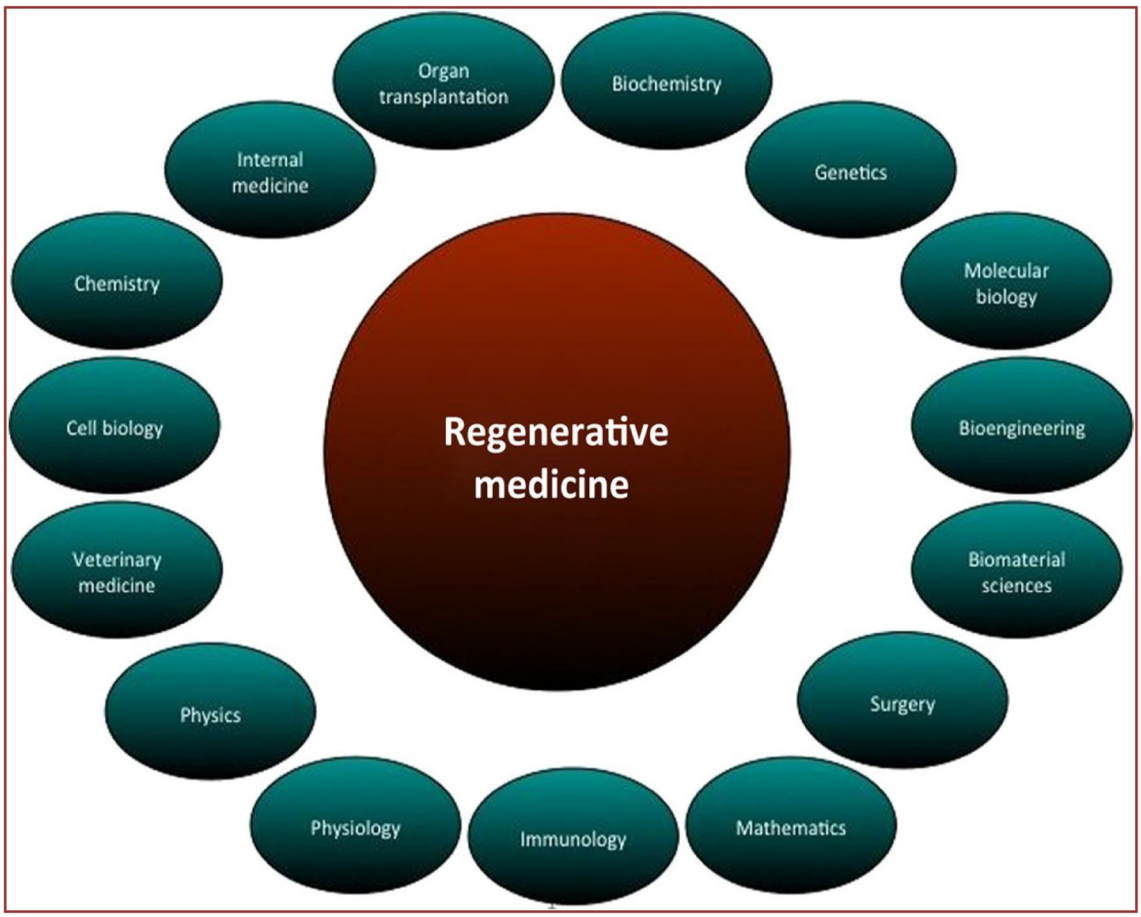

Fig. 1 Regenerative medicine. This is a superdiscipline in which scientists with a high expertise in different fields share the common goal of repair, replace, or regenerate tissues and organs through an integrated work plan

hype that often follows the reports of clinical application of RM technologies. All this generates excessive (and unmet) expectations and an erroneous perception of what RM really is and can offer" [1]. As correctly highlighted by the United Nations Educational, Scientific and Cultural Organization in 2006, RM is a superdiscipline, whose contours are still being (and need to be) defined (http://unesdoc.unesco.org/images/0014/001454/145409e.pdf), but these contours will de traced on the basis of clinical needs that are sought to be met. For instance, from a transplant perspective, the application of RM technologies should aim at either engineering marginal organs to make them transplantable and so minimize the discard rate, or at manufacturing organs from patients' own cells to maximize the donor pool and, therefore, nullify the waiting list. On the other side, the therapeutic approach contemplated by medical disciplines does not generally aim at "replacing" damaged tissues or organs, but rather at "repairing and regenerating" tissues and organs showing a more or less pronounced functional impairment. Herein, we will briefly review the three main RM technologies that are of interest to Internal Medicine, namely, tissue engineering, stem-cell treatment, and gene therapy with a focus on those tools that have already received or are going to receive approval for commercialization while highlighting the challenges that this field is facing (Table 1).

Tissue engineering

The term 'tissue engineering' refers to a field of health sciences that aims at restoring the function of injured tissues using a combination of cells, supporting scaffolding materials, molecular and physical cues that, harnessed together, will eventually recreate the damaged and functionally impaired tissue. The term 'tissue engineering' was coined in 1993 by Langer and Vacanti [2], a transplant surgeon and a basic scientist, respectively, and was conceived to address the most urgent need—at that time—of transplant medicine, namely, the lack of transplantable organs [1, 3]. Interestingly, tissue engineering proposes the use of patient's own cells to manufacture the tissue of interest, to bypass the need for anti-rejection medications; this, per se, will tackle the

Table 1 Overview on potential and hurdles of regenerative medicine strategies

Treatment strategy	Components	Source	Mechanisms of action	Risks	Limitations
Tissue engineering	Stem/progenitor cells Scaffolds Chemical/physical compounds	Autologous Allogeneic Xenogenic	Fill a gap Replace a damaged tissue/organ	Tissue survival Tissue rejection	Engraftment Vascularization
Embryonic stem cells	Embryo cells	Allogeneic	Repair Replace Regenerate	Malignancy	Ethics concerns Rejection
Induced pluripotent stem cells	Differentiated adult cells	Autologous	Repair Replace Regenerate	Malignancy	Stability Integration Dosage
Haematopoietic stem cells	Adult stem cells	Autologous Allogeneic	Recapitulate immune system Regenerate	Graft-versus-host disease Infections Malignancy	Invasiveness Donor shortage
Mesenchymal stem cells	Adult stem cells	Autologous Allogeneic	Aid the regeneration of damaged tissue/organ Immuno-modulation	Thromboembolism	Manufacturing
Epithelial stem cells	Adult stem cells	Autologous	Fill a tissue gap	Tissue survival	Engraftment
Gene therapy	Replacement Addition Subtraction Editing	Autologous	Correct defective function	Mutagenesis, immune response	Efficiency Persistence Control

other big problem that modern transplantation faces, which is the chronic toxicity that patients suffer from lifelong immunosuppression.

Depending on the degree of impairment of the tissue that needs to be replaced, two alternative strategies can be considered to build tissues ex vivo. If a tissue is only partially impaired, function can be restored through the implantation of a patch or section of the whole tissue. Instead, if the organ in question has exhausted its functional reserve, then the whole organ needs to be fabricated. Thus far, few hundreds of patients have already received body parts that were produced from their own biomaterials, with promising results that, however, still need to be validated in larges series and in more complex and challenging clinical scenarios [3]. Moreover, while the technologies that enable the manufacturing of tissue sections or patches have already reached the bedside, the implantation of more complex tissue engineered organs, like the kidney or the heart, has never been reported, not even in small animal models.

Tissue patches can either contain cells or be acellular. For example, cellular tissue patches can be obtained with the so-called "cell-sheet" technology pioneered by Teruo Okano [4, 5]. Briefly, cell layers (namely, sheets) are prepared using temperature-responsive culture dishes on whose surface temperature-responsive polymers are covalently grafted. This allows various types of cells to adhere and proliferate at 37 °C and to spontaneously detach when the temperature is reduced below 32 °C without the need for proteolytic enzymes [6]. The confluent cells are non-invasively harvested as single, contiguous cell sheet with intact cell–cell junctions and deposited extracellular matrix (ECM) and can be applied to the diseased anatomical district either as monolayer (two-dimensional) or as multilayer (two-dimensional). This technology bears immense potential to offer alternative therapeutic tools to treat a myriad of different diseases as recently reviewed [7].

However, the patch may also be acellular. In fact, ECM-based patches contain a wealth of growth factors and cytokines that are critical for tissue homeostasis [8]. These molecules will be released to attract cells from adjacent tissues that will eventually proliferate and build a new tissue. If the tissue defect is small, a patch may be applied to successfully fill the gap as reported by Nieponice et al. in patients who had undergone extended endoscopic mucosal resection [9]. In his series, four patients requiring esophageal reconstruction underwent a patch esophagoplasty using an ECM scaffold obtained from the porcine urinary bladder ECM. The full thickness esophageal defect was filled (=repaired) with an ECM patch that was sutured to the edges of the remaining oesophagus, similar to the patch angioplasty performed in vascular procedures. Reportedly, all patients had a favorable clinical outcome and oral intake was reinstated after 7 days. One patient had a micro leak at day 5 that underwent spontaneous closure 2 days after drainage. Follow-up radiological studies showed adequate esophageal emptying through the surgical segment in all patients, while endoscopy showed complete mucosal remodeling at 2 months. Interestingly, the area of the defect was indistinguishable from surrounding healthy tissue and displayed normal squamous epithelium.

The oesophagus seems to offer an ideal playground for the application of tissue engineering and RM technologies. In fact, a multidisciplinary team in London (United Kingdom) was able to grow and successfully transplant into rodents a functional oesophagus engineered from stem cells [10]. Briefly, the rat oesophagus was decellularized to obtain its acellular ECM-based counterpart that was seeded with muscular and epithelial precursors. The so-obtained construct was allowed to mature into an omental wrap to enhance vascularization, and eventually implanted in vivo. The experiment provided a fully re-populated, structurally organized, and pre-vascularized oesophageal substitute that—should translation in human beings occur—will offer an alternative treatment strategy for oesophageal defects. In the future, the plan will be to regenerate the cellular compartment using patient's own cells, either adult cells, as well as progenitors and/or stem cells.

Stem-cell therapy

Stem cells are characterized by the unique capacity to renew themselves indefinitely and to differentiate into specialized cell types through an asymmetrical division. Several types of stem cells have been identified, such as embryonic stem cells (ESC), haematopoietic stem cells (HSC), mesenchymal stem cells (MSC), and induced pluripotent stem cells (iPSC). While ESC and iPSC are totipotent, meaning that they have the ability to give rise to differentiated progeny representative of the three germ layers, HSC and MSC are multipotent and give rise to progeny of only one cell lineage. Moreover, each tissue is endowed with unipotent stem cells, i.e., cells committed to differentiate only in a tissue-specific fashion, that are responsible for both the physiologic turnover and healing process when damage occurs.

Embryonic stem cells

These are totipotent stem cells derived from the inner cell mass of blastocyst-stage embryos. Human ESC were first isolated in 1998 [11]. They carry the potential to repair, replace, and regenerate all defective or damaged cells and tissues. However, the enthusiasm for ESC suddenly collapsed because of the ethical debate surrounding their retrieval. Indeed, the process to obtain ESC requires the destruction of a human embryo that otherwise could have developed to term. Another challenge to ESC use is the need for solutions to avoid teratoma formation and immune rejection [12]. In the recent past, reducing the risk of tumor development has been aided by progress in the purification of the final cellular preparation. By contrast, the issue of immune rejection still requires to be addressed. Therefore, ESC-based therapy may currently be applied only in those immune-privileged sites, such as the retina, as already showed in a case series of patients with macular degeneration or dystrophy [13].

Induced pluripotent stem cells

This next population of totipotent stem cells was discovered in 2007 when terminally differentiated fibroblasts were reprogrammed to generate iPSC that showed the same plasticity as ESC [14]. The great advantage is that the starting cell can be patient-derived, eradicating the need for both human embryo use and immunosuppressive treatment. The first applications of iPSCs were in the generation of cardiac [15], renal [16], liver [17], and pancreatic [18] tissues. Furthermore, the recellularisation process of scaffolds was used to produce organs for transplantation. A further application of iPSC has involved deriving organoids with the capability of modeling the functional unit of a specific human tissue [19, 20]. Organoids hold the potential to unravel mechanisms involved in both human physiology and disease and to develop new drugs without the need for animal models. Therefore, iPSC have the tremendous potential to be an unlimited source of any tissue-specific cells, including HSC and MSC. However, their use has raisen several safety concerns, since the possibility for dangerous clones to emerge, the risk of contamination from still undifferentiated cells, the genomic instability, and the possibility of epigenetic aberrations, are all issues that need to be primarily solved together with the determination of the appropriate dosage [21, 22] before translating this therapeutic tool at bedside. Meanwhile, their utility in the development of tissue banks for RM purposes should not be missed. In this regard, in 2007, the International Stem-Cell Banking Initiative was established involving world leading groups in the field of iPSC banking, stem-cell biology, and regulatory bodies, with the aim to promote global harmonization and standardization of the use of these stem cells in both research and clinics [23]. The last workshop was held in 2016, where priority issues were considered the safety and the efficacy of a final cell therapy product and quality assured source materials, and particular emphasis was given to ethics matter [24].

Haematopoietic stem cells

Bone marrow contains at least two types of stem cells. One population consists of $CD34^-$ cells called MSC (see below), and the other is represented by $CD34^+$ cells named HSC and capable of differentiating into all blood cell lineages. The therapeutic application of the latter comprises both autologous and allogeneic transplantation for treatment of onco-haematological conditions and, more recently, even of refractory autoimmune conditions. In this regard, some degree of success was reported by the European Group for Blood and Marrow Transplantation showing the

achievement of sustained remission in selected cases suffering from multiple sclerosis, systemic scleroderma, rheumatoid arthritis, systemic lupus erythematosus, and other conditions [25]. The type of the disease rather than the transplant technique was the most relevant determinant of outcome in this study. Indeed, a European randomized clinical trial enrolling patients with refractory Crohn's disease, who underwent autologous HSC transplantation raised significant safety concerns and did not achieve the ambitious primary outcome of disease remission at 1 year [26]. Recently, the therapeutic target of HSC transplantation has been expanded to certain inherited neurologically devastating metabolic diseases, including lysosomal storage diseases and peroxisomal storage diseases, and to the autism spectrum disorders with some positive results [27]. In addition, the possibility to regenerate the myeloablated haematopoietic system in cancer patients [28], to give raise to a functional immune system in a wide spectrum of primary immunodeficiencies [29], and to induce tolerance to mismatched organ allografts [30] represent all new applications of HSC transplantation.

Mesenchymal stem cells

This cell population is also defined 'stromal' in view of its supportive function in creating the appropriate niche for HSC differentiation into cells of the lympho-haematopoietic system. Other than from the bone marrow, MSC have been also isolated from a variety of further adult and foetal tissues, including adipose tissue, muscles, skin, liver, kidney, spleen, placenta, amniotic fluid, and umbilical cord [31]. Because of the lack of a specific surface marker, the International Society for Cellular Therapy established the minimum criteria to be met to classify a cell as an MSC, as follows: (1) adherence to plastic under standard culture conditions; (2) expression of CD105, CD73, and CD90; (3) lack of expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and human leukocyte antigen-DR; and (4) differentiation into osteoblasts, adipocytes, and chondroblasts following in vitro culture under specific conditions [32]. In the last decade, this cell population has rapidly attracted the interest of both scientists and clinicians as evidenced by the growing number of basic and clinical publications [33]. At this point, it should be emphasized that MSC were initially thought to display only regenerative potentiality given their ability to differentiate into several cell types of the mesoderm lineage. Subsequently, they were also found to display tremendous effects on all cells involved in immune response with the end result of dampening inflammation and favouring tolerance [34]. Further reasons for their attraction in cell-based therapy are their immense plasticity, easy expansion ex vivo with maintenance of genetic stability, homing to inflamed sites and ability to repair injured tissues, and, importantly, the absence of ethical controversies. Notably, unstimulated MSC lack of expression of class II human leukocyte antigens and co-stimulatory molecules on their surface while displaying expression of class I human leukocyte antigens at very low levels. This is why MSC use does not require an immunoablative conditioning regimen, even when using an allogeneic source [35]. However, it may not suffice to fend off acquired alloimmunization, thus leading to the notion that MSC are 'immunoevasive' instead of 'immune-privileged' as previously proposed [36, 37]. At this point, it should be emphasized that MSC do not stably engraft the patient, and their therapeutic effect seems to depend on the ability of the host natural killer cells to kill MSC and generate MSC-derived apoptotic bodies that deliver the immunomodulant action directly to the target organ in both humans and experimental model of graft-versus-host disease [38]. All these findings have led to the application of MSC as new therapeutic strategy in those conditions characterized by a disregulated immune response and progressive tissue damage. The first application was to a boy suffering from steroid-refractory acute graft-versus-host disease who was rescued by intravenous infusions of haplo-identical MSC [39]. Since this, a number of clinical trials were performed, including phase III studies [40], that have led to the approval of Remestemcel-L® (Mesoblast Ltd; bone-marrow-derived MSC from healthy donors) for the treatment of this condition in Japan. MSC have also been used in those immune-mediated disorders, where autologous HSC transplantation was indicated, such as multiple sclerosis, systemic lupus erythematosus, rheumatologic, and intestinal chronic inflammatory diseases refractory to standard treatments [41], with promising results. A unique opportunity offered by MSC is the possibility of local injections. In this regard, Darvadstrocel (Alofisel®, Takeda; formerly Cx601 by TiGenix), a ready-to-use industrial preparation of adipose tissue-derived MSC from healthy donors, has recently received approval from the European Medicines Agency to treat complex perianal fistulas in Crohn's disease, following the evidence of its safety and efficacy in achieving fistula healing in the registrative trial [42]. Therefore, after more than a decade of clinical application of MSC, three overarching conclusions may be drawn. First, they are overwhelmingly safe, since the most relevant risks are transient fever [43] and thromboembolic events [44], that can be avoided with proper manufacturing and delivery of the cell preparation. Second, long-term engraftment and survival are extremely low, thus avoiding the risk of malignant transformation. Their mechanism of action, indeed, does not depend on their differentiation into specific end-organ cells, but rather on the creation of an appropriate microenvironment, where both resident and immune cells are aided in re-establishing tissue homeostasis [45]. Third, the standardization of source, dosage, delivery strategy, number, and timing of infusions in each specific clinical setting needs to be carefully assessed, together with

the identification of functional markers of potency to meet regulatory authority requirements for conduct of advanced clinical studies and their eventual registration. In this regard, the Consensus of the International Society for Cell and Gene Therapy identified three preferred analytic methods that could inform a matrix assay approach: quantitative RNA analysis of selected gene products; flow cytometry analysis of functionally relevant surface markers; and protein-based assay of secretome [46].

Epithelial stem cells

In addition, tissue-specific stem cells are being used for regenerative purposes. One product, Holoclar® (Chiesi Pharma), has recently received approval for commercialization. It consists of a sheet derived from autologous limbal stem cells, i.e., the cells responsible for renewal and repair of the cornea, plated on a fibrin matrix, and then implanted directly in the patient's eye [47]. Holoclar® is indicated for use in chemical and physical ocular burns causing limbal stem-cell deficiency. In this condition, bulbar conjunctival cells invade the corneal surface in an attempt to regenerate the epithelium. However, this process results in neovascularization, inflammation, scar formation, and corneal opacity leading to loss of vision.

Another promising use of epithelial stem cells is for treatment of a dismal and often lethal genetic disease, which is junctional epidermolysis bullosa. The defect is in the genes encoding laminin, a component of the skin basement membrane, which causes the development of chronic wounds with loss of fluids, impairment of body temperature control, and development of skin cancer. Recently, a boy suffering from a life-threatening form of this condition was rescued and definitely cured by using epidermal grafts derived from his own keratinocytes. These cells were preventively transduced with a retroviral vector to express the full length of the correct cDNA within the grafts [48].

Gene therapy

Gene therapy refers to the introduction of a gene—called transgene—into cells of the body for a therapeutic purpose (Fig. 2). It was originally conceived to replace a defective function in monogenic diseases such as muscular dystrophies, cystic fibrosis, and metabolic or lysosomal storage disorders. Currently, its applications are being broadened to multifactorial conditions including cardiovascular, neurodegenerative, infectious diseases, and cancer [49]. To deliver the transgene into target cells, a vehicle known as 'vector' is needed to target the nucleus and direct gene expression without adverse effects in the recipient. Gene transfer vectors include chemical and physical methods, biocompatible nanoparticles (non-viral vectors), or viral-derived vectors. Mammalian cells have developed physical, chemical, and biological barriers to protect their own genetic information. Since viruses have evolved strategies to overcome the barriers to gene transfer into cells, virus-derived vectors are the optimal vehicles for delivery of transgenes into human cells. Recombinant viral vectors are designed to harness the native viral infection pathways but to limit their replicative life cycles. The cycle ends with the transduction and the insertion of the recombinant viral genome into target cells. In addition to the transgene, vectors have to carry the elements necessary to express it. The transgene and its regulatory elements are referred to as 'expression cassette'. Viral vectors can be broadly categorised in integrating and non-integrating, depending on their ability to mediate integration of the vector genome into recipient cells' nuclear DNA. Efficiently integrating vectors are gammaretroviral (RV) and lentiviral vectors (LV), while adenoviral (AdV) and adeno-associated viral (AAV) vectors are classified as non-integrating vectors and will be discussed further below.

Gene therapy strategies

Gene therapy strategies can be classified into: (1) gene replacement; (2) gene addition; (3) gene subtraction; and (4) gene editing. Gene replacement/addition involves the delivery of a functional version of a mutated gene and the encoded protein that is missing or dysfunctional in target cells. This represents the typical protocol devised to treat monogenic diseases. In addition, this approach may be useful to induce drug resistance or to manipulate target cells' behavior (i.e., by delivering a cytokine gene) or to provide an anti-cancer function mediated by T cells through a chimeric antigen receptor (CAR) [50]. CAR comprises the variable portion of antibodies fused to intracellular signaling molecules with T-cell-activating function that redirect T cells against cancer cells. Gene subtraction can be achieved by down-regulating the expression of endogenous genes, exploiting RNA interference [51] or epigenetic silencing [52]. Subtraction can also disrupt the coding sequence of the endogenous gene through sequence-specific engineered endonucleases (see below). Gene subtraction strategies are intended to counteract the effect of gain-of-function mutations or fight infectious diseases, for example, by targeting directly the genome of a virus or one of its cellular receptors [53]. Gene editing is considered the holy grail of gene therapy. It would potentially ensure predictable and stable transgene expression with minimal consequences to cellular genomic regulation. Site-specific integration can be achieved by providing target cells with DNA carrying sequences homologous to the desired site and exploiting the cellular machinery of homologous recombination to insert the exogenous DNA in that specific site. To direct homologous

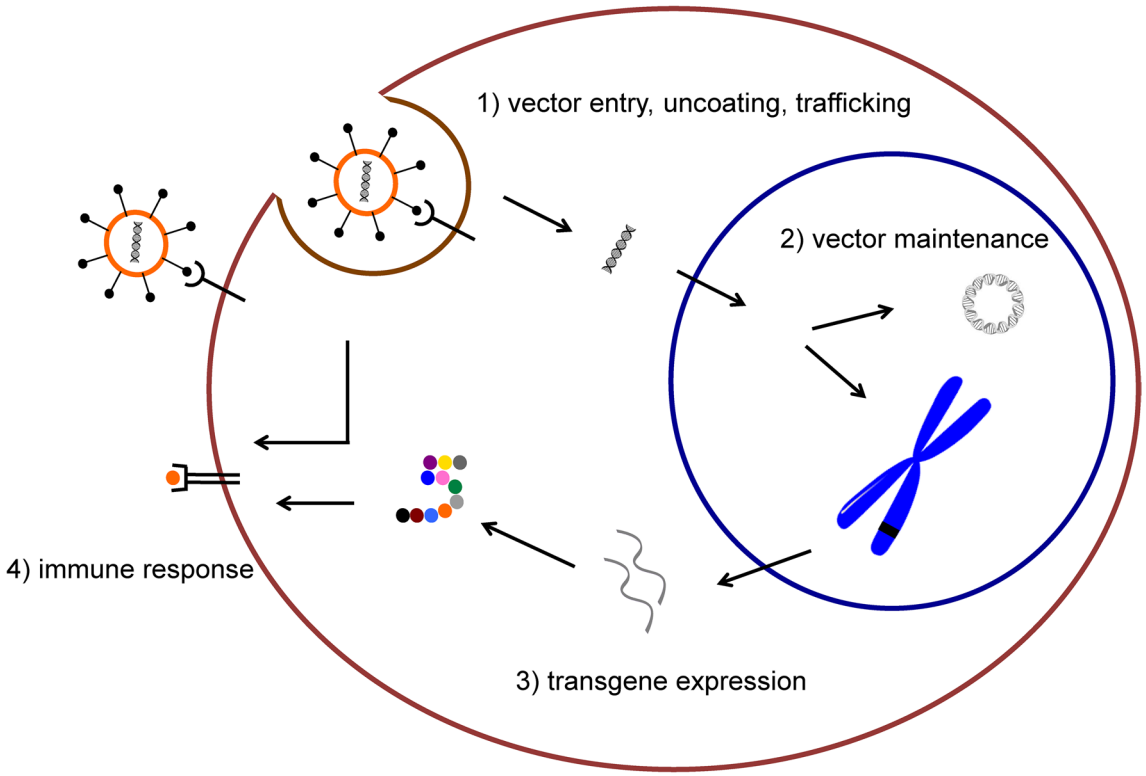

Fig. 2 Main steps to gene transfer and potential barriers. (1) Vector entry, uncoating, and trafficking: viral vectors typically enter into cells through receptor-mediated internalization. Then, uncoating occurs and the recombinant viral vector genome trafficks to the nucleus. (2) Vector maintenance: the vector genome is either maintained as an episome associated to the nuclear matrix or it is integrated into target cells' chromatin. When long-term vector persistence is desirable, integrating vectors are needed when targeting dividing cells or stable episomes can be used in tissues with relatively slow turnover such as muscle, liver, and brain. (3) Transgene expression: the vector contains the regulatory elements necessary to express the transgene; the transgene is transcribed and translated into the protein that exerts the therapeutic function. (4) Immune responses: vector- or transgene-derived antigens can be presented in class I and/or II major histocompatibility complexes and can initiate immune responses. Vector antigens are typically present only transiently in transduced cells. Ideally, immune tolerance to the transgene product is established, thus protecting the transgene protein itself and the transduced cells from immune-mediated attack

recombination at specific sites and improve the efficiency of the process, engineered sequence-specific endonucleases have been developed, such as zinc finger nucleases, transcription activator-like effector nucleases, and the more recent CRISPR/Cas9 RNA-guided nuclease system [54]. Once nucleases generate a site-specific double-strand break in the genome, target cells attempt repair by the error-prone mechanism non-homologous end joining. This form of repair can be exploited to perform gene disruption. Alternatively, the break can be repaired by recombination of homologous template DNA co-delivered with the nucleases to target cells, giving rise to the desired modification. However, gene editing is still at an early stage of development because of its technical demands. Nonetheless, it holds the promise to solve two issues of gene therapy, namely, transgene expression control and insertional mutagenesis (see below), even if DNA breaks at off-target sites and their consequences need to be carefully addressed [55, 56]. All gene therapy approaches can only target somatic cells, since the genetic modification of germline stem cells and its eventual transmission to the progeny is not allowed for ethical reasons. The desired gene therapy strategy can be accomplished by an ex vivo or in vivo approaches. Ex vivo approaches involve the isolation of stem, progenitor or differentiated cells from patients, their in vitro genetic modification and reinfusion into patients. In vivo approaches involve the administration of the vector directly to patients, either locally (e.g., in the brain, eye, muscle, joint, etc.) or systemically (intravenous).

Challenges

The major challenges for gene therapy are: (1) the efficiency of gene transfer (i.e., the fraction of cells that can be targeted and the number of transgene copies that can be transferred); (2) the persistence of gene transfer for the time needed to meet the therapeutic purpose; (3) transgene expression control; and (4) for integrating vectors, the risk of insertional mutagenesis. Vector integration may give a growth

advantage to selected cell clones that may, upon selective pressure and accumulation of secondary mutations, results in oncogenic transformation [57]. A further obstacle is the host immune response that can be mounted against the vector and/or the transgene and that may result in toxicity and loss of efficacy [58].

Gene therapy applications

Gene therapy is emerging as a realistic treatment option for several disorders, as highlighted by the recent marketing authorization of some products. The most successful applications are: (1) ex vivo gene replacement therapy with HSC for immune deficiencies; (2) ex vivo gene addition therapy with CAR-potentiated T cells for liquid tumors; and (3) in vivo gene replacement therapy with AAV vectors in the eye for retinal blindness, in the central nervous system for spinal muscular atrophy (SMA) and in the liver for hemophilia. A few ex vivo and in vivo examples are described below. Ex vivo gene replacement therapy with HSC was first successfully applied in children suffering from severe combined immune deficiency (SCID) due to adenosine deaminase deficiency (ADA-SCID), who showed restoration of normal purine metabolism and immune functions after receiving a transplant of autologous HSC transduced with an ADA-encoding RV [59]. Since 2016, this gene therapy has become a commercial medicinal product (Strimvelis®, Orchard Therapeutics). Similarly, ex vivo gene therapy with autologous HSC transduced with LV expressing the corrective transgene has yielded promising results in other immune deficiencies, such as SCID-X1 and Wiskott Aldrich syndrome [60], in the lysosomal storage disorder metachromatic leukodystrophy [61], and in β-thalassemia [62]. Moreover, autologous T cells transduced with vectors expressing CAR against the B-cell antigen CD19 have proved to be effective against B-cell malignancies [63]. This type of gene therapy has recently received marketing authorization with indications for refractory B-cell acute lymphoblastic leukemia (Tisagenlecleucel, Kymriah® from Novartis) and B-cell lymphomas (Axicabtagene Ciloleucel, Yescarta® from Kite Pharma Inc.). Additional clinical trials are ongoing in multiple myeloma [64]. Another ex vivo gene therapy that has received marketing authorization is autologous T cells transduced with a so-called suicide transgene (which can be activated by a specific molecule). This product (Zalmoxis® from MolMed) is used in patients with leukemias who undergo haplo-identical HSC transplant to promote immune reconstitution and anti-leukemia effect. Simultaneously, it aims to control potential graft-versus-host disease by transferring the suicide gene to the T cells [65].

Remarkable clinical improvement has been reported in patients with Leber's congenital amaurosis, a form of retinal blindness, following a single subretinal injection of AAV vectors carrying the retinal pigment epithelium gene necessary to reconstitute photoreceptor function (Voretigene Neparvovec, Luxturna® from Spark Therapeutics) [66].

Finally, patients with hemophilia may benefit from an in vivo gene therapy that is at an advanced stage of clinical testing. Indeed, reconstitution of nearly normal levels of clotting factor activity has been achieved by a single intravenous administration of AAV vectors expressing the functional transgene [67]. Very promising clinical results have also been achieved in children with SMA-1, after intravenous administration of AAV vectors encoding for the missing protein [68]. A different in vivo gene therapy strategy has been evaluated in Parkinson's disease, where intraputamen injection of LV expressing genes involved in dopamine biosynthesis showed some improvement in motor behavior in treated patients [69]. Overall gene therapy is poised to become a powerful weapon in the physicians' toolbox for the treatment and, perhaps, the cure of several inherited and acquired diseases.

Final remarks

Undoubtedly, RM holds an immense potential to cure a number of chronic invalidating conditions. However, it appears clear that bringing innovative therapies from the bench to bedside raises a number of ethical and technical hurdles. Furthermore, before the successful commercialization of a product is achieved, several rules should be accomplished. First, an interdisciplinary approach is necessary with synergies among stem-cell biologists, bioengineers, and material scientists. Second, well-thought-out clinical development plans are required for reducing the risk of failure, together with fully validated manufacturing processes and supply chains. Third, thoughtful regulatory bodies in the design and development plan of any RM solution are pivotal to guarantee the market release of products with real curative potential for specific clinical indications. Therefore, just like Mars has become a reality, RM represents a tangible modern therapeutic option.

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