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Published in:
Trends in Biotechnology

Link to article, DOI:
10.1016/j.tibtech.2022.11.008

Publication date:
2023

Document Version
Publisher's PDF, also known as Version of record

CellPress
OPEN ACCESS

Trends in
Biotechnology

Special issue: 40th anniversary

Review

Emerging immunomodulatory strategies for cell therapeutics

Corrine Ying Xuan Chua,[1] Allen Yujie Jiang,[2,3] Tatiane Eufrásio-da-Silva,[4] Alireza Dolatshahi-Pirouz,[4] Robert Langer,[2,3] Gorka Orive ,[5,6,7,8,9,*] and Alessandro Grattoni[1,10,11,*]

Cellular therapies are poised to transform the field of medicine by restoring dysfunctional tissues and treating various diseases in a dynamic manner not achievable by conventional pharmaceutics. Spanning various therapeutic areas inclusive of cancer, regenerative medicine, and immune disorders, cellular therapies comprise stem or non-stem cells derived from various sources. Despite numerous clinical approvals or trials underway, the host immune response presents a critical impediment to the widespread adoption and success of cellular therapies. Here, we review current research and clinical advances in immunomodulatory strategies to mitigate immune rejection or promote immune tolerance to cellular therapies. We discuss the potential of these immunomodulatory interventions to accelerate translation or maximize the prospects of improving therapeutic outcomes of cellular therapies for clinical success.

Highlights

Cellular therapies are poised to transform the field of medicine.

These therapies can potentially impact on cancer, regenerative medicine, and immune disorders.

Cellular therapies encompass stem or non-stem cells derived from autologous, allogeneic, or xenogeneic sources.

A new generation of innovative immunomodulatory interventions may accelerate translation or maximize the prospects of improving therapeutic outcomes of cellular therapies for long-term clinical success.

Modulating the immune response for successful cellular therapies

The convergence of bioengineering innovations with advances in immunology has substantially expanded the landscape of **cellular therapies** (see Glossary) [1]. Cellular therapies aim to treat or manage a disease by introducing living cells that will integrate within the host to restore or eliminate dysfunctional tissues. They typically encompass **stem cells (SCs)** or non-SCs derived from **autologous**, **allo-** or **xenogeneic** sources, either unaltered or genetically engineered. Currently, hematopoietic SC and **chimeric antigen receptor T (CAR-T) cell** therapies for hematologic disorders and cancers are the predominant clinically approved products. As of August 2022, there are more than 3000 active clinical trials of cellular therapies. These trials primarily use SCs and blood cells (leukocytes, red blood cells, and platelets) for a spectrum of therapeutic indications such as cancer, hematologic disorders as well as autoimmune, cardiovascular, degenerative, and infectious diseases. A breakdown of the current landscape of active clinical trials of cellular therapies is provided elsewhere [2].

Despite this expanding pipeline, host immune response to cellular therapies remains a challenge that could fundamentally impede clinical adoption and desirable outcomes [3]. Host immune rejection could occur even for cells of allogeneic origin with **human leukocyte antigen (HLA)** matching, attributable to mismatched minor alleles [3]. Systemic **immunosuppression** through **immunosuppressant** drugs is routinely used to reduce rejection. Immunosuppressive treatments are categorized as 'induction' (high-intensity immunosuppression immediately post-therapy), 'maintenance' (long-term immunosuppression to prevent chronic rejection), and 'rejection treatment' (used to treat acute rejection). These immunosuppressants include calcineurin inhibitors (e.g., tacrolimus and cyclosporine), corticosteroids (prednisone), monoclonal antibodies (e.g., basiliximab, adalimumab, and rituximab), inosine monophosphate dehydrogenase inhibitors (mycophenolate mofetil), mechanistic target of rapamycin (mTOR) inhibitor (rapamycin), and depleting antibodies (anti-thymocyte globulins). In general, immunosuppressive

[1]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX 77030, USA
[2]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[3]David H Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[4]Department of Health Technology, Technical University of Denmark (DTU), 2800 Kgs. Lyngby, Denmark
[5]NanoBioCel Research Group, School of Pharmacy, University of the Basque Country (UPV/EHU), 01006 Vitoria-Gasteiz, Spain
[6]Bioaraba, NanoBioCel Research Group, 01009 Vitoria-Gasteiz, Spain
[7]Biomedical Research Networking Centre in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Institute of Health Carlos III, Av Monforte de Lemos 3-5, 28029 Madrid, Spain
[8]University Institute for Regenerative Medicine and Oral Implantology-UIRMI (UPV/EHU-Fundación Eduardo Anitua), 01007 Vitoria-Gasteiz, Spain

agents are administered to target T and B cells, which are key in immune rejection. However, systemic immunosuppression is undesirable due to increased risks of infections, cancers, and organ damage.

Therefore, the clinical success of cellular therapies demands innovations in facilitating as well as maintaining immune acceptance for favorable long-term therapeutic outcome. This review highlights emerging immunomodulatory strategies to attenuate immune rejection or promote tolerance to cellular therapies, with a discussion on local, site-specific immunomodulation measures (Figure 1, Key figure). We exclude solid organ transplantation and tissue grafts, which are extensively reviewed elsewhere [4]. We provide a perspective on opportunities for accelerating translation of innovative immunomodulation strategies that synergize with cellular therapies toward achieving widespread clinical success.

[9]Singapore Eye Research Institute, The Academia, 20 College Road, Discovery Tower, Singapore 169856, Singapore
[10]Department of Radiation Oncology, Houston Methodist Research Institute, Houston, TX 77030, USA
[11]Department of Surgery, Houston Methodist Research Institute, Houston, TX 77030, USA

*Correspondence:
gorka.orive@ehu.eus (G. Orive) and
agrattoni@houstonmethodist.org
(A. Grattoni).

CRISPR-Cas9 genome editing

Genome editing technologies such as the **clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR-Cas9)** system have led to the development of 'off-the-shelf', or 'universal', engineered cell therapies with little to no immunogenicity. The CRISPR-Cas9 system generates targeted **double-strand breaks (DSBs)** in the genome. which can be repaired by the cell. For repair, cells can use **non-homologous end joining (NHEJ)**, which can effectively knockout the gene of interest. Alternatively, **homology-directed repair (HDR)** can occur if a donor DNA template is provided, which allows for targeted insertion of exogenous genes. Depending on the application, gene editing to avoid immune recognition has mainly focused on the elimination of genes encoding for immunogenic surface markers, such as HLAs and T cell receptors (TCRs). For regenerative therapies using **iPSCs**, the focus has been on deletion of the *B2M* and *CIITA* genes required for the expression of HLA class I and II genes, respectively, which typically drive the **alloimmune response** (Figure 2). Although complete HLA knockout helps to avoid recognition by host $CD4^+$ and $CD8^+$ T cells, HLA-1 deficiency can lead to activation of recipient **natural killer (NK) cells** and transplant rejection [5]. Allele-specific editing of polymorphic HLA-1 to express common HLA-C alleles which could be matched to >90% of the world's population, in addition to HLA-II elimination, was used to generate iPSCs which suppressed recognition by both NK and T cells [6]. Alternatively, overexpression of nonpolymorphic HLA-1 molecules such as HLA-E in stem and progenitor cells can also inhibit NK cell lysis activity [7,8]. Finally, iPSCs have been edited to simultaneously disrupt HLA-1 expression and overexpress CD47, a 'don't-eat-me' signal which effectively inhibits phagocytosis and thus prevents macrophage and NK cell-mediated transplant rejection [9]. We further expand on iPSCs in the section 'Stem cell-derived immunomodulatory therapeutics'.

For generating 'off-the-shelf' CAR-T cells, human T cells have been edited to eliminate both *CD7* and *TRAC*, with the latter's deletion blocking TCR-mediated signaling which causes **graft-versus-host disease (GVHD)** [10]. These doubly edited CAR-T cells were effective in killing T cell acute lymphoblastic leukemia (T-ALL) *in vivo* without evidence of xenogeneic GVHD [10]. Additionally, CAR-T cells edited to lack TCR and HLA-1 reduced alloreactivity and GVHD associated with the cell therapy with a simultaneous third edit to delete PD1 [11]. The PD1 inhibitory pathway can attenuate CAR-T cell-mediated antitumor activity. As such, the abrogation of the PD1 inhibitory pathway improved antitumor efficacy.

Furthermore, Cas9 was used to endow INS-1E, a rat insulin-secreting β-cell line, with immunomodulatory functions. Specifically, INS-1E was precisely engineered to continuously produce interleukin-10 (IL-10) cytokine in a glucose-responsive manner as the knock-in site was located in the c-peptide region [12]. The continuous local secretion of IL-10 can reduce fibrosis and

protect β cells from proinflammatory cytokine-induced cell death, with minimal systemic effect on the host immune system.

The generation and use of genetically engineered cell therapies with minimal immunogenicity come with safety concerns which must be considered. Gene editing with CRISPR-Cas systems often involves DSBs in the genome which can cause unintended large deletions, complex genomic rearrangements, or aneuploidy leading to harmful pathologies if not carefully monitored and addressed [13,14]. These risks are especially important to consider in cell therapies engineered to avoid immune recognition because malignant transformation of the engineered cells may escape sensing by host immune cells. Therefore, there is growing interest in using gene editing tools which do not induce DSBs such as base editors and prime editors, or even epigenomic editing tools, to lower immunogenicity of cell therapies [15].

RNA therapeutics for immune tolerance

RNA therapeutics, such as **RNAi** technologies, for targeted silencing of genes or *in vitro*-transcribed mRNA for transient expression of encoded peptides or proteins, hold significant potential in promoting **immune tolerance** toward cell therapies. Similar to CRISPR-Cas9, RNAi offers the ability to silence the expression of immunogenic alloantigens through mRNA transcript degradation or translation inhibition. This is achieved through the use of short, double-stranded RNA (dsRNAs) in combination with the endogenous effector RNA-induced silencing complex to facilitate homology-directed gene silencing at the post-transcriptional level. Therefore, the elimination of surface MHC molecules can be achieved through RNAi without the risks associated with gene editing as mentioned in the previous section (Figure 2).

For vascularized cell therapies, host immune responses to graft endothelial cells (ECs) expressing nonmatched HLA can lead to transplant rejection. Pretreatment of donor blood vessels *ex vivo* with siRNA targeting *CIITA* eliminated HLA-II expression in ECs and prevented rejection of donor arteries by adoptively transferred allogeneic peripheral blood mononuclear cells (PBMCs) in immunodeficient mice [16]. While the use of siRNA is promising for transient knockdown of *HLA*, which may be beneficial in promoting initial immune tolerance, permanent elimination may be more desirable to improve the likelihood of the long-term viability of cell therapy. Lentiviral delivery of short hairpin RNA (shRNA) targeting *B2M* can enable stable expression of the interfering RNA to achieve a more permanent knockdown of *HLA-1*. This approach has been used to generate *HLA-1* knocked down cells, which prevents $CD8^+$ T cell response with residual HLA-1 expression preventing NK cell lysis [17]. Stably expressed shRNA targeting *B2M* has also been used to generate HLA-1 knocked down iPSCs that can be derived into megakaryocytes capable of generating platelets following transfusion into a mouse model for platelet refractoriness [18].

With the approval of two mRNA vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and many more mRNA therapies in clinical trials, there is growing interest in the use of mRNA to promote immune tolerance toward cell therapies [19]. The focus of mRNA therapies in this area has been on the activation and expansion of regulatory T cells (Tregs) which play an important role in immunosuppression and prevention of GVHD [20]. mRNA encoding for human *IL-2* mutein was designed to preferentially bind IL-2 receptor α (IL-2Rα) receptors on Tregs and avoid proinflammatory T cell activation [21]. Delivery of this mRNA led to Treg activation and expansion in mouse and non-human primate models and effectively reduced acute GVHD in mice. However, the dual role of IL-2 in promoting both Tregs and proinflammatory T cells will require careful monitoring of T cell responses to avoid exacerbating immunogenicity toward cell therapies.

Glossary

Allogeneic: cells or tissues derived from a donor of the same species.
Alloimmune response: an immune response to non-self antigens ('alloantigens') from members of the same species. An alloimmune response can result in graft rejection.
Apoptosis: a type of programmed cell death leading to self-destruction of cells, triggered by the presence or absence of certain stimuli.
Autoimmunity: immune response against an individual's own cells or tissues through the presence of antibodies and T lymphocytes directed against self-antigens.
Autologous: cells or tissues derived from the same individual into whom they are transplanted.
Cellular therapy: living cells used for therapy.
Chimeric antigen receptor T (CAR-T) cell: a form of immunotherapy using T cells genetically modified to have a synthetic receptor that binds to a specific target (cancer cells) and mediate immune destruction. They are referred to as 'chimeric' because both antigen-binding and T cell activating functions are combined within a single receptor.
Clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR-Cas9): a genome editing technology adapted from bacteria that can be used to specifically edit DNA at precise locations. A specially designed RNA molecule is used to guide Cas9 enzyme to a targeted sequence of DNA. Cas9 cuts the targeted DNA sequence for removal, thus allowing for deletion or the addition of a new, customized DNA sequence. CRISPR-Cas9 technology holds promise for treating and preventing previously untreatable diseases such as neurodegenerative, genetic, or hereditary disorders, HIV, and cancer.
Double-strand break (DSB): occurs when both strands of DNA are cleaved by damaging agents such as ionizing radiation or certain chemicals.
Graft-versus-host disease (GVHD): GVHD is a life-threatening systemic inflammatory complication that can occur after transplantation when donor T cells in the transplant attack the recipient.
Homology-directed repair (HDR): a mechanism used by the cell to repair DSBs in DNA, relying on a homologous

Alternatively, tolerogenic mRNA vaccines may be used to induce alloantigen-specific tolerance. Such vaccines are designed with chemically modified mRNA that are carefully purified to remove dsRNA contaminants. The resulting noninflammatory mRNA vaccines can induce tolerance toward an encoded antigen when presented to T cells in the absence of co-stimulatory molecules [22–24]. Currently, the use of tolerogenic mRNA vaccines has been limited to the induction of autoantigen-specific Treg responses for the prevention of autoimmune disease onset in a mouse model of multiple sclerosis [24]. We envision that prophylactic tolerogenic mRNA vaccines encoding for donor HLA could induce tolerance toward HLA-mismatched cell therapies mediated by donor HLA-specific Tregs.

Local immune microenvironment alteration via immunomodulators or immunomodulatory cells

Local delivery of immunomodulators is an interventional approach to alter the immune microenvironment to be conducive for cellular therapy, as an alternative to systemic administration (Figure 3) [25]. Additionally, *in situ* co-deployment of immunomodulatory cells including tolerogenic dendritic cells (tolDCs) and Tregs has shown promise. Mesenchymal SCs (MSCs) can also be used in a local setting and are further reviewed in the section 'Stem cell-derived immunomodulatory therapeutics'.

Locally delivered immunomodulators

Hydrogels and **micelles** are biomaterials increasingly used for immunomodulation, including that of cellular therapies, due to their tunability, biocompatibility, and flexibility [26–30]. Similarly, biomaterial-based scaffolds have found utility as niches for local immunomodulation [29,31]. The Fas receptor/Fas ligand (FasL) pathway confers **immune privilege** and tolerance to self-antigens by triggering **apoptosis** in infiltrating lymphocytes and inflammatory cells. When allogeneic islets were cotransplanted with FasL-modified **microgel** or scaffold, long-term islet engraftment and normoglycemia were achieved in diabetic mice [32,33] and non-human primates [34], signifying a viable alternative to systemic tolerance induction. Similarly, immune checkpoint modulators, in which its blockade has proliferated in oncology settings, can be applied for transplant immunomodulation. The programmed cell death-1 (PD-1)/PD-ligand 1 (PD-L1) pathway regulates $CD8^+$ T cell anergy and induces Tregs, which are both critical for alloimmune responses and transplant tolerance. In line with this, a PD-L1-eluting microgel in combination with transient rapamycin treatment created a tolerogenic, immunosuppressive cell-enriched microenvironment for islet transplantation [35].

Alternatively, localized delivery using dexamethasone-eluting micelles in combination with systemic cytotoxic T lymphocyte-associated antigen-4-Ig (CTLA4Ig) reduced intragraft proinflammatory cytokines such as IL-10, IL-1β, and interferon gamma (IFNγ), enhanced allogeneic islet survival in diabetic mice. Similarly, islet engraftment was achieved by dexamethasone-eluting graphene scaffolds, which provided a localized immunosuppressed microenvironment for islet cotransplantation with adipose tissue-derived MSCs [36]. Graphene is a novel biomaterial in which research efforts have focused on functionalization as a scaffold for localized drug delivery, tissue engineering, or regenerative medicine [37–39]. In line with localized immunosuppression approach, the Neovascularized Implantable Cell Homing and Encapsulation (NICHE) implant has a drug reservoir for sustained elution of immunosuppressants directly into the interconnected cell transplant chamber. CTLA4Ig and/or anti-lymphocytic serum immunosuppressant(s) elution created a locally immune-protected NICHE milieu within which host vasculature provided engraftment support for long-term Leydig cells [40] or islet [41] transplantation. Moreover, islet cotransplantation with MSCs within the NICHE further provided local immunomodulation, supportive of long-term graft acceptance [41].

sequence of DNA, primarily occurring during G2 and S phases of the cell cycle.
Human leukocyte antigen (HLA): HLA genes code for cell surface proteins in MHCs, which are unique to the individual. HLAs are used by the immune system to differentiate between self and non-self.
Immune privilege: refers to certain sites in the body that are isolated from the immune system, which can tolerate foreign antigens, cells, or tissues without inducing an inflammatory immune response that can lead to rejection.
Immune tolerance: unresponsiveness of the immune system to a specific antigen or a previously encountered antigen. Transplant tolerance refers to the lack of immune responses to antigens from donor cells or grafts, which prevents immune rejection, while reactivity to other antigens remains intact.
Immunosuppressants: agents used to suppress the immune system to prevent the cells from attacking donor cells, which are seen as foreign to the host.
Immunosuppression: suppression of the body's immune system and consequently, the ability to fight infection and disease. Immunosuppression can be induced by drugs or specialized cells, as well as occur as a result of a disease state.
iPSCs: cells that are obtained by reprogramming terminally differentiated adult cells, such as skin cells, into an embryonic-like pluripotent state to be used as an unlimited source for therapeutic purposes. iPSCs can be created from cells of the same individual who will receive the transplant.
Micelles: spherical amphiphilic structures containing a hydrophobic core and a hydrophilic shell. They are used as drug carriers, as the hydrophilic shell renders micelles water soluble, whereas the hydrophobic core carries the payload.
Microgel: 3D network of polymer microfilaments comprising natural or synthetic materials that can be crosslinked using physical, chemical, or light-mediated mechanisms. Microgels are hydrogels with particle sizes bigger than 100 nm and smaller than 100 μm. Microgels are used in biomedical applications such as drug delivery, regenerative medicine, and tissue engineering. For example, microgels can be used to encapsulate drugs and engineered to swell or degrade in

Codelivery of tolDCs

TolDCs, otherwise known as DCregs, are immature immunosuppressive DC subtypes. TolDCs can induce and maintain immune tolerance by promoting T cell anergy, apoptosis, and hyposensitivity as well as favor the generation of Tregs. Because of this, tolDCs, which can be targeted *in situ* or generated *ex vivo*, have prospective values for promoting graft tolerance and survival [42,43].

To generate tolDCs *in situ*, tacrolimus-loaded microspheres and clodronate liposomes were co-administered during subcutaneous xenogeneic islet transplantation in rats [44]. DC cell surface expression of CD40, CD80, CD86, and MHCII molecules was markedly downregulated, suggestive of polarization toward a tolDC phenotype. The reduced antigen-presenting and T cell activation capacity of tolDCs, as well as the generation and maintenance of Tregs, were observed up to 520 days post-transplantation, signifying long-term maintenance of immune tolerance.

TolDCs can be generated *ex vivo* by culture of bone marrow (BM)- or blood-derived DCs with cytokines such as granulocyte–macrophage colony-stimulating factor (GM-CSF), transforming growth factor beta (TGFβ), IL-4, IL-10, or IL-3, as well as rapamycin, vitamin D3, or dexamethasone. In a study by Madelon *et al*., autologous BM-derived tolDCs was cotransplanted with rat islets under the kidney capsule of mice, achieving prolonged xenograft survival without immunosuppressants [45]. Although promising, there are concerns of low migratory activity or elimination by NK cells, as well as risks that tolDCs mature and promote alloimmunity instead of tolerance.

Cotransplantation with Tregs

Tregs ($CD4^+CD25^+FoxP3^+$) are a long-lived, immunosuppressive subset of T cells, which are indispensable for maintaining immunological self-tolerance. There are numerous active clinical trials investigating Treg cell therapy [46]. Of note, autologous Treg infusion simultaneously with allogeneic islets through the portal vein has demonstrated safety and feasibility (NCT04820270) [47]. Other studies include Treg infusion 6-week post-islet transplantation (NCT03444064), in hopes of reducing the need for immunosuppressants. Although promising for achieving immune tolerance, Treg sourcing, isolation, and manufacturing procedures (particularly for long-term immunosuppressive use) require further development.

Codelivery with Sertoli cells

Sertoli cells are involved in generating an immune evading microenvironment in the testis. They serve a primary role in the blood–testis barrier, which thwarts the transport of lymphocytes and antibodies. In light of their ability to secrete immunomodulatory molecules that inhibit IL-2 production and proliferation of B and T lymphocytes, Sertoli cells are explored for cotransplantation with exogenous cell grafts. Examples include cotransplantation with allogenic islets in models of type 1 diabetes (T1D) [48], mesencephalic tissue in a model of Parkinson's disease [49], and skin grafts, among others [50].

SC-derived immunomodulatory therapeutics

SCs can be bioengineered and differentiated into specific cell lineages or leveraged as living drugs for immunomodulation in cell therapies. In cell transplantation, SCs offer the promise of an unlimited cell source, eliminating the issues of limited donor tissue availability.

iPSC-derived cells

Human PSC (hPSC)-based therapies, including human ESCs (hESCs) and hiPSCs, are extensively investigated for various conditions such as neurodegenerative and cardiovascular diseases, T1D, and spinal cord injury. However, immune rejection of hPSC-derived cells can

response to stimuli such as temperature, pH, and light, for drug release.
Natural killer (NK) cells: cytotoxic lymphocytes of the innate immune system, which are early cellular responders to infected cells or cancers. These cells play a central role in modulating alloimmune responses.
Non-homologous end joining (NHEJ): the primary pathway to repair DNA DSBs, involving ligation of broken strands, throughout the cell cycle. NHEJ has a higher capacity for repair and is faster than HDR and does not need a repair template.
RNAi: RNAi is a process that triggers sequence-specific suppression of gene expression using double-stranded RNA, either via translational or transcriptional repression.
Stem cells (SCs): cells that have the ability to self-renew and differentiate into different specialized cell types. SCs can propagate indefinitely and thus be an unlimited source for replacing lost or diseased tissues.
Xenogeneic: cells or tissues derived from a donor from a different species.

Key figure

Cellular therapies, administration, and immunomodulatory strategies

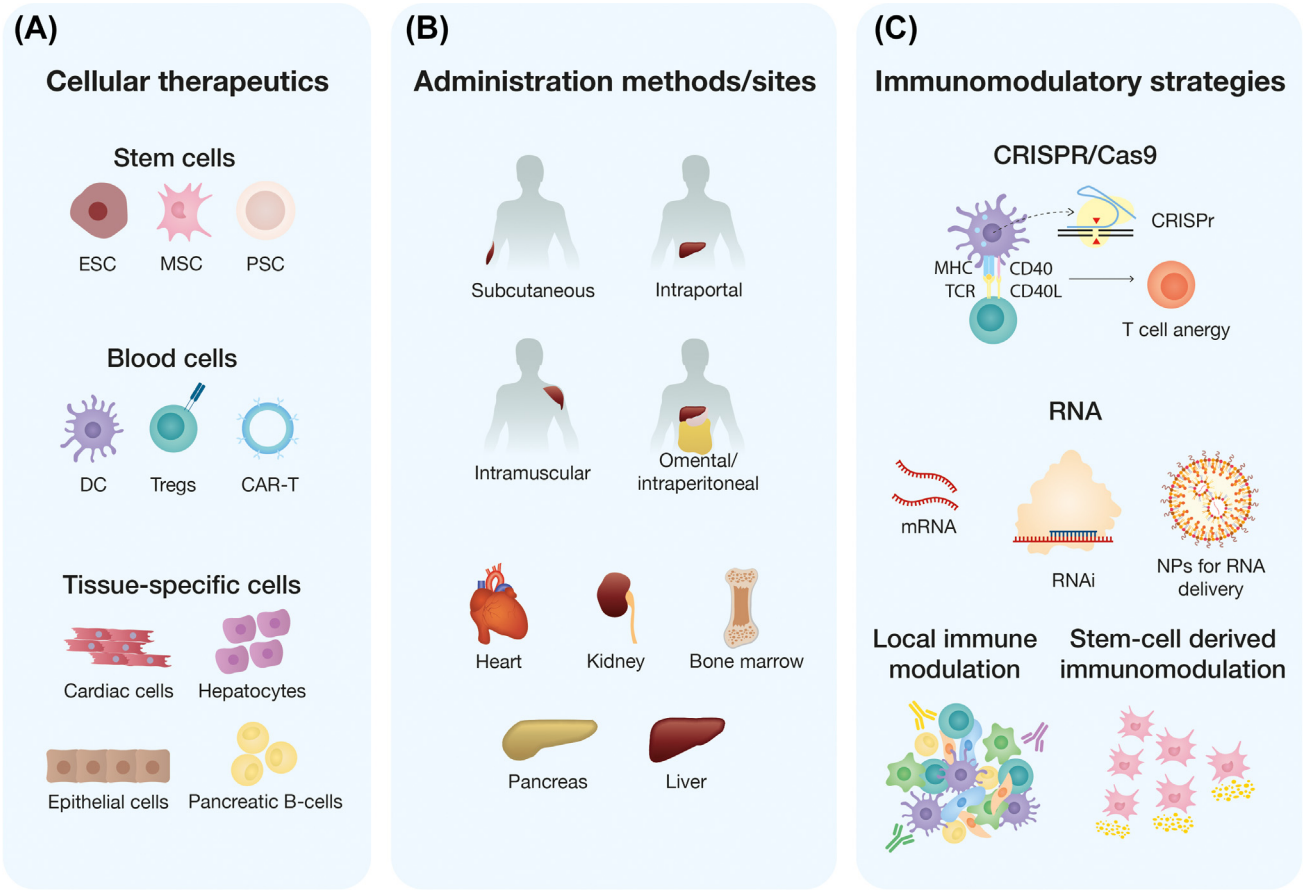

Figure 1. There are various types of cellular therapies (A) and different administration methods and sites (B), and correspondingly, diverse approaches to modulate the immune response (C) to achieve maximal therapeutic benefit. Cellular therapies comprise stem cell (SC) or non-SC-based sources. SC-based therapies include those of embryonic SC (ESC), mesenchymal SC (MSC), or pluripotent SC (PSC) origin. Non-SC-based therapies include blood cells such as dendritic cells (DCs), regulatory T cells (Tregs), and CAR-T cells, and tissue-specific cells. There are different options for administration methods and sites, depending on the type of cellular therapy and therapeutic indication. These sites could have distinct immune composition and necessitate different immunomodulatory strategies for optimal outcome. As immune rejection is a key obstacle for cellular therapy, numerous immunomodulatory interventions have emerged to improve clinical success. Clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR/Cas9) technology is used to disrupt immunogenic cell surface markers such as human leukocyte antigens (HLAs), T cell receptors (TCRs), and co-stimulatory molecules (e.g., CD40) at the genomic level, resulting in immune avoidance or T cell anergy. Similarly, RNA therapeutics, such as RNAi technologies for targeted gene silencing or mRNA for transient protein expression, using nanoparticles (NPs) as delivery vehicles, are leveraged to modulate immune activity. Immunosuppressants can be codelivered with cellular therapy to create a locally immunosuppressed microenvironment. On a similar note, SCs or cells derived from SCs can be used to induce tolerance or avoid host immunoreaction. Abbreviation: CAR-T cells, chimeric antigen receptor T cells.

occur despite autologous origin or HLA matching [51,52], which hampers clinical application. Additionally, the high costs associated with personalized cell production for individual patients could pose a barrier for clinical developments. Further, **autoimmunity**, as in the case for T1D, remains a clinically significant impediment.

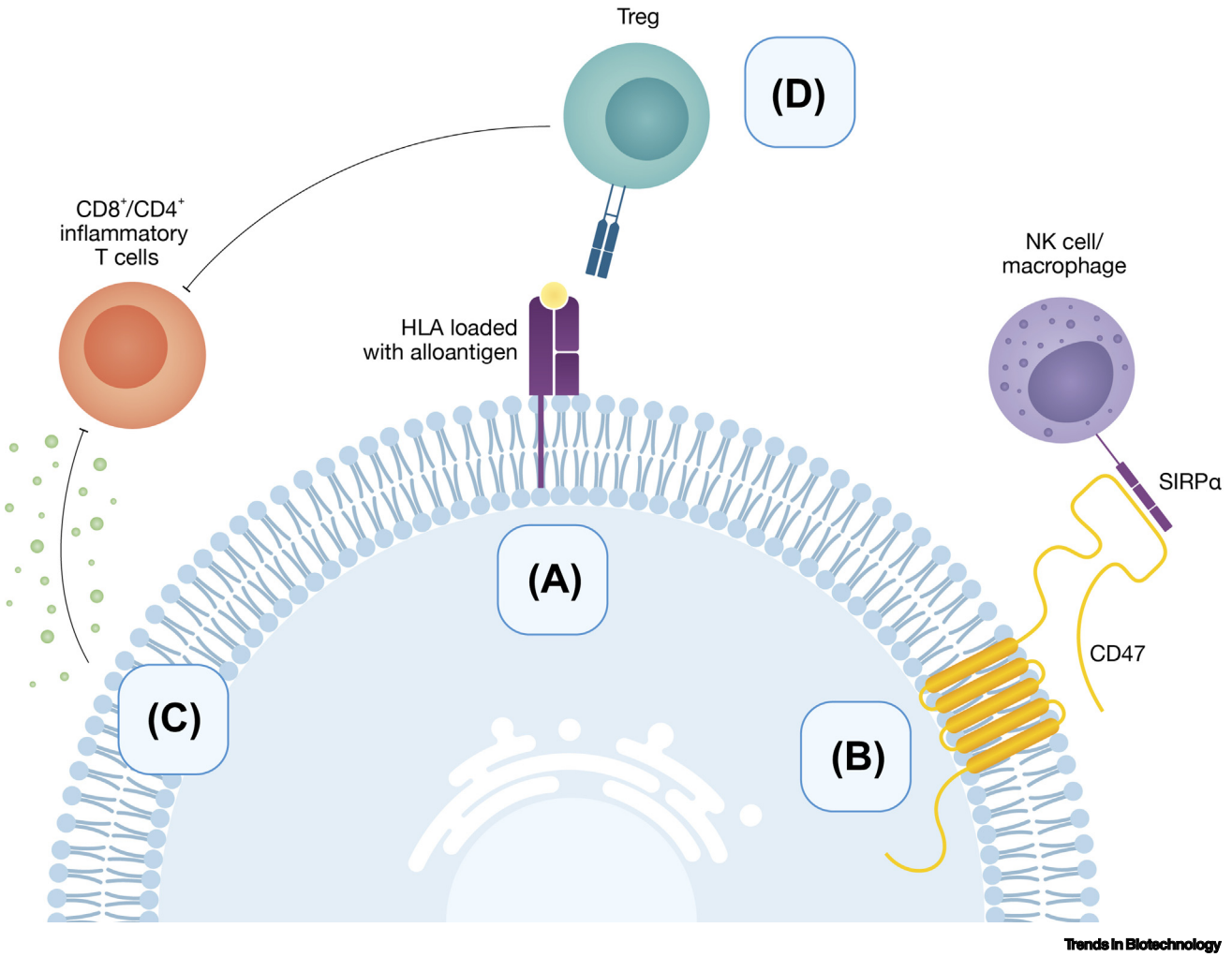

Figure 2. CRISPR- and RNA-based technologies for immunomodulation. These technologies can be applied to either (A–C) reduce the immunogenicity of cell therapies or (D) induce tolerance toward the cell therapy by the host immune system. Strategies to reduce donor immunogenicity include (A) CRISPR-Cas9 editing of donor cells to express common or nonpolymorphic HLAs or knockout of immunostimulatory HLA gene through CRISPR-Cas9 or RNAi therapies. Additionally, donor cells can also be genetically engineered to (B) overexpress immunosuppressive surface markers such as CD47 or (C) secrete anti-inflammatory cytokines such as IL-10 to modulate the local immune environment. (D) Additionally, recipient tolerance toward allogeneic cell therapies can be achieved through targeted activation of Tregs using mRNA-based IL-2 production or tolerogenic vaccines. Abbreviations: CRISPR-Cas9, clustered regularly interspaced short palindromic repeats-associated protein 9; HLA, human leukocyte antigen; IL, interleukin; NK, natural killer; Tregs, regulatory T cells.

To this end, advances in immune engineering have paved the way for the generation of hypoimmune (HIP) cells. Cai *et al*. demonstrated in a T1D murine model that deletion of the *Rnls* gene via CRISPR rendered iPSC-derived beta cells resistant to autoimmunity and did not impact cell function [53]. In another study, lentiviral manipulation of hiPSCs to achieve PD-L1 overexpression protected islet-like xenografts from immune rejection and restored normoglycemia in T1D immunocompetent mice [54]. Exploring applications in cardiovascular regeneration, HIP-iPSCs were engineered MHC class I and class II expression depletion and CD47 overexpression [9,55]. HIP-iPSCs were differentiated into iPSC-derived ECs (HIP-iECs) and injected into the infarct zone in an allogeneic murine model of myocardial infarction [55]. The cells engrafted within the heart and significantly improved cardiac output with no measurable immune response against the graft. Taken together, these studies highlight the promise of genetic manipulation of PSCs to abrogate auto- and alloimmune rejection of cell therapeutics.

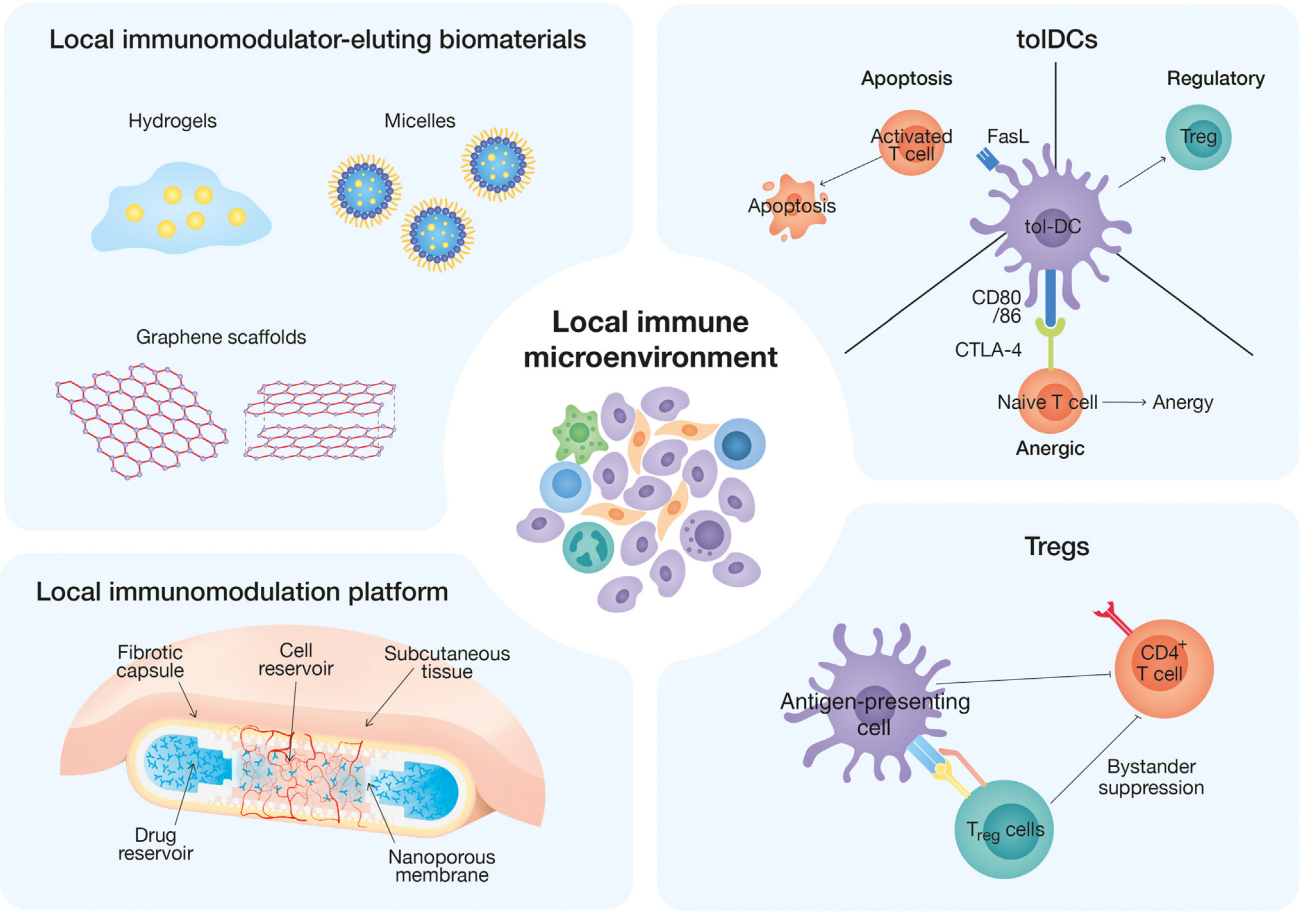

Figure 3. Local immune microenvironment alteration via immunomodulators or immunomodulatory cells. The local immune microenvironment where cellular therapies are administered plays a key role in engraftment success. Various strategies have emerged to modulate local immune responses inclusive of drug-eluting biomaterials such as hydrogels (microgels), micelles, and graphene scaffolds. These drug-eluting biomaterials are functionalized to allow for local release of immunosuppressants and are codelivered with cellular therapy, creating an immunosuppressed milieu. Local immunomodulatory subcutaneous implant, the NICHE, allows simultaneous cell encapsulation with immunosuppressant elution creating a physically immune-protected microenvironment conducive for engraftment. Other strategies include codelivery of tolerogenic dendritic cells (tolDCs) with cellular therapy, which can induce and maintain immunological suppression by promoting Tregs or T cell anergy to maintain immunological self-tolerance, thus protecting transplanted cells. Abbreviation: NICHE, Neovascularized Implantable Cell Homing and Encapsulation.

DC-like cells

SC-derived DC-like (DCL) cells have been investigated as an approach to induce tolerance. Todorova *et al.* derived DCL cells from CTLA4-Ig/PD-L1 expressing hESC [51]. DCL cells induced long-term tolerance to hPSC-derived smooth muscle and cardiomyocyte allografts by maintaining in an immature, tolerogenic state akin to tolDCs. Uniquely, only T cells specific for the DCL-expressing alloantigens were immune tolerated, thus avoiding systemic immunosuppression and its associated toxicities and risks.

MSCs

MSCs secrete cytokines, chemokines, and growth factors responsible for regulation of inflammation and immune response [56]. MCSs can inhibit allogeneic T cell responses, promote Tregs, trigger DC differentiation into tolDCs, transform proinflammatory M1 macrophages to anti-inflammatory M2 phenotype, as well as inhibit NK cell proliferation. These immunosuppressive

properties of MSCs have rendered them attractive for codelivery in cellular therapies, including for that of islets (NCT02384018).

In a murine model of retinal degenerative disease, cotransplantation of MSCs with fetal retinal pigment epithelial (RPE) cells suppressed host immunoreaction, allowing prolonged graft survival for preserving retina function [57]. The co-encapsulation of hepatocyte nuclear factor-4 alpha (HNF4α) overexpressing MSCs with hepatocytes promoted M2 macrophage polarization and alleviated inflammation in an acute liver failure murine model [58]. Yoshida *et al.* showed that syngeneic MSCs induced immune tolerance to iPSC-derived cardiomyocytes by promoting Tregs and triggering $CD8^+$ T cell apoptosis [59]. The combination of iPSC-derived cardiomyocytes and MSCs yielded improved cardiac function in a murine model of myocardial infarction, compared with single-cell population transplant alone [60]. In the context of diabetes, good manufacturing practice-compliant human umbilical cord perivascular MSCs cotransplantation with islets in diabetic mice achieved T cell suppression and maintenance of tight glycemic control [61]. Furthermore, MSCs engineered to express PD-L1/CTLA4-Ig (eMSCs) can induce local immunosuppression and support allogeneic rat islet engraftment without systemic immunosuppression [62].

Despite their promise, challenges with SCs include challenges with achieving full functional maturation, unclear long-term fate, immunogenicity, and cost and complexity of large-scale manufacturing. On that note, quality control between different SC sources, ease of procurement, and upscaling while maintaining stable phenotype are important criteria for clinical translatability [63]. We refer the readers to the section 'Concluding remarks and future perspectives, for considerations regarding clinical translation.

Site-specific immune modulation for cell delivery

Immunological surveillance is inherently suppressed in certain organs or tissues, rendering these immune privileged spaces ideal for administration of cellular therapy [64]. Typically, these anatomical niches have limited or slow regeneration capacity, such as the eye, central nervous system (CNS), testes, and placenta. SC niches including that of hematopoietic or hair follicle are also known as immune privileged sites. However, immune privilege oftentimes does not extend across all tissues within an organ. The blood–ocular barrier confers protection of intraocular compartments (anterior chamber, vitreous cavity, and subretinal space) [65], whereas the blood–brain barrier immunologically shields the parenchyma. As such, cell replacement therapy is commonly investigated in these immune privileged sites for protection against rejection.

iPSC-derived dopamine neural progenitor cells

Autologous or allogeneic iPSC-derived dopamine neural progenitor cells were transplanted into the brain parenchyma of a rhesus macaque model of Parkinson's disease [66]. Despite transplantation in an immune privileged site, only autologous transplantations yielded signs of motor and depressive recovery over the 2-year study duration without immunosuppression. This study underscores that even in immune privileged sites, immunosuppression could still be required for successful cell therapy using nonautologous transplants. On this note, in a first-of-its-kind study, autologous iPSC-derived dopamine neural progenitor cells were intracranially administered in a patient with progressive idiopathic Parkinson's disease [67]. Improvements were noted over the 24-month follow-up period, suggesting successful implantation of the cell therapy without immunosuppression throughout the course of treatment.

hESC-derived RPE

Investigations of cell replacement therapies for retinal diseases are widespread owing to the retinal-immune barrier [68]. In a Phase 1/2 study, retinal transplantation of allogeneic hESC-RPE

(OpRegen) in legally blind patients with dry age-related macular degeneration (AMD) and geographic atrophy demonstrated improvement and maintenance of visual acuity over 15 months (NCT02286089). In a separate study, hESC-derived RPE (MA09-hRPE) was transplanted in the retina of patients with advanced Stargardt disease for macular repair (NCT01344993) [69]. Subretinal hyperpigmentation developed over the follow-up period of 12 months, indicating survival of transplanted cells. Although both transplantation studies occurred in an immune privileged site, systemic immunosuppression was administered to mitigate risks of rejection. Retinal transplantation of a patch containing hESC-RPE in patients with AMD, resulted in gain of visual acuity [70]. In this study, local immunosuppression was administered using intravitreal fluocinolone acetonide implant. In addition, human retinal progenitor cells (hRPCs) are clinically investigated through intravitreal or retinal injection without immunosuppression for retinitis pigmentosa (NCT02464436, NCT03073733).

Islet or beta cell transplantation in the anterior chamber of the eye

In a study of T1D, intraocular islet allografts engrafted immunosuppression free in mice, whereas transient immune intervention was required for a baboon model [71]. Results of this study led to a clinical trial of pancreatic islet transplantation into the anterior chamber of the eye (ACE) of legally blind T1D patients (NCT02846571). Islet revascularization in the ACE can break the immune barrier as vasculature network extends from the iris. In line with this, maintenance immunosuppression is administered for 2 years in the trial participants. To avoid systemic immunosuppression, there are studies investigating local protection, including one that leverages sustained-release rapamycin microparticles, which enhanced islet allograft survival in the ACE of mice [72].

Site-specific immunomodulatory role of ECs

Various studies have highlighted the ability of ECs to serve immunomodulatory roles beyond their function in immune cell recruitment, immune tolerance, and alloimmunity [73,74]. Specifically, tissue-specific subsets of ECs serve immune activities and present properties typical of immune cells, such as the ability to induce apoptosis in other cells, secrete cytokines, and express co-inhibitory or co-stimulatory receptors. Their fundamental role in maintaining tissue-specific immunity is exemplified at the blood–brain barrier, where ECs express low levels of adhesion molecules and lower levels of cytokines, ultimately impairing immune cell migration. As new findings elucidate the tissue-specific immune function of EC subgroups, new opportunities may emerge from leveraging these cells for immunomodulatory therapeutic purposes. However, this field is in its infancy.

Concluding remarks and future perspectives

The wide breadth of investigations in the field are exemplified by the numerous clinical trials exploring immunomodulatory strategies for cell therapy, some of which are listed in Table 1. The first-in-human and open-label, clinical trial of CAR-Tregs for inducing and maintaining immune tolerance to kidney transplants was initiated in 2021 (NCT04817774). Recently, a clinical trial in Japan suggested that cord blood transplantation combined with intra-BM injection of MSCs could prevent GVHD without engraftment inhibition [75]. Further, some innovative immunomodulatory interventions include clinical investigations of CRISPR-Cas9- and mRNA-based therapeutics to mitigate immune rejection or promote tolerance (NCT05210530).

As with all novel biomedical technologies, safety and ethical concerns must be addressed prior to clinical translation. Further, critical considerations for translation of cellular therapies include reproducibility, large-scale production [76], and standardization and quality control protocols [77,78] (see Outstanding questions). To resolve these challenges, various public and private programs have established fundamental guidelines for good manufacturing processes in the

Outstanding questions

Will the added factor of immunomodulation for cellular therapies, which already require laborious, large-scale manufacturing with rigorous quality standards, impact commercialization costs and be economically feasible?

Will genetic cell manipulation alone allow for complete graft immune evasion?

What types of gene manipulation are most feasible for a wide spectrum of cell therapeutics?

Will potential genotoxicity caused by traditional CRISPR-Cas9 gene editing push the field toward newer CRISPR-based technologies or alternative gene editing approaches?

Will tolerogenic vaccines improve transplant outcomes and what will be the key alloantigen(s) to target for vaccination?

Will nanotheranostics and optogenetics better support investigation and monitoring of localized immunomodulation?

How to better identify if acute rejection is due to the cell therapy or other factors?

Table 1. Selected clinical trials involving cell therapy and immunomodulation for regenerative therapy[a]

Disease	Type of cellular therapy	Administration method	Rationale/target	Monitoring parameters	Status	Country	Clinicaltrials.gov/Trialsearch.who.int
Multiple sclerosis	Tolerogenic fibroblasts	Intravenous injection	Anti-inflammatory, immunomodulatory factors (IL-10 and TGF-β) and neurotrophic mediators	Inflammation markers; Gd-MRI-brain and cervical scans	Completed (early Ph1)	Mexico	NCT05080270
Multiple sclerosis	Autologous BM-MSCs	Intravenous; intrathecal injections	Immunomodulation	MRI, immunological factors measuring	Not informed (Ph1/Ph2)	Jordan	NCT03069170
Multiple sclerosis	Autologous BM-MSCs	Intravenous and intrathecal injection	Induce neuroregeneration and protection	Migration of transplanted cells by SPIONs-MRI; immune cells and markers	Completed (Ph1/Ph2)	Israel	NCT00781872
Amyotrophic lateral sclerosis	Autologous MSCs	Intravenous and spinal cord injection	Safety and immunomodulation	Immunological factors measuring	Recruiting (Ph2)	Iran	IRCT20160809029275N2
Epidermal necrolysis; Lyell syndrome	ADSCs	Intravenous injection	Paracrine ADSC effects, wound healing immunomodulation	Th1/Th2 associated and anti-inflammatory chemokines; skin biopsy	Not yet recruiting (Ph1/Ph2)	France	NCT04711200
Ulcerative colitis; autoimmune and Crohn's diseases	Treg cells	Intravenous injection	Autoimmune diseases	Define changes in the effector T cell and Treg in the gut	Recruiting (Ph1)	Germany	NCT04691232
Crohn's disease	Autologous-BM-MSCs	Intravenous injection	Immune response and modulation	Immune cells and function monitoring	Completed (Ph1 and Ph2)	Belgium	NCT01540292
COPD	ADSCs	Intravenous injection	Immunomodulation	Not clear	Not informed (Ph1/Ph2)	Vietnam	NCT02645305
Acute respiratory distress syndrome	BM-MSCs	Not informed	Recovery of organ function; immunomodulation	Mortality; immune cells/cytokine/ miRNA monitoring	Not informed (Ph1)	Sweden	NCT02215811

Kidney transplantation	Tregs; BM-MSCs; tocilizumab	Intravenous injection	Immunomodulation in kidney transplant	Leukocyte monitoring	Recruiting (Ph1/Ph2)	Austria	NCT03867617
Kidney transplantation	Mitomycin C-treated donor PBMCs (MICs)	Intravenous injection	Safety and immunosuppression	Biomarkers and biopsy	Completed (Ph1/Ph2)	Germany	NCT02560220
Kidney transplant	CAR-Tregs TX200-TR101 ($CD4^+$/$CD45RA^+$/$CD25^+$/ $CD127^{low/neg}$)	Intravenous injection	Induce and maintain immunological tolerance	Biomarkers	Recruiting (Ph1/Ph2)	USA, UK, Netherlands, Belgium	NCT04817774
Kidney transplantation	Treg cell therapy	Intravenous injection	Immunosuppression	Immunological factors measuring; biopsy	Recruiting (Ph2)	UK	ISRCTN11038572
Liver transplantation	Treg cell therapy		Tolerance induction	Biopsy	Recruiting (Ph1/Ph2)	Japan	JPRN-UMIN000015789
Liver transplantation	MultiStem® (hBM-MSCs)	First: intra-portal at liver transplantation; second: intravenous infusion	Regulate immune system; immunomodulation; tissue repair	Ultrasound, biopsy; toxicity measuring	Completed (Ph1)	Germany	NCT01841632
Lung transplantation	ADSCs	Intravenous injection; reduce long-term graft rejection and dysfunction	Immunomodulation; reduce rejection and ischemic reperfusion injury after surgery; access the primary graft dysfunction	Inflammatory markers	Recruiting (Ph1/Ph2)	Denmark	NCT04714801
Bronchiolitis obliterans; lung transplantation	BM-MSCs	Intravenous	Immune tolerance and cardiopulmonary compromise	Pulmonary observation	Completed (Ph1)	USA	NCT02181712
Intestinal transplantation	$CD34^+$ stem cells	Infusion	Immunosuppression, tolerance induction	Graft survival and retention rate	Recruiting (Ph1)	USA	NCT04804891
Heart transplantation	Pediatric-autologous thyTreg	Intravenous injection; graft rejection: children heart-transplanted	thyTreg (>95% of $CD25^+$ $Foxp3^+$ cells)	Treg cell monitoring	Recruiting (Ph1/Ph2)	Spain	NCT04924491
Central retinal vein occlusion	Autologous BM $CD34^+$ stem cells	Intravitreal injection	Safe, feasible, and beneficial in eyes with vision loss	Ophthalmological observation	Recruiting (Ph1/Ph2)	USA	NCT03981549
Dry eye disease; Sjögren's syndrome	ADSCs	Injection into lacrimal gland	Increases tear production and reduce inflammation	Ophthalmological observations; biomarkers	Not recruiting (Ph2)	Denmark	NCT04615455

(continued on next page)

Table 1. (continued)

Disease	Type of cellular therapy	Administration method	Rationale/target	Monitoring parameters	Status	Country	Clinicaltrials.gov/Trialsearch.who.int
Macular degeneration	WT1 peptide-pulsed dendritic cell	Intradermal or subcutaneous injection	Safety, immunotherapy	Immunological factors measuring; retinal thickness, choroidal neovascularization		Japan	JPRN-jRCTc030210068
Macular degeneration	hESC-RPE seeded on a polymeric substrate implanted	Subretinal injections; subretinal space	Cell therapy and biomaterial safety implant	Ophthalmological observation	Completed (Ph1/Ph2)	Brazil	NCT02903576
Macular degeneration	hESC-RPE		Long-term observation	Observation, biomarkers	Enrolling by invitation (Ph1/Ph2)	USA, UK	NCT03167203
Knee osteoarthritis	BM-MSCs	Intra-articular injection	Safety and efficacy of single intra-articular injection of MSCs; reconstruction	MRI – XRD	Completed (Ph1/Ph2)	Taiwan	NCT03589287
Knee osteoarthritis	BM-MSCs, corticosteroid	Intra-articular, subchondral injections	Safety and efficacy	MRI – XRD	Not yet, recruiting (Ph1/Ph2)	USA	NCT05288725
Knee osteoarthritis	ADSCs	Knee injection	Therapeutic effect	MRI	Recruiting (Ph3)	Iran	IRCT20210307050611N1
Shoulder osteoarthritis	ADSCs and PRP	Intra-articular injection	Degeneration or chronic injury	MRI	Recruiting (Ph2)	USA	NCT02844738
Ischemic heart failure	ADSCs	Intracoronary injection	Heart regeneration	Echocardiography, Tc99m-SPECT or cardiac MR	Completed (Ph1)	Japan	JPRN-jRCTb040190115
TDM1	MSCs; mononuclear cells	Intravenous injection; localized infusion in pancreatic arteries and capillaries	Safety transplantation and immunomodulation	Exogenous insulin; anti-GAD titers; C-peptide and HbA1c level monitoring	Completed (Ph1/Ph2)	Jordan	NCT02644759
TDM1	Immunoregulatory DCs (engineered)	Intradermal injection-peri-umbilical	Immune response	Plasma C-peptide/-tolerogenic/Treg/B cells/DCs/insulin HbA1c level monitoring	Not informed (Ph2)	USA	NCT024354911

[a]Abbreviations: ADSCs, adipose-derived stem cells; BM-MSCs, bone marrow mesenchymal stem cells; COPD, chronic obstructive pulmonary disease; hESC-RPE, human embryonic stem cell-derived retinal pigmented epithelium; Ph1, Phase 1; Ph2, Phase 2; SPIONs, superparamagnetic iron oxide; thyTreg, thymus-derived Tregs.

biofabrication industry. A relevant example of these efforts is the BioFabUSA program established at the Advanced Regenerative Manufacturing Institute (ARMI). BioFabUSA is a public–private partnership consisting of industry, academia, government, and nonprofit organizations. This unique partnership focuses on directing science and engineering resources toward enabling scalable, consistent, and cost-effective manufacturing of cellular therapies. Within this, advances in robotics, information technology, computational sciences, and artificial intelligence infrastructures [79] will be fundamental to rendering cellular therapies more personalized, accessible, and affordable. In addition, as cellular therapies are essentially living drugs, mastering the supply chain from appropriate temperature regulated transportation logistics and storage to proper thawing and administration is important for widescale implementation in a reproducible manner.

Another significant challenge is developing safe and effective delivery strategies for cell therapeutics. Transplantation of pancreatic islets or SC-derived β cells provides a clear example of the importance of the delivery approach on the viability and function of the graft. Despite more than 70 years of research and development in the field, the ideal technological solution for the delivery of these cells has yet to be identified. To this end, novel discoveries in biomaterials and nanomedicine will continue to support these efforts in providing new molecular and cell engineering tools [80,81].

Finally, new research opportunities reside in developing effective strategies to track cell therapeutics upon delivery in the body [82], noninvasively monitor their viability and function, as well as modulate immunological response *ad hoc*. Here, innovative real-time imaging technologies and optogenetic approaches to manipulate cells using light stimuli at specific wavelengths may pave the way for novel discoveries [83].

These translational challenges are massive and require the convergence of multidisciplinary expertise and capabilities. As exemplified by the global response to the SARS-CoV-2 pandemic via the collective actions of academia and industry, which resulted in the ultra-rapid vaccine development and regulatory approval, concerted efforts could enable cellular therapies to reach their full potential.

Acknowledgments

We received funding support from National Institutes of Health (NIH) grant R01DK132104 (A.G.), Juvenile Diabetes Research Foundation (JDRF) [2-SRA-2022-1224-S-B (A.G.); 2-SRA-2021-1078-S-B (A.G.)], Vivian L. Smith Foundation (A.G. and C.Y.X.C.), and Men of Distinction Foundation (A.G. and C.Y.X.C.). A.Y.J. is supported by a NIH grant 4UH3HL147367-04. This work was also supported by the Spanish Ministry of Economy, Industry, and Competitiveness (PID2019-106094RB-I00/AEI/10.13039/501100011033). The authors wish to thank the graphic designer Virginia Facciotto (virginia.facciotto@gmail.com) for the figure preparations.

Declaration of interests

No interests are declared.

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