Stem Cell Research & Therapy

REVIEW

Open Access

CRISPR-mediated engineering of mesenchymal stromal/stem cells: a summary of recent progress in immunological applications for regenerative medicine and cancer therapy

Mohammadreza Dashti[1], Morteza Mohammaddust Sarab[2,3], Faezeh Shad[2,3] and Sajad Dehnavi[4,5*]

Abstract

Mesenchymal stromal/stem cells (MSCs) have introduced as a cornerstone of regenerative medicine, owing to their immunomodulatory properties and therapeutic potential in autoimmune and inflammatory disorders. Although, their clinical application is often restricted due to immune rejection and heterogeneity in immunoregulatory responses. The advent of Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9 (CRISPR/Cas9) technology has revolutionized MSC engineering, enabling precise genetic modifications to enhance their immunological efficacy. This review explores how CRISPR-mediated editing of MSCs can mitigate immunogenicity, amplify anti-inflammatory functions, and repurpose MSCs for targeted immunotherapy. Key strategies include knockout of β2-microglobulin to evade T-cell recognition, augmentation of anti-inflammatory mediators like interleukin (IL)-10 and TNF-alpha stimulated gene/protein 6 (TSG-6), and disruption of pro-inflammatory pathways such as toll-like receptor 4 (TLR4)/NF-κB. In addition, CRISPR-engineered MSCs demonstrate promise in reshaping tumor microenvironments and combating bacterial infections through enhanced innate immunity. Despite challenges including off-target effects and delivery optimization, CRISPR-tailored MSCs represent a transformative approach to overcoming immunological barriers, paving the way for universal, off-the-shelf therapies in rheumatoid arthritis, cancer, and beyond.

Keywords Mesenchymal stromal/stem cell, CRISPR/Cas9, Immune system, Immune cells

*Correspondence:
Sajad Dehnavi
sjd72dehnavi@gmail.com; dehnavis@mums.ac.ir
[1]Asadabad School of Medical Sciences, Asadabad, Iran
[2]Kashmar School of Medical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran
[4]Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[5]Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Introduction

In recent years, cell-based therapeutic approaches have gained prominence as an innovative strategy, drawing considerable research interest due to its regenerative potential [1]. A central component of this approach involves mesenchymal stromal/stem cells (MSCs)—a unique population of multipotent stromal cells known for their self-renewing capability, plasticity, and adherent growth. These cells, primarily found in mesenchymal tissues, are defined by their surface marker profile, including high surface expression of CD105, CD90, and CD73, alongside minimal or absent levels of hematopoietic and immunogenic markers such as human leukocyte antigen (HLA)-DR, CD11b/CD14, CD34, CD45, and CD79a/CD19 [2, 3]. MSCs are a fundamental component of regenerative medicine and cell-based therapies [4]. The processes through which MSCs implement their effects include their remarkable multipotency, broad tissue repair capabilities, and, most significantly, their potent intrinsic immunomodulatory properties [5, 6]. These plastic-adherent stem cells, which can be isolated from various tissues, exert their therapeutic effects through a variety of mechanisms [7]. These mechanisms include differentiation, paracrine signaling, and direct cell-to-cell interactions [8, 9]. Collectively, these mechanisms dampen excessive inflammation, promote tissue repair, and maintain immune homeostasis [10–12]. While MSCs inherently possess low immunogenicity, the full therapeutic potential of allogeneic MSCs often encounters significant limitations stemming from the complex and dynamic immune responses in pathological microenvironments [13, 14]. These responses can compromise the survival, engraftment, and desired functional outcomes of the MSCs [15]. The inherent heterogeneity of MSC populations, coupled with their variable responsiveness to diverse disease cues and potential vulnerability to hostile inflammatory microenvironments, often necessitates strategies to augment their therapeutic robustness and precision [16].

Gene editing represents a transformative biotechnology that enables precise modifications of genomic sequences, including insertions, deletions, and base substitutions, offering unprecedented control over genetic material [17, 18]. This technology holds prominent therapeutic potential, especially for monogenic disorders and diseases driven by aberrant gene expression, as it allows direct intervention at the genetic level [19].

The Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9 (CRISPR/Cas9) system, now a cornerstone of genetic engineering, originated as an adaptive immune mechanism in bacteria and archaea, defending against viral and plasmid invasions [7, 8]. During initial exposure to foreign DNA, prokaryotes integrate short segments of the invader's sequence into their CRISPR arrays. Upon re-exposure, these sequences are transcribed and processed into single-guide RNAs (sgRNAs), which direct Cas9 to cleave complementary invading DNA. Target recognition by sgRNAs depends on protospacer-adjacent motifs (PAMs), typically guanine-rich sequences such as the NGG motif recognized by Streptococcus pyogenes Cas9 (SpCas9) [9]. The ubiquity of NGG sites across diverse genomes has propelled CRISPR/Cas9's widespread adoption in biomedical research, agriculture, and therapeutic development [10–14]. The emergence of research in gene editing has given rise to a novel editing platform known as CRISPR/ Cas9 [20]. In contrast to earlier platforms such as zinc finger nucleases (ZFNs) and transcriptional activator-like nucleases (TALENs), this platform applies a programmable guide RNA (gRNA) to direct Cas9 to specific genomic locations, thereby significantly elevating the efficiency and versatility of editing in comparison to previous platforms [21]. By simply redesigning the gRNA sequence, CRISPR/Cas9 can be directed to virtually any genomic locus, enabling precise correction of disease-causing mutations or silencing of pathogenic genes [22]. This capability provides a wide variety of genetic manipulations, such as targeted gene knockout, precise gene insertion (knock-in), and gene expression modulation (CRISPR activation, CRISPRa; or repression, CRISPRi) without altering the underlying DNA sequence [23]. However, its efficacy may be limited in highly condensed chromatin regions [24]. Despite this constraint, CRISPR-based therapies show remarkable promise for treating cancers, cardiovascular disorders, sickle cell anemia, and neurodegenerative diseases, heralding a new era in precision medicine [25, 26]. While the majority of applications of MSC engineering have utilized the canonical CRISPR/Cas9 system for targeted DNA cleavage, additional CRISPR platforms are increasingly being explored (Table 1). Catalytically dead Cas9 (dCas9) has been demonstrated to facilitate transcriptional regulation without the requirement of DNA cleavage, thereby providing a versatile tool for gene activation or repression [27]. Cas12 (Cpf1), with its distinct PAM recognition and staggered DNA cleavage, offers alternative editing options [28]. Cas13, a specific RNA targeting mechanism, enables the transient modulation of gene expression and the regulation of post-transcriptional processes [29]. More recently, CIRTS (CRISPR-Cas–inspired RNA targeting system) has emerged as another RNA-targeting platform with potential applications in MSC engineering [30]. The incorporation of these systems serves to expand the CRISPR toolkit, thereby potentially unveiling novel pathways for the refinement of MSC functions within both immunological and regenerative contexts.

The necessity of CRISPR in advancing MSC-based therapies stems from its unparalleled ability to overcome

Table 1 Overview of different CRISPR platforms and their potential applications in cell engineering

CRISPR System	Editing / Function	Key Features	Evidence in MSCs	Refs.
Cas9	DNA double-strand break (knockout/knock-in)	Most widely used; NGG PAM requirement	Applied for knockout of immunogenicity-related genes (β2M, CIITA) in MSCs → reduced HLA expression and lower immunogenicity	[31, 32]
dCas9 (CRISPRi/a)	Transcriptional repression (CRISPRi) or activation (CRISPRa)	Catalytically inactive Cas9 fused to repressors/activators	Used in MSCs to activate anti-inflammatory genes (e.g., IL-10, TSG-6)	[33–35]
Cas12a (Cpf1)	DNA cleavage with staggered ends	Distinct PAM (TTTV); shorter gRNAs; sticky-end cuts	No direct data in MSCs yet; validated in mammalian cells, potential for MSC editing	[36, 37]
Cas13	RNA targeting and cleavage	Targets RNA instead of DNA; transient and reversible	No direct MSC data; high potential for modulation of cytokine or immune transcripts	[29]
CIRTS	RNA binding/editing	Cas-independent, synthetic RNA-targeting system	No direct MSC data; potential tool for immune transcriptome regulation in MSCs	[38]

key challenges. It permits for the precise and efficient modifying MSCs to enhance specific therapeutic traits, standardize cell product quality, and, crucially, overcome immunological barriers that hinder their efficacy [39]. By precisely altering genes involved in immune recognition, inflammatory signaling, or production of immunomodulatory mediators, CRISPR offers a powerful means to fine-tune MSCs' interaction with the host immune system, rendering them more resilient, less immunogenic, or more potently immunomodulatory for specific diseases [40, 41]. This profound capability renders CRISPR an indispensable tool for realizing the therapeutic promise of MSCs in the complex realm of immune-mediated disorders and regenerative medicine [42].

The present narrative review aims to comprehensively explore the cutting-edge applications of CRISPR/Cas9 in engineering MSCs, with a specific focus on the profound impact of these genetic modifications on their immunological attributes. The subsequent discussion will explore the application of CRISPR technology in the context of immune evasion, anti-inflammatory responses, the repurposing of MSCs for targeted anti-tumor immunity, and the identification of novel immunological applications. This exploration will contribute to the advancement of next-generation MSC-based immunotherapies.

Overcoming immunogenicity and immune rejection: engineering "Immune Stealth" MSCs

MSCs can be administered in either an autologous manner, in which the cells are derived from the same individual, or an allogeneic manner, in which the cells are derived from a donor and administered to a different recipient. While autologous MSCs generally avoid immune rejection, allogeneic MSCs are more practical for clinical use as "off-the-shelf" products. However, they raise critical questions regarding immunogenicity and host immune responses [43]. A paramount challenge in the broad application of allogeneic MSC therapies is the potential for host immune recognition and subsequent rejection, despite their inherently low immunogenicity [44]. This rejection is primarily induced by the expression of Major Histocompatibility Complex Class I (MHC-I) molecules on the MSC surface, which, particularly upon upregulation in inflammatory microenvironments, can trigger alloreactive T-cell responses [45, 46]. CRISPR/Cas9 technology has introduced as a transformative approach to precisely address this immunological barrier, enabling the creation of "immune stealth" MSCs designed to evade host immune surveillance (Fig. 1).

A prominent strategy involves the targeted knockout of beta-2 microglobulin (β2M), the crucial light chain of the MHC-I complex [47]. Studies have compellingly demonstrated that CRISPR-mediated deletion of β2M in various MSC sources, including umbilical MSCs (UMSCs) and induced pluripotent stem cell (iPSC)-derived MSCs, significantly abrogates HLA class I surface expression [48, 49]. This genetic modification profoundly impacts MSCs' interaction with the host immune system: by largely eliminating MHC-I presentation, these β2M-deficient MSCs become less recognizable to alloreactive CD8 + T cells, leading to a marked suppression of T-cell proliferation, activation, and infiltration into transplanted tissues. For instance, in models of cardiac repair, β2M-deleted UMSCs effectively suppressed CD8 + T cell activation and infiltration, modulated the immune microenvironment by reducing pro-inflammatory mediators like IFN-γ and TNF-α, and consequently enhanced stem cell survival and engraftment [49]. Another study conducted by Kwon et al. developed an approach to generate iPSC-derived MSCs with a disrupted HLA-I gene [50]. First, dermal fibroblasts were reprogrammed into iPSCs using Yamanaka's four factors (Oct4, Sox2, Klf4, c-Myc). Subsequently, CRISPR/Cas9-mediated gene editing was employed to knockout the polymorphic HLA-A, B, and C alleles. These edited iPSCs were then differentiated into MSCs using standard MSC culture conditions, yielding pseudo-homozygous HLA class I knockout iPSC-derived MSCs (KO iMSCs). The resulting KO iMSCs

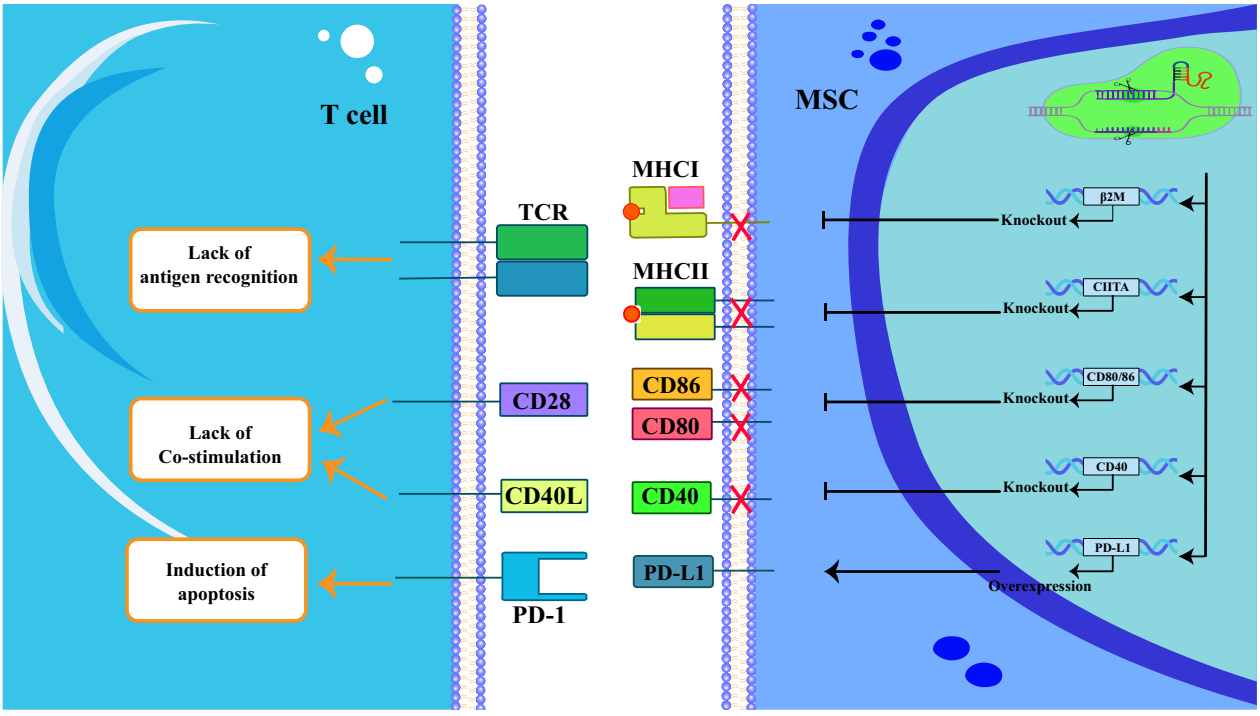

Fig. 1 CRISPER/Cas9-based approaches to reduce the immunogenicity of MSCs

retained multipotency, demonstrated in vivo safety, and maintained the capacity for chondrogenic differentiation. Importantly, unlike complete HLA class I-null cells, KO iMSCs evaded natural killer (NK) cell-mediated cytotoxicity. These findings suggest that KO iMSCs could serve as an immunologically compatible, "off-the-shelf" cell therapy product. The ability to generate such hypoimmunogenic MSCs without impairing their crucial reparative functions or differentiation potential represents a significant leap towards successful allogeneic transplantation without the need for extensive HLA matching. Furthermore, Romano et al. reveal the specific genetic modification involved using a single guide RNA (sgRNA) targeting the third exon of the CIITA gene. This process resulted in the insertion of a single nucleotide on both alleles. This insertion led to a frameshift mutation, which consequently generated a premature stop codon. The functional consequence of this knockout is the loss of CIITA protein, which is necessary for the expression of MHC class II molecules. The removal of MHC class II from the cell surface of engineered iPSCs has been demonstrated to facilitate their evasion of immune detection and rejection by the host's immune system. This approach offers a promising solution for the production of "off-the-shelf" allogeneic cell products. The study confirmed that the resulting cell line maintained typical pluripotent morphology, expressed pluripotency markers, and could differentiate into all three germ layers, all while having a normal karyotype [51]. In a separate study, researchers employed the CRISPR-Cas9 system to excise the major histocompatibility complex (MHC) class I and II genes, specifically B2M and CIITA. Furthermore, the murine protein CD47 was incorporated into the cells to impede xenograft formation, and hSEAP was added to monitor cell trafficking in vivo. The results of the in vivo experiments demonstrated that, despite these genetic modifications, the cells were completely rejected within 11 days after transplantation into mice with an active immune system. T-lymphocyte infiltration was clearly observed on day 8. The study's findings indicate that the deletion of the B2M and CIITA genes, in conjunction with the expression of CD47, is inadequate in preventing graft rejection within a foreign and immune-active environment. These results suggest the involvement of additional mechanisms in the process of graft rejection [52].

In addition to MHC-I modulation via β2M knockout, co-stimulatory molecules such as CD80 (B7-1), CD86 (B7-2), and CD40 represent key targets for enhancing MSC immune evasion [53]. These molecules are generally expressed at low basal levels on MSCs; however, they can be upregulated in inflammatory microenvironments, thereby promoting T-cell activation and alloreactivity through interactions with CD28/CTLA-4 (for CD80/CD86) and CD40L (for CD40) on T cells [54]. In order to counteract the aforementioned process, the use of immunosuppressive MSCs has been proposed. The mechanism of action of these cells involves the downregulation of CD80, CD86, and CD40 expression in dendritic cells

(DCs). This process has been shown to inhibit the maturation of DCs and to suppress the production of pro-inflammatory cytokines, such as IL-12. The net effect of these processes is to foster an anti-inflammatory milieu and to reduce immunogenicity. For instance, MSCs have been demonstrated to maintain DCs in an immature state by suppressing MHC-II, CD80, CD86, and CD40, thereby constraining T-cell priming and effector responses.

CRISPR/Cas9 provides a precise mechanism for amplifying this natural process through targeted gene knockouts [55]. While direct applications in MSCs are in their nascent stages, analogous strategies in dendritic cells (DCs)—a pivotal player in MSC-immune cross-talk—demonstrate feasibility. Specifically, the simultaneous CRISPR-mediated knockout of CD80, CD86, and CD40 in DCs using nanoparticle-delivered Cas9/sgRNA complexes has triggered Treg expansion and prevented autoimmune responses in type 1 diabetes models by impairing co-stimulation and antigen presentation [56]. The extension of this concept to MSCs has the potential to synergize with β2M editing, resulting in the creation of hypoimmunogenic stealth cells that exhibit minimal T-cell engagement. Preliminary evidence suggests that such multiplex edits preserve the multipotency of MSC while enhancing the efficacy of allogeneic engraftment, thereby paving the way for off-the-shelf therapies [55].

Furthermore, the safe and efficient delivery of CRISPR components is critical for minimizing unintended immune responses to the gene-editing machinery itself [57]. Innovations in non-viral delivery methods, such as ribonucleoprotein (RNP) complexes and advanced electroporation techniques, including tube electroporation, have proven instrumental in achieving high editing efficiencies with minimal toxicity [58, 59]. These approaches are crucial for ensuring the overall immunocompatibility of the engineered MSC product, avoiding potential immune reactions against residual viral vectors or off-target effects that could compromise the cell's therapeutic efficacy or safety in clinical applications. In this regard, a novel tube electroporation method has been developed in Xu et al. study that significantly enhances the efficiency of homology-directed repair (HDR) and overall gene ablation in these difficult-to-transfect cell types while maintaining minimal toxicity [59]. Of particular significance is the demonstration of the platform's immunological implications through successful gene editing in key immune cells. The method demonstrated a high success rate in the knockout of β2M in MSCs (37.3% to 80.2%), a target that has been identified as being relevant for modulating cellular immunogenicity and transplantation outcomes. Furthermore, the efficient ablation of PD-1 in primary human T cells (42.6% to 58.6%) underscores its direct applicability in cancer immunotherapy, offering a robust strategy to overcome immune checkpoint inhibition, akin to established antibody treatments. This high-efficiency, low-toxicity gene delivery system represents a substantial advancement toward the clinical translation of precision gene-edited cellular immunotherapies.

In another study, Han et al. used CRISPR/Cas9 technology to delete the β2M gene to reduce the immunogenicity of hMSCs [40]. In this study, they systematically compared plasmid DNA and RNP delivery methods for CRISPR-Cas9 genome editing in MSCs. Their results demonstrate that RNP-mediated editing achieves significantly higher indel (insertion-deletion)) frequencies with minimal cellular toxicity compared to plasmid-based approaches. Flow cytometry results showed a decrease in MHC-I expression on the surface of edited MSCs. Also, to evaluate the immunological function, modified MSCs were co-cultured with $CD8^+$ T cells. The results highlighted that the death rate of modified MSCs was much less than that of the control group, and T cell proliferation was also suppressed. Among the canonical immunosuppressive mediators of MSCs, indoleamine 2,3-dioxygenase (IDO) and prostaglandin E2 (PGE2) play central roles in dampening immune activation. IDO contributes to suppression of T-cell and NK-cell function through tryptophan depletion and kynurenine accumulation, while PGE2 modulates cytokine production and reduces cytotoxic activity [60, 61]. Examination of the levels of IDO-1 and PGE2 in the supernatant by ELISA and Western blot showed a significant increase compared to normal MSCs, which is directly related to the immunosuppressive ability [40]. Finally, this study showed that deletion of β2M in MSCs not only significantly reduced the stimulation of the host immune system, but also increased their survival in an inflammatory environment. This property is very valuable for applications in cell therapies, transplantation, and autoimmune diseases. By focusing on both precise genomic modification and immunologically inert delivery, CRISPR/Cas9 engineering is paving the way for truly allogeneic and widely applicable MSC therapies. Recent studies have increasingly unveiled the potential of CRISPR-engineered MSCs to bolster their inherent anti-inflammatory and immunomodulatory properties. These advancements offer novel strategies to address immunological challenges across various diseases [62].

Augmenting MSCs' intrinsic anti-inflammatory and Immunomodulatory capacities

Enhancing anti-inflammatory cytokine and protein secretion

As shown in the Table 2, CRISPR/Cas9 engineering has endowed MSCs with the ability to profoundly recalibrate inflammatory microenvironments. For instance, CRISPRa of Interleukin (IL)-10), a pivotal anti-inflammatory mediator, in bone marrow-derived MSCs led

Table 2 CRISPR enhancement of MSC Immunomodulatory & Anti-Inflammatory functions

Target/Pathway	Gene type / Category	Direction of regulation (CRISPR strategy)	Cell Source	Functional / Molecular Change	Immunological / Therapeutic Effect	Disease Model	Refs.
IL-10	Cytokine (anti-inflammatory)	Overexpression	BM-MSCs	↑ IL-10 secretion	↓ TNF-α, IL-1β, IL-6, MCP-1; reduced CD68+/CD11b + macrophage infiltration; enhanced anti-inflammatory phenotype	Diabetic myocardial infarction	[63]
TSG-6	Cytokine-like protein / EV cargo	Overexpression / EV enrichment	Generic MSCs	↑ Anti-inflammatory EV cargo (miR-146a, TSG-6, IL-10)	↓ IL-8 & COX-2 in disc cells; attenuated disc inflammation	Intervertebral discitis	[35]
Keap1 (Nrf2 pathway)	Signaling regulator (oxidative stress sensor)	Knockout (Keap1 deletion → constitutive Nrf2 activation)	Rat AD-MSCs	Nrf2 pathway activation; ↑ antioxidant enzymes	↑ Oxidative stress resistance; ↑ survival in inflammatory microenvironment; promotion of anti-inflammatory phenotype	Oxidative stress models	[65]
TLR4	Pattern-recognition receptor (innate immunity)	Knockout / downregulation	Human AD- & cardiac MSCs	↓ NF-κB signaling	↓ IL-6, TNF-α, MCP-1; ↓ cardiac fibrosis & inflammatory infiltration	Myocardial infarction	[67]
TLR3 / IFN-β / JAK1 axis	Pattern-recognition receptor / signaling pathway	Knockout / modulation	hUCB-MSCs	↓ Senescence-associated secretory phenotype (SASP)	Preserved immunomodulatory function after expansion	Senescence prevention	[68]
miR-2861	Non-coding RNA (miRNA)	Overexpression	Human MSCs	↓ HDAC4/5 → epigenetic reprogramming	Acquisition of immunoprivileged phenotype; ↑ osteogenic potential	Inflammatory conditions	[71]
CD14	Pattern-recognition receptor (LPS receptor)	Overexpression	BALB-MSCs	↑ LPS recognition; accelerated NF-κB activation	↓ E. coli growth; enhanced antibacterial response	Bacterial infection	[72]
sTNFR1 feedback	Soluble cytokine receptor	Overexpression (inducible)	hUC-MSCs	TNF-α-responsive sTNFR1 secretion	Neutralized TNF-α; ↓ iNOS + macrophages; ↑ motor function	Spinal cord injury	[73]
PDGF-B	Growth factor	Overexpression	hBM-MSCs	↑ Angiogenesis; ↑ BDNF expression	Accelerated wound healing; ↓ chronic inflammation	Diabetic wounds	[74]

AD-MSCs: Adipose-derived MSCs, BM-MSCs: Bone marrow-derived MSCs, CCL2: C-C motif chemokine ligand 2, CXCL12: C-X-C motif chemokine ligand 12, EVs: Extracellular vesicles, hUCB-MSCs: Human umbilical cord blood MSCs, hUC-MSCs: Human umbilical cord MSCs, IL: Interleukin, KO: Knockout, Nrf2: Nuclear factor erythroid 2-related factor 2, PD-L1: Programmed death-ligand 1, RNP: Ribonucleoprotein, SASP: Senescence-associated secretory phenotype, TSG-6: TNF-α stimulated gene/protein 6

to a dramatic decrease in pro-inflammatory cytokine levels, specifically IL-1β, IL-6, TNF-α, and MCP-1, in cardiac tissue following diabetic myocardial infarction [63]. Furthermore, the engineered MSCs effectively suppressed the recruitment of inflammatory CD68+/CD11b + cells and CD68 + macrophages into the infarct region, thereby mitigating the detrimental immune cell accumulation associated with post-MI injury. Beyond this profound immunomodulation, the treatment concurrently improved critical cardiac function indicators (e.g., EF and FS), diminished cellular apoptosis, and promoted angiogenesis, collectively driving enhanced cardiac repair. It is important to note that these findings were derived from preclinical animal models (primarily murine models of MI, and therefore, they should be interpreted with caution in terms of clinical translation. While these results support the therapeutic potential of CRISPR-engineered MSCs, challenges such as off-target genome edits, long-term safety in vivo, and scalability for clinical-grade manufacturing remain significant. In addition, a limited number of independent groups have validated these findings, thereby underscoring the necessity for replication across various laboratories and models. A comprehensive review of the extant literature reveals that the current body of research underscores the promising potential of CRISPR technology to enhance the reparative capacity of MSCs. However, a thorough examination of these studies also illuminates the necessity of addressing significant translational and safety challenges prior to the realization of clinical applications.

Extending the therapeutic efficacy of MSC-derived extracellular vehicles (EVs) as potent immunomodulatory agents, another study masterfully leverages CRISPR-Cas9 activation to induce the expression of TNFα-stimulated gene/protein 6 (TSG-6) in MSCs [35]. This approach, critically, operates by activating gene expression without

direct DNA cleavage, offering a significant safety advantage over traditional gene knockout or knock-in strategies, thereby minimizing potential immunogenic responses associated with genomic alterations. The strategic overexpression of TSG-6 profoundly reprograms the anti-inflammatory cargo within MSC-derived EVs. In robust functional assays utilizing human intervertebral disc (IVD) cells—a critical target for inflammatory interventions—pre-stimulated with the potent pro-inflammatory cytokine IL-1β, treatment with EVs from TSG-6-activated MSCs (MSCa EVs) elicited a significant attenuation of inflammatory responses. This was evidenced by a marked decreased expression of key pro-inflammatory genes, notably IL-8 and COX-2, when compared to control EVs. These findings firmly establish that EVs generated from MSCs with augmented TSG-6 expression serve as powerful, non-cellular anti-inflammatory biotherapeutics. Their capacity to precisely modulate and control inflammation positions them as highly promising therapeutic modalities for conditions such as intervertebral disc degeneration and other inflammatory musculoskeletal and neurological diseases, capitalizing on their enhanced immunological signaling. This method represents a novel approach to enhance EV application from MSCs with the aim of improving immunogenicity and therapeutic efficacy.

Targeting inflammatory signaling pathways

CRISPR/Cas9 enables the precise targeting of inflammatory signaling pathways within MSCs [64]. Hu et al. investigated a CRISPR/Cas9-based strategy to enhance the intrinsic anti-oxidative capacity of rat adipose-derived MSCs (Ad-MSCs) by disrupting the Keap1 gene [65]. Normally, Keap1 sequesters Nrf2 in the cytoplasm, inhibiting its crucial role as a master regulator of antioxidant and anti-inflammatory genes [66]. By targeting either the start codon or a critical interaction site (376th codon) of Keap1 with CRISPR/Cas9, the researchers successfully induced Nrf2 nuclear localization, thereby activating its protective pathways. Genetically modified Ad-MSCs demonstrated significantly enhanced resistance to oxidative stress, evidenced by reduced malondialdehyde (MDA) content and modulated expression of apoptotic (Bax-1) and anti-apoptotic (Bcl-2) genes after hydrogen peroxide treatment [65].

In another study, Schary and colleagues edited human adipose and cardiac-derived MSCs applying the CRISPR/Cas9 system to delete the TLR4 gene, a key player in inflammatory pathways [67]. CRISPR transfer was performed non-virally (electroporation), and the editing rate was reported to be up to 68%. After TLR4 knockout, these cells still retained MSC markers including CD73, CD90, and CD105. Molecular analyses showed that TLR4 deletion significantly reduced NF-κB signaling and the expression levels of pro-inflammatory mediators such as IL-6, TNF-α, and MCP-1 in MSC cells. Flow cytometry results also confirmed the reduction of TLR4 levels on the cell surface and the reduction of inflammatory secretory activity. In animal experiments, these edited MSCs were injected into a mouse model of myocardial infarction and resulted in increased survival by 90%, reduced cardiac fibrosis, improved left ventricular function indices (EF, FS), and reduced inflammatory cell infiltration. Histopathological findings also showed reduced scarring and preservation of heart wall thickness. This highlights that even without altering other MSC properties, their therapeutic potential can be enhanced by inhibiting a single inflammatory receptor like TLR4.

Furthermore, the therapeutic promise of hUCB-MSCs in immunomodulation and regenerative medicine is significantly constrained by their propensity for in vitro senescence, a process driven by a critical immunological axis [68]. This study elucidates that the continuous ex vivo expansion of hUCB-MSCs induces a detrimental upregulation of toll-like receptor 3 (TLR3), a pivotal pattern recognition receptor that, when activated, initiates a potent autocrine IFN-β signaling loop. This self-reinforcing IFN-β signaling cascade subsequently activates JAK1, a key mediator in cytokine-driven immune responses, ultimately driving the senescence phenotype in hUCB-MSCs. Furthermore, this TLR3-mediated senescence is profoundly associated with the acquisition of a Senescence-Associated Secretory Phenotype (SASP), characterized by the release of pro-inflammatory cytokines and chemokines. This shift fundamentally compromises the MSCs' intrinsic immunomodulatory capabilities and can even induce a pro-inflammatory state, thereby undermining their therapeutic potential. Disrupting this TLR3/IFN-β/JAK1 pathway offers a compelling strategy to preserve MSC immune functionality and enhance their clinical viability.

Epigenetic reprogramming for immune-privileged phenotypes

CRISPR has been employed for targeted epigenetic modulation in MSCs to overcome immunological barriers [69]. HDAC4, a class IIa histone deacetylase, modulates inflammatory and metabolic pathways through histone and non-histone protein deacetylation, with multi-level regulation making it a promising therapeutic target [70]. By upregulating miR-2861 in human MSCs, precise control over immunomodulatory HDACs (HDAC4/5) was achieved. This miR-2861-mediated HDAC suppression created an immunoprivileged MSC phenotype, evidenced by reduced HDAC5 expression and enhanced osteogenic potential [71]. Importantly, this CRISPR approach avoided plasmid-associated cytotoxicity while establishing stable epigenetic reprogramming, resulting

in an MSC population with improved immunomodulatory capacity that maintained functionality under inflammatory conditions [71].

Repurposing MSCs for potent anti-tumor immunotherapy

Eliminating immunosuppressive capabilities

In contrast to the use of MSC-mediated immunosuppression in transplantation or autoimmune disorders, where such immunosuppression is therapeutically desirable, in the field of oncology, this same property can facilitate tumor progression by dampening anti-tumor immunity [75]. Consequently, CRISPR-based strategies have been investigated for their potential to eliminate or reverse the immunosuppressive phenotype of MSCs within the tumor microenvironment [62]. Among the various targets of interest, PD-L1 has received particular attention, as its expression on MSCs contributes to T-cell inhibition and immune escape mechanisms [76]. The reprogramming of MSCs to enhance T-cell activation and IFN-γ secretion can be achieved by deleting or modulating PD-L1, thereby augmenting anti-tumor immune responses.

In a study, Dunavin and colleagues administered the CRISPR/Cas9 gene editing technique to delete the PD-L1 gene in MSCs extracted from umbilical cord Wharton's jelly [77]. PD-L1 is among the best-known immunosuppressive agents that, by interaction to the PD-1 receptor expressed on T cells, reduces their activity and turns off the immune response against tumors [78]. Therefore, the presence of PD-L1 in MSCs could help create an immunosuppressive microenvironment around tumors [79]. The researchers used CRISPR to delete a significant portion of the PD-L1 gene and then examined the effect of this modification on the function of MSCs and their secreted exosomes on the activation of immune cells, specifically $CD4^+$ (T helper) and $CD8^+$ (cytotoxic T cells) [77]. The results showed that the edited MSCs were no longer able to inhibit T cell activation. In other words, the natural inhibition previously exerted by MSCs was significantly reduced after PD-L1 deletion [77]. In examining the function of the immune system, it was observed that the phosphorylation of key signaling proteins including Zap-70 and Syk was improved in T cells, which indicates the restoration of the immune system's response capacity. Also, the secretion of IFN-γ, one of the important cytokines in the anti-tumor response, was significantly increased in $CD8^+$ T cells. This confirms that the deletion of PD-L1 in MSCs can lead to an increase in the power of the immune system in fighting cancer cells. Another important point is that the exosomes secreted from the edited MSCs also maintained this property and were no longer suppressive of the immune response, which also indicates the importance of using MSC secretory products in anti-cancer therapies [77]. As previously mentioned, the targeting of regulatory molecules by CRISPR could serve as a promising approach towards activating MSCs. However, this strategy represents a double-edged sword: while PD-L1 deletion enhances immune activation in cancer settings, it may compromise MSC survival and efficacy in contexts like allogeneic transplantation or chronic inflammatory diseases (e.g., rheumatoid arthritis) [79]. In such scenarios, the expression of PD-L1 by MSCs is critical for the maintenance of their immunomodulatory functions, including the suppression of excessive inflammation through interactions with PD-1 on immune cells. Consequently, the therapeutic application of PD-L1-edited MSCs must be meticulously tailored to the disease context, balancing immune activation with the need for MSC persistence and immunoregulatory effects.

Modulating tumor microenvironment and immune cell trafficking

CRISPR-mediated genetic modifications of MSCs can actively modulate the tumor microenvironment and immune cell trafficking [80]. CXCL12/SDF-1 has been identified as a pleiotropic mediator of macrophage plasticity, dynamically regulating their polarization along the M1-M2 spectrum in a microenvironment-dependent manner, with significant implications for both neoplastic and autoimmune pathologies [81, 82]. Babazadeh and et al. applied CRISPR/Cas9 technology to delete the CXCL12 gene in bone marrow-derived MSCs [83]. They aimed to investigate the role of this chemokine (CXCL12) in altering the phenotype of macrophages and their effect on tumor growth. After generating CRISPR-modified MSCs (CXCL12−/−), they were co-cultured with bone marrow-derived macrophages (BMDM) in a co-culture medium to examine their effect on the immunological phenotype. In the presence of normal MSCs (CXCL12+/+), macrophages were driven towards the M2 phenotype. Increased expression of cytokines IL-4, IL-10, TGF-β and the marker CD206 (M2-specific) and decreased expression of IL-6, TNF-α and iNOS (M1-specific) were observed. On the other hand, in the presence of CRISPR-modified MSCs (CXCL12−/−), the phenotype of macrophages shifted towards M1, i.e. increased expression of inflammatory cytokines and decreased expression of suppressor factors were observed, and also, the phagocytic activity of macrophages was increased. In addition, co-culture of 4T1 mammary tumor cells and macrophages primed by (CXCL12−/−) or (CXCL12+/+) To investigate the behavior of tumor cells in the face of primed macrophages, limiting dilution assays were performed in BALB/c mice. The results showed that macrophages primed with normal MSCs (CXCL12+/+) led

to increased tumor formation and increased tumor size. In contrast, macrophages primed with modified MSCs caused a significant reduction in the size and number of tumor cells. Also, in the 3D culture of 4T1 cells with primed macrophages, the number and size of tumor spheroids (MCTS) in the face of macrophages primed with CXCL12−/− showed a significant reduction compared to macrophages primed with CXCL12+/+.

CCL2 is crucially involved in the process of prostate cancer bone metastasis by recruiting tumor cells to the osseous microenvironment, activating PI3K/Akt-mediated proliferation, and inducing cytoskeletal remodeling through p70-S6 kinase phosphorylation [84, 85]. Bui et al. used CRISPR/Cas9 technology to delete the CCL2 gene in mouse bone marrow-derived MSCs to investigate its effect on prostate cancer growth [86]. After generating CCL2−/− MSCs, they co-injected them with prostate cancer cells (TRAMP-C2) in a syngeneic mouse model. The findings showed that deletion of CCL2 led to a severe reduction in the migration of macrophages and monocytes into tumor tissue, resulting in a significant reduction in tumor size and weight. To analyze the immune populations in tumor tissue, markers CD45, CD11b, and Ly6G were used to examine monocytes and neutrophils. The results showed an increase in $CD45^{+}CD11b^{+}Ly6G^{-}$ cells, i.e., anti-tumor monocytes, in tumors of animals receiving modified MSCs. The increase in $CD45^{+}CD11b^{+}Ly6G^{-}$ (mononuclear immune cells) in the tumor environment supported an inflammatory and anti-cancer environment. Also, deletion of CCL2 via CRISPR/Cas9 inhibited the migration of macrophages into the tumor area, which resulted in a reduction in tumor diameter and weight in prostate syngeneic mice. In conclusion, the results of this study demonstrated that modified MSCs are able to alter the immune balance of the tumor microenvironment to induce a more potent anti-tumor response. In a separate study, researchers Tang et al. investigated a novel strategy for enhancing antitumor activity. This approach utilizes genetically engineered induced pluripotent stem cell-derived mesenchymal stem cells (iMSCs) to address the aforementioned challenges. Researchers created a homogeneous stem cell line called NKG2D-CAR-iMSCs. To achieve this objective, the researchers engineered induced pluripotent stem cells (iPSCs) by inserting a chimeric antigen receptor (CAR) containing the NKG2D extracellular domain into the B2M gene locus. These modified iPSCs were then differentiated into NKG2D-CAR-iMSCs. The results demonstrated significant advancements in the precision of tumor targeting. In a laboratory setting, the NKG2D-CAR-iMSCs demonstrated enhanced migration toward and adhesion to various solid tumor cells that expressed NKG2D ligands. This enhanced targeting was substantiated by RNA sequencing, which revealed that genes associated with cell adhesion and migration were significantly overexpressed in the modified cells [87]. In a living model involving A549 xenograft mice, the NKG2D-CAR-iMSCs demonstrated a significant 57% increase in their capacity to home to tumors when compared to standard iMSCs. The study's findings indicate that the NKG2D-CAR modification effectively enhances the targeting specificity of iMSCs in both in vitro and in vivo settings.

Smart therapies with feedback-controlled immunomodulation

Innovative approaches involve engineering MSCs with feedback-controlled CRISPR systems to achieve targeted anti-inflammatory effects. hUC-MSCs have been engineered using a CRISPR/Cas9 plasmid to self-regulate and feedback the secretion of soluble TNF-α receptor protein (sTNFR1) in response to increased TNF-α [73]. The aim of this design was to reduce inflammation in spinal cord injury (SCI). MSCs were administered indirectly via secreted exosomes; these EVs were conjugated with the CAQK peptide to target the spinal cord injury site. In vitro and in vivo experiments demonstrated that these engineered EVs efficiently accumulated in spinal cord tissue, neutralized TNF-α, reduced inflammatory cytokines, and reduced the presence of $iNOS^{+}$ macrophages at the injury site. ELISA and immunofluorescence data confirmed the increase in sTNFR1 and the decrease in TNF-α. In an animal model, treatment with these EVs resulted in significant improvements in motor function and neuronal regeneration. The innovative aspect of this study was the use of a feedback-controlled CRISPR plasmid system that is activated only in the presence of TNF-α. This approach produced a targeted anti-inflammatory effect without the need for direct MSC injection, demonstrating that MSC exosomes can serve as smart CRISPR carriers for neuro-inflammatory therapies.

While CRISPR-mediated strategies in transplantation and autoimmune diseases are primarily designed to reduce immunogenicity and enhance immunosuppressive or tolerogenic properties of MSCs, the therapeutic goal in oncology is fundamentally different (Table 3). In the tumor microenvironment, the inherent immunosuppressive functions of MSCs are often deleterious, as they can facilitate tumor progression and immune evasion. Consequently, engineering approaches in this context focus on eliminating or reversing these immunosuppressive properties to potentiate anti-tumor immunity. As mentioned, the deletion of PD-L1 in MSCs has been demonstrated to enhance T cell activation and increase IFN-γ secretion, thereby promoting anti-tumor responses. However, this modification also introduces a critical trade-off: loss of PD-L1 simultaneously increases MSC immunogenicity, which may compromise their persistence and survival in vivo. Furthermore, the knockout

Table 3 Context-Dependent effects of CRISPR-Edited MSCs by therapeutic goal

Therapeutic Goal	Genetic Target	Modification	Advantages	Challenges	Primary Context	Refs.
Immune Evasion (Transplantation/Autoimmunity)	β2M	Knockout	↓ MHC-I; enhanced MSC survival; reduced T-cell rejection	Potential NK-cell activation	Allogeneic transplantation, rheumatoid arthritis	[34, 36, 37]
	IL-10	Overexpression	↑ Anti-inflammatory effects; ↓ pro-inflammatory cytokines	May suppress anti-tumor immunity	Autoimmune diseases (e.g., rheumatoid arthritis)	[42]
	PD-L1	Overexpression	↑ Immunosuppression; protects MSCs from immune rejection	May promote tumor growth	Transplantation, chronic inflammation	[57]
Immune Activation (Cancer Therapy)	PD-L1	Knockout	↑ T-cell activation ($CD4^+$/$CD8^+$); ↑ IFN-γ; enhanced anti-tumor immunity	↓ MSC survival in inflammatory settings	Tumor microenvironment reprogramming	[54, 57]
	CXCL12	Knockout	↑ M1 macrophage polarization; ↓ tumor growth	Limited role in non-cancer settings	Cancer immunotherapy	[61]

of CXCL12 and CCL2 has been shown to reduce the recruitment of immunosuppressive myeloid cells, thereby reprogramming the tumor microenvironment toward a more immunostimulatory state. Collectively, these findings demonstrate that the CRISPR-based engineering of MSCs for cancer therapy constitutes a conceptually antithetical strategy in comparison to immune-stealth approaches in transplantation or autoimmune disorders. It is imperative to acknowledge this contrast and the concomitant trade-offs to comprehensively grasp the context-dependent nature of CRISPR applications in MSCs.

Other CRISPR-based functional enhancements of MSCs

Antimicrobial-related" vs. "tissue repair-related"

CD14, initially characterized as a monocyte marker, is a 55-kDa GPI-anchored PRR that recognizes diverse microbial products including LPS [88, 89]. It exists in both membrane-bound and soluble forms, mediating pathogen recognition and immune responses [90]. CRISPRa has been successfully leveraged to augment antibacterial immunity in MSCs [72]. MSCs exhibit considerable heterogeneity in their antibacterial capacities, a factor limiting their therapeutic consistency. This study identified that MSCs with superior antibacterial activity, such as C57BL/6-derived MSCs (C57-MSCs), possess elevated baseline expression of CD14, a crucial co-receptor for lipopolysaccharide (LPS) recognition. Conversely, BALB/c-derived MSCs (BALB-MSCs) showed attenuated antibacterial responses and lower CD14 levels.

Leveraging CRISPRa, endogenous CD14 expression was successfully augmented in BALB-MSCs. This targeted genetic modification significantly enhanced the antibacterial properties of BALB-MSCs, even without LPS priming, mirroring the robust activity observed in natural C57-MSCs. Mechanistically, CD14 overexpression accelerated the kinetics of NF-κB nuclear translocation in response to LPS, indicating a more rapid initiation of downstream immune signaling pathways. Single-cell transcriptional profiling further confirmed this expedited LPS response at a global molecular level. Transcriptomics and membrane surface proteomics analysis revealed that the edited MSCs had higher levels of CD14, which induced a faster response to LPS. Also, specific bacteria (e.g., E. coli) showed a significant reduction in growth after exposure to CD14-enhanced MSCs in culture medium, indicating the efficacy of this novel treatment. These findings underscore the potential of CRISPR-based approaches to precisely modulate MSC phenotypes, thereby augmenting their intrinsic immunological responsiveness for therapeutic applications.

CRISPR-engineered MSCs, even those primarily targeting regenerative outcomes, can indirectly contribute to immune regulation by resolving inflammation more rapidly or enhancing tissue repair in inflammatory contexts [91]. Genetically engineering hBM-MSCs offers a promising avenue to enhance their therapeutic efficacy for wound healing [74]. This study describes a novel platform utilizing CRISPR/Cas9 and recombinant adeno-associated virus serotype 6 (rAAV6) for precise, targeted gene insertion into hBM-MSCs, ensuring stable overexpression of desired therapeutic proteins without altering fundamental MSC characteristics. Applying this innovative approach, hBM-MSCs were engineered to constitutively overexpress and secrete platelet-derived growth factor B (PDGF-B), a crucial regulator of tissue repair. Subsequent evaluation in a diabetic murine excisional wound model demonstrated that a single, local administration of these PDGF-B-secreting hBM-MSCs significantly accelerated wound closure kinetics and reduced the overall time to complete wound healing compared to control groups. Also, increased blood vessel growth (angiogenesis) was observed in the repaired tissue, which was confirmed by CD31 staining. These findings highlight the potential of

CRISPR-based gene editing to enhance the regenerative capacity of MSCs, thereby offering a more potent cell-based therapy for challenging wound healing scenarios.

Also, hMSCs hold considerable promise for treating neurodevelopmental disorders, particularly when engineered to enhance specific therapeutic protein secretion [92]. A study conducted by kim et al. investigates the efficacy of hMSCs overexpressing Brain-Derived Neurotrophic Factor (BDNF) in improving outcomes in mouse models of Rett Syndrome, a severe neurological condition characterized by motor, cognitive, and respiratory deficits [93]. While hMSCs possess inherent immunomodulatory properties, the primary focus of this research centers on the neurotrophic benefits derived from enhanced BDNF secretion. The engineered BDNF-secreting hMSCs demonstrated significant therapeutic effects, including improvements in motor function, normalization of respiratory patterns, and amelioration of anxiety-like behaviors and learning/memory impairments in the Rett Syndrome mouse models. These findings underscore the potential of genetically modified MSCs as a neurotrophic delivery platform to directly address neurological dysfunctions. Although hMSCs generally exhibit immunomodulatory capacities, this specific investigation did not delve into a detailed immunological mechanism, but rather highlighted the direct neuroprotective and restorative roles of BDNF.

Table 4 Key challenges and potential CRISPR-Based solutions for the clinical translation of MSC therapies

Challenge	Functional Change	Therapeutic Effect	Refs.
Immune rejection of allogeneic MSCs	β2M/CIITA knockout; HLA-E/HLA-G knock-in; PD-L1 or CD47 overexpression	Reduced T-cell/NK-cell mediated clearance; enhanced immune evasion	[48, 49, 51]
Donor heterogeneity & inconsistent potency	IL-10 or IDO overexpression; stabilization of PGE2 pathway	More consistent immunomodulatory activity across donors	[34, 40]
Limited survival in inflammatory microenvironments	Keap1 knockout → Nrf2 activation; TSG-6 overexpression	Increased oxidative stress resistance; improved persistence in vivo	[65]
Tumor-promoting immunosuppression	PD-L1, CXCL12, or CCL2 knockout	Enhanced anti-tumor immune responses; reduced M2 macrophage recruitment	[77, 83, 86]
Long-term fate & uncontrolled biological activity	Incorporation of suicide genes (iCasp9, HSV-TK) as safety switches	Controlled elimination of MSCs in case of adverse events; reduced tumorigenicity risk	[107, 108]
Scalability & regulatory barriers	Creation of universal donor MSC lines with standardized edits	Improved reproducibility; compliance with GMP manufacturing	[112, 113]

Challenges and outlook

Despite the remarkable progress, the clinical translation of CRISPR-engineered MSCs faces several challenges (Table 4). These include ensuring long-term genomic stability and preventing off-target edits, which could lead to unintended immunogenic responses or altered cellular functions (Fig. 2) [94, 95]. The development of safe and efficient non-viral delivery methods, such as electroporation and ribonucleoprotein (RNP) complexes, is crucial for minimizing immunogenicity and cytotoxicity associated with viral vectors, a key advantage for clinical safety and long-term therapeutic success [96]. Regulatory aspects for genetically modified cell therapies are also complex, requiring rigorous safety and efficacy testing. Future directions will likely involve further refining gene-editing precision, developing more sophisticated feedback-controlled systems for localized and on-demand immunomodulation, and exploring combination therapies where CRISPR-engineered MSCs work synergistically with other treatments to address complex immune disorders and enhance therapeutic efficacy [97–99]. The ability to generate MSCs with low immunogenicity through β2M deletion, while retaining their stem potential and differentiation capabilities, holds great promise for allogeneic cell transplantation without the need for HLA matching [47]. Furthermore, advancements in single-cell genetic engineering of MSCs, as demonstrated by the precise deletion of the RANKL gene without affecting other MSC properties, could be instrumental for future therapeutic cell production [100].

Another critical challenge for the clinical translation of CRISPR-engineered MSCs is the uncertainty regarding their long-term fate after administration [101]. Although MSCs are generally considered safe, genetic modifications may alter their persistence, differentiation potential, or immune interactions [102]. Potential risks include uncontrolled proliferation, phenotypic drift, genomic instability, tumorigenic transformation, and unintended immune activation [103, 104]. Furthermore, the process of CRISPR-based editing itself introduces the possibility of off-target mutations, which could further compromise genomic integrity and long-term safety [105]. These concerns underscore the pressing need for precise preclinical studies with prolonged follow-up to assess the durability and safety of engineered MSCs in vivo. In order to mitigate the aforementioned risks, the incorporation of suicide genes or inducible safety switches has been proposed [106]. Although suicide genes such as HSV-TK were originally developed for cancer gene therapy to induce

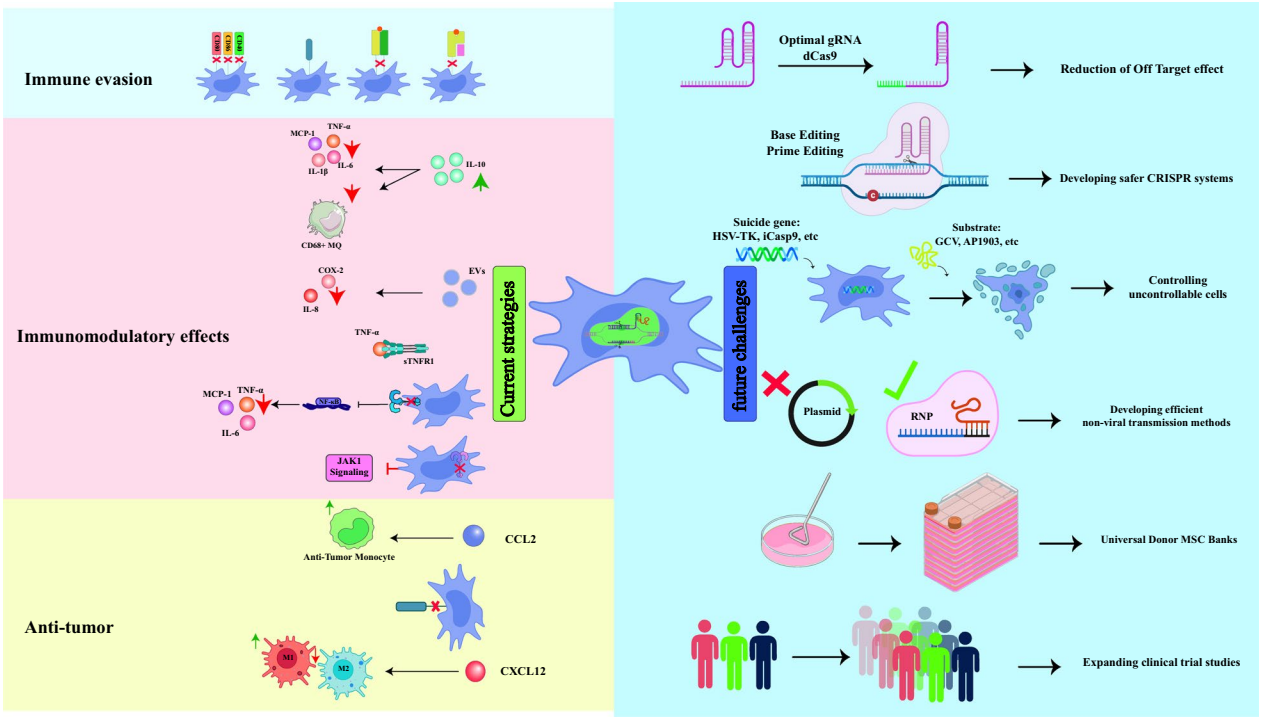

Fig. 2 Future prospects versus current strategies for MSC genome editing by CRISPR

tumor cell death, they have been increasingly adopted as safety switches in cell-based therapies [107]. When suicide genes integrated into engineered MSCs, these systems enable the controlled elimination of the administered cells in the event of adverse outcomes, thereby mitigating the risks of uncontrolled biological activity [108]. For instance, the inducible caspase-9 (iCasp9) system has been demonstrated to induce rapid apoptosis of engineered cells upon administration of a small-molecule dimerizer [109]. Similarly, the herpes simplex virus thymidine kinase (HSV-TK) approach has been shown to confer selective sensitivity to ganciclovir [107]. These strategies, which have already been validated in adoptive T-cell therapies, are increasingly recognized as important safeguards for stem cell–based interventions [110]. It is imperative that these risks be acknowledged collectively and that robust safety circuits be developed to ensure the responsible and clinically viable application of CRISPR-modified MSCs.

In other hand, the majority of current evidence is derived from preclinical in vitro and animal models, with limited independent validation across diverse experimental settings. Human clinical data remain scarce, with most MSC-related CRISPR applications still in early preclinical stages, far from clinical translation. For instance, the SAGES1 Phase 1 trial (NCT06506461), initiated by St. Jude Children's Research Hospital in March 2025, evaluates the safety and efficacy of autologous CRISPR/Cas9-edited CD34 + hematopoietic stem and progenitor cells (HSPCs) in 25 young adults (18–24 years) with severe sickle cell disease (SCD) [111]. HSPCs are mobilized with motixafortide or plerixafor, edited ex vivo to induce fetal hemoglobin (HbF) via BCL11A targeting, and infused post-busulfan conditioning. Primary endpoints include neutrophil/platelet engraftment by days + 42/+60, multilineage engraftment at 1 year, and off-target editing risks over 3 years, while secondary endpoints assess reductions in vaso-occlusive events, hemoglobin/HbF increases, and transfusion needs. Although focused on HSPCs, this trial highlights the translational potential and challenges of CRISPR-based cell therapies, such as off-target effects and engraftment variability, which are equally relevant to MSC engineering. The paucity of MSC-specific clinical trials and the need for long-term safety data underscore the importance of rigorous validation and robust clinical studies to bridge the gap between promising preclinical results and therapeutic applications.

Conclusion

The review meticulously delineates the manner in which CRISPR/Cas9 gene editing has fundamentally transformed the therapeutic potential of MSCs. While MSCs are recognized for their regenerative capacities, their profound immunomodulatory properties are particularly vital for allogeneic (off-the-shelf) applications. Historically, a significant impediment to the widespread implementation of allogeneic MSC therapy has been host immune rejection, primarily driven by MHC-I

expression. The precise CRISPR/Cas9-mediated knockout of β2M represents a significant advancement. This modification effectively eliminates MHC-I expression, rendering the MSCs largely "invisible" to alloreactive T-cells and significantly suppressing immune responses. This is a critical step towards realizing truly universal allogeneic MSC therapies, which has the potential to eliminate the significant burden of HLA matching. The emphasis on non-viral delivery methods, such as RNP complexes and advanced electroporation, ensures high editing efficiency with minimal toxicity, addressing critical safety concerns. Notably, the deletion of β2M has been observed to augment the production of immunosuppressive factors, such as IDO-1 and PGE2. This observation signifies a substantial synergistic effect, wherein engineered MSCs not only evade detection but also proactively enhance their intrinsic immunoregulatory capacity. This dual benefit is instrumental in ensuring the sustained therapeutic efficacy of the cells.

Beyond enabling immune evasion, the review demonstrates CRISPR's ability to profoundly enhance and precisely modify MSCs' intrinsic anti-inflammatory and immunomodulatory functions. The strategic application of CRISPRa to upregulate anti-inflammatory cytokines like IL-10 in MSCs exemplifies a sophisticated approach to actively reprogram pathological microenvironments, reducing pro-inflammatory cytokines and promoting tissue repair. Leveraging CRISPRa to enhance TSG-6 expression in MSCs, thereby enriching the anti-inflammatory cargo of their EVs, presents a particularly promising non-cellular biotherapeutic strategy, capitalizing on MSCs' natural communication pathways with potentially reduced risks. Furthermore, CRISPR's precision in targeting specific inflammatory signaling pathways within MSCs is remarkable. Disrupting Keap1 to activate the master anti-oxidant regulator Nrf2, or the deliberate deletion of the TLR4 gene, fundamentally reconfigures the MSCs' inflammatory response threshold. This shows a profound ability to transform an otherwise reactive cell into one inherently more resilient to inflammatory cues, leading to tangible therapeutic gains. The ability to preserve MSC viability and function during ex vivo expansion by modulating pathways like TLR3/IFN-β/JAK1, and the application of epigenetic reprogramming via CRISPRa, further underscore the depth of control achieved in enhancing MSC immunomodulation.

The most conceptually groundbreaking aspect of this review is the potential of CRISPR to repurpose MSCs in ways that counter their traditional immunosuppressive tendencies, especially in the context of anti-tumor immunotherapy. This finding marks a significant departure from the prevailing view that MSCs can, on occasion, inadvertently promote tumor progression. As elucidated by Dunavin et al., the deletion of PD-L1 in MSCs is an exacting process. This meticulous procedure re-engineers the cells to release the "brakes" on anti-tumor T cell activation. The result is enhanced IFN-γ secretion and restored immune surveillance. The critical finding that PD-L1-deficient MSC-derived exosome retains this activating property is highly significant, as it opens promising avenues for safer, more manageable cell-free cancer immunotherapies. Furthermore, the deliberate CRISPR-mediated modulation of chemokine expression, such as the deletion of CXCR12 or CCL2, illustrates a sophisticated strategy to actively sculpt the tumor microenvironment. By directly influencing the recruitment and polarization of immune cells, such as macrophages (shifting them towards an M1, anti-tumor phenotype), these engineered MSCs transition from a state of mere passive presence to a state of active participation in orchestrating a potent anti-tumor immune response. This paradigm shift indicates that MSCs are not merely a delivery vehicle; rather, they are an intelligent, programmable therapeutic agent that can be meticulously tailored to modify intricate immunological landscapes.

Despite the transformative potential of CRISPR/Cas9 for engineering MSCs into precision immunotherapies, significant challenges persist. Current evidence largely stems from preclinical in vitro and animal models, with scarce human clinical data and limited independent validation, hindering clinical translation. Additional hurdles, including off-target effects, long-term genomic stability, and complex regulatory frameworks, underscore the need for rigorous clinical trials. Nevertheless, CRISPR/Cas9's unparalleled capacity to create tailored cell products positions it as a foundational technology for next-generation MSC-based therapies, urging researchers to prioritize robust human studies to bridge the preclinical-clinical gap.

Abbreviations

MSCs	Mesenchymal stromal/stem cells
IFN-γ	Interferon-gamma
β2M	Beta-2 Microglobulin
MHC-I	Major Histocompatibility Complex Class I
RNP	Ribonucleoprotein
HLA	Human Leukocyte Antigen
TGF-β	Transforming Growth Factor-beta
IDO	Indoleamine 2,3-dioxygenase
PGE2	Prostaglandin E2
HLA-G	Human Leukocyte Antigen-G
PD-L1	Programmed Death-Ligand 1
CXCR4	C-X-C Motif Chemokine Receptor 4
CCL2	C-C Motif Chemokine Ligand 2
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
Cas9	CRISPR-associated protein 9
RNA	Ribonucleic Acid
DNA	Deoxyribonucleic Acid
EVs	Extracellular Vesicles
IL-10	Interleukin-10
TSG-6	TNF-α Stimulated Gene/Protein 6
COX-2	Cyclooxygenase-2
Keap1	Kelch-like ECH-associated protein 1
Nrf2	Nuclear factor erythroid 2-related factor 2

AD-MSCs	Adipose-derived mesenchymal stromal/stem cells
TLR4	Toll-like Receptor 4
NF-κB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
IL-6	Interleukin-6
Indel	Insertion-deletion
TNF-α	Tumor Necrosis Factor-alpha
MCP-1	Monocyte Chemoattractant Protein-1
TLR3	Toll-like Receptor 3
IFN-β	Interferon-beta
JAK1	Janus Kinase 1
hUCB-MSCs	Human Umbilical Cord Blood Mesenchymal stromal/stem Cells
SASP	Senescence-Associated Secretory Phenotype
miR-2861	microRNA-2861
HDAC4/5	Histone Deacetylase 4/5
CD14	Cluster of Differentiation 14
LPS	Lipopolysaccharide
BM-MSCs	Bone marrow-derived mesenchymal stromal/stem cells

AI use declaration
The authors declare that they have not use AI-generated work in this manuscript.

Author contributions
MRD, MMS, and FS contributed in Writing-draft preparation; and SD contributed in Writing-review and editing.

Funding
Not applicable.

Data availability
No data was used for the research described in the article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 5 August 2025 / Accepted: 5 October 2025
Published online: 29 October 2025

References

1. Hoang DM, Pham PT, Bach TQ, Ngo ATL, Nguyen QT, Phan TTK, et al. Stem cell-based therapy for human diseases. Signal Transduct Target Ther. 2022;7(1):272.
2. Via AG, Frizziero A, Oliva F. Biological properties of mesenchymal stem cells from different sources. Muscles Ligaments Tendons J. 2012;2(3):154–62.
3. Hoogduijn MJ, Popp F, Verbeek R, Masoodi M, Nicolaou A, Baan C, et al. The Immunomodulatory properties of mesenchymal stem cells and their use for immunotherapy. Int Immunopharmacol. 2010;10(12):1496–500.
4. Rehman A, Nigam A, Laino L, Russo D, Todisco C, Esposito G, et al. Mesenchymal stem cells in soft tissue regenerative medicine: a comprehensive review. Medicina (Kaunas Lithuania). 2023;59(8).
5. Dashti M, Mohammadi M, Dehnavi S, Sadeghi M. Immunomodulatory interactions between mesenchymal stromal/stem cells and immune cells in psoriasis: therapeutic potential and challenges. Stem Cell Res Ther. 2025;16(1):241.
6. Fu X, Liu G, Halim A, Ju Y, Luo Q, Song GJC. Mesenchymal Stem Cell Migration Tissue Repair. 2019;8(8):784.
7. Orbay H, Tobita M, Mizuno HJS. Mesenchymal stem cells isolated from adipose and other tissues: basic biological properties and clinical applications. 2012;2012(1):461718.
8. Huang Y, Wu Q, Tam PKH. Immunomodulatory mechanisms of mesenchymal stem cells and their potential clinical applications. Int J Mol Sci. 2022;23(17):10023.
9. Yang G, Fan X, Liu Y, Jie P, Mazhar M, Liu Y, et al. Immunomodulatory mechanisms and therapeutic potential of mesenchymal stem cells. Stem Cell Reviews Rep. 2023;19(5):1214–31.
10. Li J, Wu Z, Zhao L, Liu Y, Su Y, Gong X, et al. The heterogeneity of mesenchymal stem cells: an important issue to be addressed in cell therapy. 2023;14(1):381.
11. Jasim SA, Yumashev AV, Abdelbasset WK, Margiana R, Markov A, Suksatan W, et al. Shining the light on clinical application of mesenchymal stem cell therapy in autoimmune diseases. Stem Cell Res Ther. 2022;13(1):101.
12. Gholami M, Ghorban K, Sadeghi M, Dadmanesh M, Rouzbahani NH, Dehnavi S. Mesenchymal stem cells and allergic airway inflammation; a therapeutic approach to induce immunoregulatory responses. Int Immunopharmacol. 2023;120:110367.
13. Li Y, Jin M, Guo D, Shen S, Lu K, Pan R, et al. Unveiling the immunogenicity of allogeneic mesenchymal stromal cells: challenges and strategies for enhanced therapeutic efficacy. Biomed Pharmacother; 2024;180:117537.
14. Chen W, Lv L, Chen N, Cui E. Immunogenicity of mesenchymal stromal/stem cells. Scand J Immunol. 2023;97(6):e13267.
15. Cequier A, Vázquez FJ, Romero A, Vitoria A, Bernad E, Garcia-Martinez M, et al. The immunomodulation–immunogenicity balance of equine mesenchymal stem cells (MSCs) is differentially affected by the immune cell response depending on inflammatory licensing and major histoCompatibility complex (MHC). Compatibility. 2022;9:957153.
16. Costa LA, Eiro N, Fraile M, Gonzalez LO, Saá J, Garcia-Portabella P, et al. Functional heterogeneity of mesenchymal stem cells from natural niches to culture conditions: implications for further clinical uses. Cell Mol Life Sci. 2021;78(2):447–67.
17. Pacesa M, Pelea O, Jinek M. Past, present, and future of CRISPR genome editing technologies. Cell. 2024;187(5):1076–100.
18. Manghwar H, Lindsey K, Zhang X, Jin S. CRISPR/Cas system: recent advances and future prospects for genome editing. Trends Plant Sci. 2019;24(12):1102–25.
19. Liu W, Li L, Jiang J, Wu M, Lin PJP. Applications and challenges of CRISPR-Cas gene-editing to disease treatment in clinics. 2021;4(3):179–91.
20. Hsu Patrick D, Lander Eric S, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014;157(6):1262–78.
21. Gaj T, Gersbach CA, Barbas CF. III. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. 2013;31(7):397–405.
22. Asmamaw M, Zawdie B. Mechanism and applications of CRISPR/Cas-9-Mediated genome editing. Biologics: Targets Therapy. 2021;15:353–61.
23. La Russa MF, Qi LS. The new state of the art: Cas9 for gene activation and repression. Mol Cell Biol. 2015;35(22):3800–9.
24. Daer RM, Cutts JP, Brafman DA, Haynes KA. The impact of chromatin dynamics on Cas9-Mediated genome editing in human cells. ACS Synth Biol. 2017;6(3):428–38.
25. Hazafa A, Mumtaz M, Farooq MF, Bilal S, Chaudhry SN, Firdous M, et al. CRISPR/Cas9: A powerful genome editing technique for the treatment of cancer cells with present challenges and future directions. Life Sci. 2020;263:118525.
26. Dehnavi S, Sadeghi M, Tavakol Afshari J, Mohammadi M. Interactions of mesenchymal stromal/stem cells and immune cells following MSC-based therapeutic approaches in rheumatoid arthritis. Cell Immunol. 2023;393–394:104771.
27. Cai R, Lv R, Shi X, Yang G, Jin J. CRISPR/dCas9 tools: epigenetic mechanism and application in gene transcriptional regulation. Int J Mol Sci. 2023;24(19).
28. Paul B, Montoya G. CRISPR-Cas12a: functional overview and applications. Biomedical J. 2020;43(1):8–17.
29. Abudayyeh OO, Gootenberg JS, Essletzbichler P, Han S, Joung J, Belanto JJ, et al. RNA targeting with CRISPR–Cas13. Nature. 2017;550(7675):280–4.
30. Rauch S, He E, Srienc M, Zhou H, Zhang Z, Dickinson BC. Programmable RNA-Guided RNA effector proteins built from human parts. Cell. 2019;178(1):122–e3412.
31. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Sci (New York NY). 2012;337(6096):816–21.
32. Chen J, Zheng C-X, Jin Y, Hu C-H. Mesenchymal stromal cell-mediated immune regulation: A promising remedy in the therapy of type 2 diabetes mellitus. Stem cells (Dayton. Ohio). 2021;39(7):838–52.

33. Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell. 2013;152(5):1173–83.
34. Meng X, Zheng M, Liu J, Bai W, Zuo L, Bu X, et al. Transplantation of CRISPRa system engineered IL10-overexpressing bone marrow-derived mesenchymal stem cells for the treatment of myocardial infarction in diabetic mice. J Biol Eng. 2019;13.
35. Martinez-Zalbidea I, Wagner G, Bergendahl N, Mesfin A, Puvanesarajah V, Hitzl W, et al. CRISPR-dCas9 activation of TSG-6 in MSCs modulates the cargo of MSC-Derived extracellular vesicles and attenuates inflammatory responses in human intervertebral disc cells in vitro. Cell Mol Bioeng. 2025;18(1):83–98.
36. Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell. 2015;163(3):759–71.
37. Zetsche B, Heidenreich M, Mohanraju P, Fedorova I, Kneppers J, DeGennaro EM, et al. Multiplex gene editing by CRISPR-Cpf1 using a single CrRNA array. Nat Biotechnol. 2017;35(1):31–4.
38. Smargon AA, Shi YJ, Yeo GW. RNA-targeting CRISPR systems from metagenomic discovery to transcriptomic engineering. Nat Cell Biol. 2020;22(2):143–50.
39. Hazrati A, Malekpour K, Soudi S, Hashemi SM. CRISPR/Cas9-engineered mesenchymal stromal/stem cells and their extracellular vesicles: A new approach to overcoming cell therapy limitations. Biomed Pharmacother. 2022;156:113943.
40. Han AR, Shin HR, Kwon J, Lee SB, Lee SE, Kim EY, et al. Highly efficient genome editing via CRISPR-Cas9 ribonucleoprotein (RNP) delivery in mesenchymal stem cells. BMB Rep. 2024;57(1):60–5.
41. Golchin A, Shams F, Karami F. Advancing mesenchymal stem cell therapy with CRISPR/Cas9 for clinical trial studies. Adv Exp Med Biol. 2020;1247:89–100.
42. Crippa S, Conti A, Vavassori V, Ferrari S, Beretta S, Rivis S, et al. Mesenchymal stromal cells improve the transplantation outcome of CRISPR-Cas9 gene-edited human HSPCs. 2023;31(1):230–48.
43. Li C, Zhao H, Cheng L, Wang B. Allogeneic vs. autologous mesenchymal stem/stromal cells in their medication practice. Cell Bioscience. 2021;11(1):187.
44. Chen W, Lv L, Chen N, Cui EJSJI. Immunogenicity Mesenchymal stromal/stem Cells. 2023;97(6):e13267.
45. Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. Nat Biotechnol. 2014;32(3):252–60.
46. Lohan P, Treacy O, Griffin MD, Ritter T, Ryan AE. Anti-Donor immune responses elicited by allogeneic mesenchymal stem cells and their extracellular vesicles: are we still learning? Front Immunol. 2017;8:1626.
47. Zhang Y, Wang Y, Shao L, Pan X, Liang C, Liu B, et al. Knockout of beta-2 microglobulin reduces stem cell-induced immune rejection and enhances ischaemic hindlimb repair via exosome/miR-24/Bim pathway. 2020;24(1):695–710.
48. Zha S, Tay JC-K, Zhu S, Li Z, Du Z, Wang S. Generation of mesenchymal stromal cells with low immunogenicity from human PBMC-Derived β2. Microglobulin Knockout Induc Pluripotent Stem Cells. 2020;29:0963689720965529.
49. Shao L, Zhang Y, Pan X, Liu B, Liang C, Zhang Y, et al. Knockout of beta-2 microglobulin enhances cardiac repair by modulating exosome imprinting and inhibiting stem cell-induced immune rejection. Cell Mol Life Sci. 2020;77(5):937–52.
50. Kwon D, Ahn HJ, Han MJ, Ji M, Ahn J, Seo KW, et al. Human leukocyte antigen class I Pseudo-Homozygous mesenchymal stem cells derived from human induced pluripotent stem cells. Stem Cell Rev Rep. 2020;16(4):792–808.
51. Romano E, Trionfini P, Giampietro R, Benigni A, Tomasoni S. Generation of a homozygous CIITA knockout iPS cell line using the CRISPR-Cas9 system. Stem Cell Res. 2021;57:102580.
52. Frederiksen HR, Glantz A, Vøls KK, Skov S, Tveden-Nyborg P, Freude K, et al. CRISPR-Cas9 immune-evasive hESCs are rejected following transplantation into immunocompetent mice. Front Genome Editing. 2024;6:1403395.
53. Jiang W, Xu J. Immune modulation by mesenchymal stem cells. Cell Prolif. 2020;53(1):e12712.
54. Raicevic G, Najar M, Stamatopoulos B, De Bruyn C, Meuleman N, Bron D, et al. The source of human mesenchymal stromal cells influences their TLR profile as well as their functional properties. Cell Immunol. 2011;270(2):207–16.
55. Chen C, Zhong W, Du S, Li Y, Zeng Y, Liu K, et al. Intelligent nanotherapeutic strategies for the delivery of CRISPR system. Acta Pharm Sinica B. 2023;13(6):2510–43.
56. Luo YL, Liang LF, Gan YJ, Liu J, Zhang Y, Fan YN, et al. An All-in-One nanomedicine consisting of CRISPR-Cas9 and an autoantigen peptide for restoring specific immune tolerance. ACS Appl Mater Interfaces. 2020;12(43):48259–71.
57. Lino CA, Harper JC, Carney JP, Timlin JA. Delivering CRISPR: a review of the challenges and approaches. Drug Delivery. 2018;25(1):1234–57.
58. Zhang S, Shen J, Li D, Cheng Y. Strategies in the delivery of Cas9 ribonucleoprotein for CRISPR/Cas9 genome editing. Theranostics. 2021;11(2):614–48.
59. Xu X, Gao D, Wang P, Chen J, Ruan J, Xu J, et al. Efficient homology-directed gene editing by CRISPR/Cas9 in human stem and primary cells using tube electroporation. Sci Rep. 2018;8(1):11649.
60. Hui Y, Jiao X, Yang L, Lu D, Han Y, Yang W, et al. Indoleamine-2,3-dioxygenase: an important controller in maintaining mesenchymal stem cell-mediated Immunomodulatory homeostasis. Acta Pharm Sinica B. 2025;15(7):3404–18.
61. Yudhawati R, Shimizu K. PGE2 produced by exogenous MSCs promotes immunoregulation in ARDS induced by highly pathogenic influenza A through activation of the Wnt-β-Catenin signaling pathway. Int J Mol Sci. 2023;24(8).
62. Hazrati A, Malekpour K, Soudi S, Hashemi SM. CRISPR/Cas9-engineered mesenchymal stromal/stem cells and their extracellular vesicles: a new approach to overcoming cell therapy limitations. Biomed Pharmacother. 2022;156:113943.
63. Meng X, Zheng M, Yu M, Bai W, Zuo L, Bu X, et al. Transplantation of CRISPRa system engineered IL10-overexpressing bone marrow-derived mesenchymal stem cells for the treatment of myocardial infarction in diabetic mice. J Biol Eng. 2019;13:49.
64. Kim S, Hupperetz C, Lim S, Kim CH. Genome editing of immune cells using CRISPR/Cas9. BMB Rep. 2021;54(1):59–69.
65. Hu Y, Liu S, Zhu B-M. CRISPR/Cas9-induced loss of Keap1 enhances antioxidation in rat adipose-derived mesenchymal stem cells. 2020;10:2019.
66. Kaspar JW, Niture SK, Jaiswal AK. Nrf2:INrf2 (Keap1) signaling in oxidative stress. Free Radic Biol Med. 2009;47(9):1304–9.
67. Schary Y, Rotem I, Caller T, Lewis N, Shaihov-Teper O, Brzezinski RY, et al. CRISPR-Cas9 editing of TLR4 to improve the outcome of cardiac cell therapy. Sci Rep. 2023;13(1):4481.
68. Lee HJ, Choi B, Kim Y, Lee SE, Jin HJ, Lee HS, et al. The upregulation of Toll-Like receptor 3 via autocrine IFN-β signaling drives the senescence of human umbilical cord Blood-Derived mesenchymal stem cells through JAK1. Front Immunol. 2019;10:1659.
69. Wu Y, Xie L, Wang M, Xiong Q, Guo Y, Liang Y, et al. Mettl3-mediated m(6)A RNA methylation regulates the fate of bone marrow mesenchymal stem cells and osteoporosis. Nat Commun. 2018;9(1):4772.
70. Kang H, Park Y-K, Lee J-Y, Bae M. Roles of histone deacetylase 4 in the inflammatory and metabolic processes. Diabetes Metab J. 2024;48(3):340–53.
71. Park S-H, Kim J, Yang H-J, Lee JY, Kim CH, Hur JK, et al. CRISPR activation identifies a novel miR-2861 binding site that facilitates the osteogenesis of human mesenchymal stem cells. J Orthop Surg Res. 2024;19(1):730.
72. Hirakawa MP, Tjahjono N, Light YK, Chintalapudi P, Butler KS, Branda SS, et al. Augmentation of antibacterial activity in mesenchymal stromal cells through systems-level analysis and CRISPR-mediated activation of CD14. bioRxiv. 2020:2020.10.14.338020.
73. Wang B, Chang M, Zhang R, Wo J, Wu B, Zhang H, et al. Spinal cord injury target-immunotherapy with TNF-α autoregulated and feedback-controlled human umbilical cord mesenchymal stem cell derived exosomes remodelled by CRISPR/Cas9 plasmid. Biomaterials Adv. 2022;133:112624.
74. Srifa W, Kosaric N, Amorin A, Jadi O, Park Y, Mantri S, et al. Cas9-AAV6-engineered human mesenchymal stromal cells improved cutaneous wound healing in diabetic mice. Nat Commun. 2020;11(1):2470.
75. Minev T, Balbuena S, Gill JM, Marincola FM, Kesari S, Lin F. Mesenchymal stem cells - the secret agents of cancer immunotherapy: Promises, challenges, and surprising twists. Oncotarget. 2024;15:793–805.
76. Huang D, Miller M, Ashok B, Jain S, Peppas NA. CRISPR/Cas systems to overcome challenges in developing the next generation of T cells for cancer therapy. Adv Drug Deliv Rev. 2020;158:17–35.
77. Dunavin N, Li M, Atay S, Mitchell J, Soder R, Abhyankar S, et al. CRISPR/Cas9-Mediated disruption of PD-L1 reduces the T cell suppressive effect of wharton's jelly mesenchymal stromal cells and their extracellular vesicles. Blood. 2018;132:5095.
78. Jiang X, Wang J, Deng X, Xiong F, Ge J, Xiang B, et al. Role of the tumor microenvironment in PD-L1/PD-1-mediated tumor immune escape. Mol Cancer. 2019;18(1):10.
79. Chen Z, Yao MW, Ao X, Gong QJ, Yang Y, Liu JX, et al. The expression mechanism of programmed cell death 1 ligand 1 and its role in Immunomodulatory

ability of mesenchymal stem cells. Chin J Traumatol = Zhonghua Chuang Shang Za Zhi. 2024;27(1):1–10.
80. Keshavarz S, Nassiri SM, Siavashi V, Alimi NS. Regulation of plasticity and biological features of endothelial progenitor cells by MSC-derived SDF-1. Biochimica et biophysica acta (BBA) -. Mol Cell Res. 2019;1866(2):296–304.
81. Meiron M, Zohar Y, Anunu R, Wildbaum G, Karin N. CXCL12 (SDF-1alpha) suppresses ongoing experimental autoimmune encephalomyelitis by selecting antigen-specific regulatory T cells. J Exp Med. 2008;205:2643–55.
82. Zhang W, Wang M, Ji C, Liu X, Gu B, Dong T. Macrophage polarization in the tumor microenvironment: emerging roles and therapeutic potentials. Biomed Pharmacother. 2024;177:116930.
83. Babazadeh S, Nassiri SM, Siavashi V, Sahlabadi M, Hajinasrollah M, Zamani-Ahmadmahmudi M. Macrophage polarization by MSC-derived CXCL12 determines tumor growth. Cell Mol Biol Lett. 2021;26(1):30.
84. Zhang J, Patel L, Pienta KJ. CC chemokine ligand 2 (CCL2) promotes prostate cancer tumorigenesis and metastasis. Cytokine Growth Factor Rev. 2010;21(1):41–8.
85. Mizutani K, Sud S, McGregor NA, Martinovski G, Rice BT, Craig MJ, et al. The chemokine CCL2 increases prostate tumor growth and bone metastasis through macrophage and osteoclast recruitment. Neoplasia (New York NY). 2009;11(11):1235–42.
86. Bui QT, Lee KD, Fan YC, Lewis BS, Deng LW, Tsai YC. Disruption of CCL2 in mesenchymal stem cells as an anti-tumor approach against prostate cancer. Cancers. 2023;15(2).
87. Tang S, Zhang Y, Wang P, Tang Q, Liu Y, Lu F, et al. NKG2D-CAR-targeted iPSC-derived MSCs efficiently target solid tumors expressing NKG2D ligand. iScience. 2025;28(5).
88. Goyert SM, Ferrero EM, Seremetis SV, Winchester RJ, Silver J, Mattison AC. Biochemistry and expression of myelomonocytic antigens. J Immunol. 1986;137(12):3909–14.
89. Pugin J, Heumann ID, Tomasz A, Kravchenko VV, Akamatsu Y, Nishijima M, et al. CD14 is a pattern recognition receptor. Immunity. 1994;1(6):509–16.
90. Ulevitch RJ, Tobias PS. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. Annu Rev Immunol. 1995;13:437–57.
91. Lee K, Lee N, Shin E, Lee N, Chang J, Na D, et al. Enhancing the therapeutic potential of mesenchymal stem cell-based therapy via CRISPR/Cas9-based genome editing. Cytotherapy. 2020;22(5):S15.
92. Wechsler ME, Rao VV, Borelli AN, Anseth KSJAhm. Engineering the MSC secretome: a hydrogel focused approach. 2021;10(7):2001948.
93. Kim HJ, Bayarsaikhan D, Lee J, Bayarsaikhan G, Lee B. Brain-Derived neurotrophic factor secreting human mesenchymal stem cells improve outcomes in Rett syndrome mouse models. Front NeuroSci. 2021;15:725398.
94. Liu W, Li L, Jiang J, Wu M, Lin P. Applications and challenges of CRISPR-Cas gene-editing to disease treatment in clinics. Precision Clin Med. 2021;4(3):179–91.
95. Yang Y, Xu J, Ge S, Lai L. CRISPR/Cas: advances, limitations, and applications for precision cancer research. 2021;8:2021.
96. Zhang S, Shen J, Li D, Cheng YJT. Strategies in the delivery of Cas9 ribonucleoprotein for CRISPR/Cas9 genome editing. 2021;11(2):614.
97. Wang S-W, Gao C, Zheng Y-M, Yi L, Lu J-C, Huang X-Y, et al. Current applications and future perspective of CRISPR/Cas9 gene editing in cancer. Mol Cancer. 2022;21(1):57.
98. Golchin A, Shams F, Karami F. Advancing mesenchymal stem cell therapy with CRISPR/Cas9 for clinical trial studies. 12472019.
99. Filho DM, de Carvalho Ribeiro P, Oliveira LF, Dos Santos ALRT, Parreira RC, Pinto MCX et al. Enhancing the therapeutic potential of mesenchymal stem cells with the CRISPR-Cas system. 2019;15(4):463–73.
100. Gross T, Jeney C, Halm D, Finkenzeller G, Stark GB, Zengerle R, et al. Characterization of CRISPR/Cas9 RANKL knockout mesenchymal stem cell clones based on single-cell printing technology and emulsion coupling assay as a low-cellularity workflow for single-cell cloning. PLoS ONE. 2021;16(3):e0238330.
101. Hoseinzadeh A, Esmaeili S-A, Sahebi R, Melak AM, Mahmoudi M, Hasannia M, et al. Fate and long-lasting therapeutic effects of mesenchymal stromal/stem-like cells: mechanistic insights. Stem Cell Res Ther. 2025;16(1):33.
102. Kim JS, Lee JH, Kwon O, Cho JH, Choi JY, Park SH, et al. Rapid deterioration of preexisting renal insufficiency after autologous mesenchymal stem cell therapy. Kidney Res Clin Pract. 2017;36(2):200–4.
103. Fitzgerald JC, Hanley S, Barry F. Phenotypic instability of mesenchymal stromal cells isolated and expanded in different growth media. Cytotherapy. 2020;22(5):S84.
104. Antoon R, Overdevest N, Saleh AH, Keating A. Mesenchymal stromal cells as cancer promoters. Oncogene. 2024;43(49):3545–55.
105. Fu Y, Foden JA, Khayter C, Maeder ML, Reyon D, Joung JK, et al. High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. Nat Biotechnol. 2013;31(9):822–6.
106. Yagyu S, Hoyos V, Del Bufalo F, Brenner MK. An inducible Caspase-9 suicide gene to improve the safety of therapy using human induced pluripotent stem cells. Mol Ther. 2015;23(9):1475–85.
107. Kenarkoohi A, Bamdad T, Soleimani M, Soleimanjahi H, Fallah A, Falahi S. HSV-TK expressing mesenchymal stem cells exert inhibitory effect on cervical cancer model. Int J Mol Cell Med. 2020;9(2):146–54.
108. Ramos CA, Asgari Z, Liu E, Yvon E, Heslop HE, Rooney CM, et al. An inducible caspase 9 suicide gene to improve the safety of mesenchymal stromal cell therapies. Stem Cells. 2010;28(6):1107–15.
109. Rossignoli F, Grisendi G, Spano C, Golinelli G, Recchia A, Rovesti G, et al. Inducible Caspase9-mediated suicide gene for MSC-based cancer gene therapy. Cancer Gene Ther. 2019;26(1):11–6.
110. Falcon C, Smith L, Al-Obaidi M, Abu Zaanona M, Purvis K, Minagawa K ,et al. Combinatorial suicide gene strategies for the safety of cell therapies. 2022;13:2022.
111. Hospital SJCsR. Gene editing for sickle cell disease. 2025.
112. Strecanska M, Sekelova T, Smolinska V, Kuniakova M, Nicodemou A. Automated manufacturing processes and platforms for Large-scale production of Clinical-grade mesenchymal Stem/ stromal cells. Stem Cell Rev Rep. 2025;21(2):372–89.
113. Calascibetta F, Martorana A, Lo Pinto M, Carcione C, D'Arpa S, Amico G, et al. GMP-compliant, serum-free cultures preserve therapeutic potential of extracellular vesicles from human mesenchymal stromal cells. 2025;13:2025.

Publisher's Note