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Review Article

Comparing the Benefits and Drawbacks of Stem Cell Therapy Based on the Cell Origin or Manipulation Process: Addressing Immunogenicity

Sung-Ho Chang [1], **Chung Gyu Park** [2,3,4,5,*]

[1]Department of Immunology and Molecular Microbiology, School of Dentistry and Dental Research Institute, Seoul National University, Seoul 03080, Korea
[2]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul 03080, Korea
[3]Transplantation Research Institute, Medical Research center, Seoul National University College of Medicine, Seoul 03080, Korea
[4]Cancer Research Institute, Seoul National University College of Medicine, Seoul 03080, Korea
[5]Department of Microbiology and Immunology, Seoul National University College of Medicine, Seoul 03080, Korea

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***Correspondence to**
Chung Gyu Park
Department of Microbiology and Immunology, Seoul National University College of Medicine, 101 Daehak-ro, Jongno-gu, Seoul 03080, Korea.
Email: chgpark@snu.ac.kr

ORCID iDs
Sung-Ho Chang
https://orcid.org/0000-0002-1558-9896
Chung Gyu Park
https://orcid.org/0000-0003-4083-8791

Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
ABMR, antibody-mediated rejection; ADSC, adipose-derived mesenchymal stem cell; B2MKO, β2-microglobulin knockout; BM-MSC, bone marrow-MSC; CIITA, class II MHC transactivator; CNI, calcineurin inhibitors; DC, dendritic cell; FCGS, feline chronic gingivostomatitis; GE, genome edited; GFP, green fluorescent protein; IAI,

ABSTRACT

Mesenchymal stem cells (MSCs) are effective in treating autoimmune diseases and managing various conditions, such as engraftment of allogeneic islets. Additionally, autologous and HLA-matched allogeneic MSCs can aid in the engraftment of human allogeneic kidneys with or without low doses of tacrolimus, respectively. However, HLA alloantigens are problematic because cell therapy uses more HLA-mismatched allogeneic cells than autologous for convenience and standardization. In particular, HLA-mismatched MSCs showed increased Ag-specific T/B cells and reduced viability faster than HLA-matched MSCs. In CRISPR/Cas9-based cell therapy, Cas9 induce T cell activation in the recipient's immune system. Interestingly, despite their immunogenicity being limited to the cells with foreign Ags, the accumulation of HLA alloantigen-sensitized T/B cells may lead to allograft rejection, suggesting that alloantigens may have a greater scope of adverse effects than foreign Ags. To avoid alloantigen recognition, the β2-microglobulin knockout (B2MKO) system, eliminating class-I MHC, was able to avoid rejection by alloreactive CD8 T cells compared to controls. Moreover, universal donor cells in which both B2M and Class II MHC transactivator (*CIITA*) were knocked out was more effective in avoiding immune rejection than single KO. However, B2MKO and *CIITA* KO system remain to be controlled and validated for adverse effects such as the development of tumorigenicity due to deficient Ag recognition by CD8 T and CD4 T cells, respectively. Overall, better HLA-matching or depletion of HLA alloantigens prior to cell therapy can reduce repetitive transplantation through the long-term survival of allogeneic cell therapy, which may be especially important for patients seeking allogeneic transplantation.

Keywords: Immunogenicity; HLA-matched/mismatched allogeneic cell therapy; CRISPR/Cas9; B2MKO; Universal cell therapy; Opportunity cost

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intra-articular injection; iEC, differentiation of iPSCs into endothelial cell; iPSC, induced pluripotent stem cell; IV, Intravenous; KTp, kidney transplantation; MSC, mesenchymal stem cell; OLP, oral lichen planus; SIBG, standard iliac bone graft; SLE, systemic lupus erythematosus; SRG, severe refractory gingivostomatitis; UC-MSC, umbilical cord-MSC.

Author Contributions

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INTRODUCTION

Mesenchymal stem cells (MSCs) can be obtained from a variety of tissues, such as bone marrow, adipose tissue, and umbilical cord blood, and organs, such as, the spleen, thymus, and kidney, with adipose-derived mesenchymal stem cells (ADSCs), bone marrow-MSCs (BM-MSCs), and umbilical cord-MSCs (UC-MSCs) being the most commonly used (1). In addition to their immunomodulatory effects, MSCs are multipotent progenitor cells that can differentiate into a variety of cell types and have been shown to be therapeutic in a variety of diseases (2-6). However, the HLA-ABC alloantigens expressed on allogeneic MSCs are highly immunogenic (7-9), which is a common issue in cell therapy utilizing allogeneic cells (10). Mechanistically, in alloantigen recognition, T cells of the recipient immune system can recognize and act on HLA alloantigens through direct or indirect pathways (**Supplementary Fig. 1**) (11). Ab-mediated rejection (ABMR) by alloreactive B cells is a widely recognized cause of allograft rejection (11,12).

CRISPR/Cas9 is the most widely used method for epigenome editing, owing to its efficiency and convenience (13). Specifically, cell therapies involving MSCs and induced pluripotent stem cells (iPSCs) use CRISPR/Cas9 for genome editing (14). However, it is a system of bacterial origin, which raises concerns regarding its potential immunogenicity (15). The expression of foreign Ags from genome-edited cell therapy can trigger an immune response in the recipient's immune system, which can lead to a decrease in therapeutic efficacy and accumulation of sensitized immune cells (16,17). These foreign Ags can be recognized by T cells via class I and II MHC pathways on Ag presenting cells (18).

This study first examined the various efficacies of MSCs for disease treatment and whether there were differences based on their origin. Second, cell therapy-induced immunogenicity and its causes were investigated in detail. Third, we investigated the benefits and disadvantages of HLA-matched allogeneic cell therapies. Fourth, we discuss the ability of the β2-microglobulin knockout (B2MKO) and universal cell therapies system to evade recipient immune responses to HLA alloantigens. We also review the currently available options for suppressing Ag-specific recipient immune responses. This review of immunogenic-cell therapy will contribute to the identification of the most effective therapeutic approaches while minimizing their adverse effects.

THE UTILITY OF MSCs AS A TREATMENT FOR A VARIETY OF DISEASES

MSCs have therapeutic effects in various diseases, including autoimmune diseases such as systemic lupus erythematosus, severe refractory gingivostomatitis, and oral lichen planus (OLP) (**Table 1**; reports from 2016 to 2022). Specifically, feline chronic gingivostomatitis (FCGS), an autoimmune disease similar to OLP, is a painful and debilitating inflammatory disease of the oral mucosa that requires lifelong treatment with antibiotics and corticosteroids; however, when MSCs were used to treat FCGS, 4 out of 7 animals (57%) reported clinical improvement (**Table 1**). HLA-mismatched MSCs (cured by 12–20 months) were also effective in the treatment of FCGS, but less effective than autologous MSCs (cured by 3–9 months) (concentrations of serum IFNγ and neutrophil counts, both $p=0.057$) (19). In addition, MSCs contributed to improved treatment of ankle non-union in diabetic patients (improving non-unions, $p=0.04$; reduced infection rate, $p<0.01$), engraftment of allogeneic

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Table 1. Effects of MSCs on the treatment of various diseases

Authors	Trial types/Object/ Period	MSCs (subject)/Dose/ Number of doses/Injected site	MHC matching/ Serum types	Results
Effects of cell therapy on autoimmune diseases				
Arzi et al. (2016) (20)	· Animal trial (feline) · Treatment of SRG (similar disease to human OLP) · 6 months	· ADSCs (n=7), control (n=6) · 5×10^6 cells/kg · 2 times · IV	· Autologous · FBS	· Therapeutic effect: 1) reduction of circulating $CD8^+$ T cells 2) normalization of the CD4/CD8 ratio 3) reduction of neutrophils.
Arzi et al. (2017) (19)	· Animal trial (feline) · Treatment of SRG · 6 months	· ADSCs (n=7) · 5×10^6 cells/kg · 2 times · IV	· MHC mismatching · FBS	· Clinical improvement by ADSCs treatment (4 of 7 [57%]).
Kamen et al. (2022) (21)	· Human clinical trial · SLE · 12 months	· UC-MSCs (n=6) · 1×10^6 cells/kg · 1 time · IV	· HLA mismatching · HSA	· Allogeneic MSCs is effective for refractory SLE patients (phase I trial). · Effective in reducing Lupus Impact Tracker in SLE patients (52 wk, p=0.007) (22). · Autologous MSCs from SLE patients were not effective.
Effect of cell therapy in various disease treatment				
Hernigou et al. (2015) (23)	· Human clinical trial · Ankle non-unions in diabetic patients · 12 months	· BMSCs (n=86), control (n=86) · 61,000±18,000 · 1 time · Ankle non-union	· Autologous · No use of serum	· Therapeutic effect: BMSCs (82.1%), SIBG Control (62.3%) (p=0.04). · Infection rate: BMSCs (1%), SIBG control (20%) (p<0.01). · Skin necrosis: BMSCs (1%), SIBG control (11%) (p=0.01).
Oliveira et al. (2017) (24)	· Animal trial (mouse) · Engraftment of islets · 30 days	· ADSCs (n=40) · 2×10^5 cells · 1 time · Subcutaneous injections at the kidney	· MHC mismatching, syngeneic · FBS	· Survival of allogeneic ADSCs: 100% on day 7(6 out of 6 mice), 33.3% on day 14, and 0% on day 28. · Rejection time of allogeneic islet: Prolonged survival up to 19 days in the islet + allogeneic MSCs group (up to 13 days in the islet alone group).
Chen et al. (2021) (4)	· Human clinical trial · Knee osteoarthritis · 96 wk	· ADSCs (n=17), control (n=8) · 16×10^6 cells · 1 time · IAI	· HLA mismatching · Unknown	· Reduction of pain scores by ADSCs treatment (wk 12, p=0.0026). · Improvement of KSCRS score (objective knee indicator, symptoms, and functional activities) (wk 48, p=0.0234).
Lynggaard et al. (2022) (3)	· Human clinical trial · Radiation-induced xerostomia · 4 months	· ADSCs (n=10), control (n=10) · 5×10^7 cells · 1 time · Submandibular and parotid gland	· HLA mismatching · Human platelet lysate	· Improvement of UWS flow rate by ADSCs treatment: increase of 0.06 ml/min (p=0.0009). · Improvement of XQ score: 22.6 units reduced (p=0.0004). · ADSCs (Off-the-Shelf)

HSA, human serum albumin; IAI, intra-articular injection; KSCRS, Knee Society Clinical Rating System; UWS, unstimulated whole saliva; XQ, xerostomia questionnaire.

islets (improving graft survival, p<0.01), knee osteoarthritis (p<0.05), and radiation-induced xerostomia (increase of 0.06 ml per minute, p=0.0009) (**Table 1**).

MSCs IN ENGRAFTMENT OF HUMAN KIDNEY TRANSPLANTS: AUTOLOGOUS & HLA-MATCHED ALLOGENEIC CELLS

In human organ transplants, calcineurin inhibitors (CNI; cyclosporine [1980s], tacrolimus [1990s]) provide better protection against acute rejection (25). However, the long-term use of these inhibitors increases the risk of infection and malignancy, which is an obstacle to long-term survival; therefore, MSCs treatment has been utilized as an alternative to CNI (**Table 2**; reports from 2016 to 2022). Human MSCs can reduce or replace the use of CNI in the engraftment of HLA-matched allogeneic kidneys and suppress rejection for a long time. Specifically, autologous BM-MSCs were able to prevent rejection after the tacrolimus withdrawal, and HLA-matched allogeneic BM-MSCs were able to prevent rejection with a low dose of tacrolimus for two years (**Table 2**).

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Table 2. Effect of autologous- or HLA-matched allogeneic-MSCs on engraftment of human kidney transplantation

Authors	Object/Period	MSCs (subject)/Dose/ Number of doses/Injected site	HLA matching/Serum types/Injection point	Results
Pan et al. (2016) (26)	· Improvement of kidney transplant · 24 months	· BMSCs (n=32) · 5×10^6 cells (1st), 2× 10^6 cells/kg (2nd) · 2 times · 1st: kidney artery, 2nd: IV	· Matched allogeneic (donor-derived MSCs) · No use of serum · Unknown	· The combination of low-dose tacrolimus and MSCs was as effective as standard dose tacrolimus (at after 2 years): urea, urine protein, urinary RBC, urinary WBC, 24-h urine protein, and creatinine clearance rates.
Dreyer et al. (2020) (27)	· Improvement of kidney transplant · 12 months	· BMSCs (n=10) · 1.5×10^6/kg · 2 times · IV	· Matched allogeneic · FBS · 6 months after KTp	· BMSCs injection prevented kidney rejection even with the use of low-dose tacrolimus (at 12 months).
Meucci et al. (2021) (5)	· Improvement of kidney transplant · 24 wk	· BMSCs (n=27), control (n=27) · 1–2×10^6/kg · 2 times · IV	· Autologous · Unknown · 6–7 wk after	· BMSCs replaces tacrolimus without kidney rejection · BMSCs ameliorates cardiovascular complications by discontinuation of tacrolimus (at 24 wk).
Reinders et al. (2021) (28)	· Improvement of kidney transplant · 24 wk	· BMSCs (n=29), control (n=28) · 1.5×10^6/kg · 2 times · IV	· Autologous · No use of serum · 6–7 wk after KTp	· BMSCs replaced tacrolimus without kidney rejection and was safe. · BMSCs increased the number of regulatory T cells compared to the control group (at 24 wk, p=0.014).
Večerić-Haler et al. (2022) (29)	· Treatment of Ab-mediated kidney graft rejection · 12 months	· BMSCs (n=3) · 3×10^6 cells/kg · 3 times · IV	· Autologous · Human serum · 5 wk after KTp	· Even standard therapy combined with autologous BMSCs did not improve AMR in KTRs.

AMR, antibody-mediated rejection; KTR, kidney transplant recipient.

STEM CELL THERAPY IMMUNOGENICITY

Causes of immunogenicity: HLA-alloantigens, xenogeneic molecules, and foreign Ags from genome editing system

Cell therapy is promising for the treatment of various diseases; however, immunogenicity remains challenging (**Table 3**; reports from 2015 to 2023). Thus, understanding the causes and impact of immunogenicity is essential for the safe use of cell therapy. Kol et al. (30) reported that HLA-mismatched MSCs induce an immune response in alloreactive Abs or cytotoxic CD8 T cells. These results are supported by reports that HLA-ABC alloantigens expressed by MSCs induce alloreactive CD8 T cell activity primarily through a direct pathway (**Supplementary Fig. 1**) (8). Interestingly, rejection of HLA-mismatched MSCs can be analyzed both in vivo and in vitro approximately 3 weeks after injection, compared to mixed lymphocyte responses in donors and recipients, which are detectable at approximately 7 days (8,31,34,41). These results correlate with MSCs having immunomodulatory activity and relatively low expression of HLA alloantigens, which may be a hallmark of MSCs, leading to a later onset of rejection (11). However, T cells specific for alloantigens on allogeneic MSCs do not respond to immunosuppressants (36,38). Večerić-Haler et al. (29) also reported that autologous MSCs used for immunomodulation were ineffective as inhibitors of chronic ABMR, suggesting that, even if MSCs have immunomodulatory effects, they are limited in suppressing Ag-specific rejection (**Tables 1–4**) (8). These results are also supported by reports that HLA-mismatched allogeneic cell therapy results in a lower survival rate than autologous cell therapy (**Table 3**). In iPSC cell therapy, it has been reported that HLA-matched iPSC-neurons have a higher survival rate than mismatched cells in the brain, a tissue known to have a low immune response, proving that the same applies to allogeneic cell therapy (17,48).

Other causes of immunogenicity include foreign Ags and xenogeneic molecules used in the genetic manipulation and culture of cell therapy, respectively. First, the Cas9 protein from CRISPR/Cas9, which is used to generate iPSCs, can induce the activity of CD8 T cells as well as Ag-specific CD4 T cells (36). This is further supported by recent reports that plasmacytoid

Table 3. Cell therapy immunogenicity

Authors	Trial types/Object/Period	MSC types/Dose/ Number of doses/Injected site	MHC matching/ Serum types	Results
Immunogenic cell therapy				
Kol et al. (2015) (30)	· Animal trial (horse) · Safety and lymphocyte response tests · 35 days	· ADSCs/BMSCs · 25×10^6 cells · 3 times · IV	· Mismatched allogeneic · No use of serum	· No organ toxicity or systemic inflammatory response. · Repeated injections of allogeneic MSCs may result in a cytotoxic response with an increase of circulating $CD8^+$ T cells.
Owens et al. (2016) (31)	· Animal trial (horse) · Detection of alloreactive Ab · 600 days	· ADSCs/BMSCs · $2.5–8\times10^7$ cells · 4 times · 4 sites including IV	· Mismatched allogeneic · FBS	· Anti-MSCs Abs were detected in 7 out of 19 horses (37% of the study horses). · Abs to MSCs may develop 3–4 wk after MSCs injection.
Kawamura et al. (2016) (32)	· Animal trial (monkey) · Immunogenicity evaluation for MHC matched iPSC-derived CM · 2 months	· iPSC-CM (GFP) · Aggregation or sheet type: 3.3×10^6 cells (3 sheets/animal). · 1 time · Anterior wall of the left ventricle, Backs of recipient animals (for sheets)	· Matched/mismatched allogeneic · Both autologous and xeno-free serum (33)	· Matched iPSC-CM grafts had better survival compared to mismatched those ($p<0.05$).
Morizane et al. (2017) (17)	· Animal trial (monkey) · Immunogenicity for MHC matched/mismatched iPSC-derived neurons in brain · 4 months	· iPSC-neurons · 4.8×10^6 cells (8×10^5 cells/ tract, 6 tracts) · 1 time · One side of the putamen	· Matched/mismatched allogeneic · Both autologous and xeno-free serum (33)	· Rate of allogeneic iPSC-neuron induced inflammation: MHC-matching < MHC-mismatching (at 3 months, $p=0.005$). · More iPSC-neurons survived in MHC-matched grafts compared to MHC-mismatched grafts (at 4 months, $p=0.004$).
Chang et al. (2020) (34)	· *Ex vivo* (human) · Immunogenicity evaluation for human MSCs · 3 wk	· ADSCs · 1×10^3 cells/well · 2 times · Allogeneic-Ag stimulation	· Mismatched allogeneic · Autologous serum (comparison with FBS)	· Allogeneic ADSCs induce alloreactive CD8 T cell-mediated cytotoxicity (8). · Allogeneic ADSCs cause significant production of memory-CD8 T cells in allogeneic-Ag stimulation (34).
Immunogenicity for xenogeneic molecular				
Joswig et al. (2017) (35)	· Animal trial (horse) · Osteoarthritis therapy · 36 days	· BMSCs · 10×10^6 · 2 times · Intra-articular	· Autologous, mismatched allogeneic · Both FBS and autologous serum	· Autologous BMSCs exposed to xeno-serum (FBS) induced a significant adverse response in joints and increased synovial total nucleated cell counts ($p=0.0007$).
Wagner et al. (2019) (36)	· *Ex vivo* (human) · Evaluation of T cells reactive to Cas9 · 5 days (response with PBMC and Cas9 protein)	· Using Cas9 whole protein · 1×10^7 PBMCs · 1 time · Proliferation assay for Cas9 protein	· Human PBMCs · Human serum	· CRISPR/Cas9 for gene-editing induces activity of preexisting reactive CD4/CD8 T cells. · Reactive T cells responding to virus-related gene therapy are not well regulated by immunosuppressive agents such as anti-CTLA4 and low dose prednisone (37). · Cas9-specific reactive Treg are effective in regulating these reactive T cells.
Chang and Park (2019) (8)	· *Ex vivo* (human) · Evaluation of alloreactive memory T cells · 3 wk	· ADSCs · 1×10^3 ADSCs/well · 2 times · Allogeneic-Ag stimulation	· Mismatched allogeneic · Autologous serum (comparison with FBS)	· ADSCs grown in xenogeneic medium cause faster T cell-mediated cytotoxicity through a direct pathway.
Deuse et al. (2019) (38)	· *Ex vivo* (human) · Mechanism of autologous iPSC rejection · 90 h (response with effector cells and target cells)	· iPSC/iEC · 4×10^5 iEC · 1 time · *In vitro* T cell-mediated rejection	· Autologous · FCS	· Autologous iPSCs and their derivatives also induce immune activation. · Gene reprogramming has up to 9 times higher mutation rates than conventional culture conditions (39,40). · Mutations in the mitochondrial DNA of iPSCs cause the production of neoepitopes.

dendritic cells (DCs), as well as conventional DCs, which are known to be potent cross-presenting dendritic cells, can cross-present extracellular Ags to Class I MHC (18). However, it is encouraging that Wagner et al. (36) have shown that Ag-specific Treg cells are effective in suppressing the activity of Cas9-specific T cells. Second, fetal bovine serum, commonly used for the culture and proliferation of MSCs, promotes cytotoxic CD8 T cell activation and kills HLA-mismatched MSCs more rapidly than human serum (8,35). These results correlate with reports that albumin can bind to a variety of tissues and cells and be absorbed intracellularly

Table 4. Comparative analysis of immunogenicity for autologous-versus allogeneic-cell therapy

Authors	Trial types/Object/Period	MSC types/Dose/Number of doses/Injected site	MHC matching/ Serum types	Results
Comparison of autologous and MHC-mismatched allogeneic MSCs				
Hare et al. (2012) (42)	· Human clinical trial · Functional safety & adverse effects · 13 months	· BMSCs · 2×10^7 (1^{st}), 10×10^7 (2^{nd}), 20×10^7 (3^{rd}) · 3 times · Myocardium	· Autologous, mismatched allogeneic · Human serum albumin (Pa, 0), FBS (Pa, 1)	· Both allogeneic and autologous cells showed their safety by reducing infarct size ($p<0.001$) in patients with ICM. · More than 30% of patients tested showed sensitization to HLA Ags (8 of 27) at baseline. · A majority of the sensitized patients (7 of 8 [87.5%]) showed sensitization at all time points with alloreactive Ab.
Pigott et al. (2013) (43)	· Animal trial (horse) · Immune response to MSCs from various sources · 120 days	· BMSCs · 15×10^6 cells · 1 time · IAI	· Autologous, mismatched allogeneic, and xenogeneic · FBS	· The number of perivascular cellular cuffs and vascular microthrombi was increased in all MSC-injected joints ($p=0.036$). · No presence of any MSCs was confirmed in the synovium at day 60.
Isakova et al. (2014) (44)	· Animal trial (monkey) · Comparison of autologous and allogeneic MSCs · 7 months	· BMSCs · 2.5×10^6 cells (1^{st}), 1×10^6 cells (2^{nd}) · 2 times · 1^{st}: ICI, 2^{nd}: SCi	· Autologous, mismatched allogeneic · Unknown	-Primary injection · A significant increase of circulating leukocytes, neutrophils and eosinophils was observed only at 10 or 30 days in the allogeneic group ($p<0.05$). · Levels of circulating $CD3^+$ T cells and NK were found to be significantly elevated at 60 days in the allogeneic group ($p<0.05$, $p=0.0005$, respectively). · B cells were always significantly increased in the allogeneic group ($p<0.05$). -Secondary injection · The number of lymphocytes showed a significant increase at 7 days after the second transplant in the allogeneic group ($p<0.05$).
Arzi et al. (2017) (19)	· Animal trial (feline) · Treatment of SRG · 6 months	· ADSCs (n=7) · 5×10^6 cells/kg · 2 times · IV	· HLA mismatching · FBS	· Allogeneic ADSCs have been shown to have lower clinical efficacy compared to autologous ADSCs.
Oliveira et al. (2017) (24)	· Animal trial (mouse) · Islet transplantation · 30 days	· ADSCs · 2×10^5 cells · 1 time · SCi at the kidney	· Syngeneic, mismatched allogeneic · FBS	· Survival rates of allogeneic GFP^+ ADSCs: day 7 (100%, 6 out of 6 mice), day 14 (33.3%), day 28 (0%) · Rejection time of allogeneic islet: 19 days in islet plus allogeneic MSC group (13 days in islet alone group).
Colbath et al. (2020) (45)	· Animal trial (horse) · Comparison of autologous and allogeneic MSCs · 2 wk	· BMSCs · 1×10^7 cells · 1 time · IAI	· Autologous, mismatched allogeneic · FBS	· Lymphocytes increased significantly only in the allogeneic group (at 24 h after injection, $p=0.04$).
Hwang et al. (2020) (46)	· Animal trial (mouse) · Comparison of immune responses to MSCs of various origins · 7 days	· ADSCs · 2×10^5 cells · 1 time · Left caudate putamen injection	· Syngeneic, allogeneic, and xenogeneic · FBS	· Infiltration of $CD45^+$ Leukocytes: xenogeneic (43.1%), allogeneic (23.3%), and syngeneic (2.8%) ($p<0.001$ vs. xenogeneic). · Infiltration of CD8 T cells: allogeneic (5.1%), xenogeneic (0.09%), and syngeneic (0.02%) ($p<0.001$, allogeneic vs. xenogeneic).
Immunogenicity for the treatment of repeated allogeneic cell therapy				
Joswig et al. (2017) (35)	· Animal trial (horse) · Clinical response to repeated injection of allogeneic versus autologous MSCs · 4 wk	· BMSCs · 10×10^6 · 2 times · Intra-articular injection	· Autologous, mismatched allogeneic · Both FBS and autologous serum	· Even when using autologous serum, repeated IAI injections of allogeneic MSCs induce an alloimmune response ($p=0.0009$).
Rowland et al. (2021) (47)	· Animal trial (horse) · Effects of repeated injection · 36 days	· BMSCs · 10×10^6 · 2 times · IAI	· Autologous, matched/ mismatched allogeneic · FBS	· The repeated injection increased peri-articular edema and synovial effusion in the mismatched group (all $p<0.05$; matched vs. mismatched). · The mismatched group consistently induced an increase of IFNγ in the joint ($p=0.01$; matched vs. mismatched). · Mismatched group are susceptible to donor-specific anti-MHC Abs and complement-mediated cytotoxicity.

Pa, passage; ICM, ischemic cardiomyopathy; ICI, intracranial injection; SCi, subcutaneous injection.

(49,50). This means that fetal bovine serum must be replaced with human serum or xenofree material before use (8,51).

Comparison of immunogenicity in autologous– versus HLA-mismatched allogeneic–cell therapy

Data comparing the immunogenicity of allogeneic and autologous cells in MSCs therapy have been examined (**Table 4**; reports from 2012 to 2023). The results showed that HLA-mismatched MSCs induced an increase in B cells and the production of alloreactive Abs compared to autologous MSCs (42,44). In addition, HLA-mismatched MSCs induce significant T lymphocyte infiltration compared to autologous MSCs (44-46). Ultimately, the immunogenicity of HLA-mismatched MSCs resulted in a significant decrease in the survival rate compared to that of autologous MSCs (8,24). Interestingly, even immunogenic HLA-mismatched MSCs were effective in prolonging the survival of allogeneic islets without serious adverse effects (**Table 1**) (19,24). These results suggest that HLA-mismatched MSCs may partially contribute their immunomodulatory effects, even if they trigger Ag-specific immune responses in the recipient immune system. However, immunogenic-cell therapy may eventually be eliminated by rejection by the recipient's immune system, which may cause routine repeated administration and result in adverse effects (**Tables 3** and **4**).

BENEFITS OR DEFECTS OF HLA-MATCHED ALLOGENEIC-CELL THERAPY

Autologous-derived cell therapies may be the most effective choice from an immunological safety and functional standpoint. However, they have the disadvantage of not being immediately available. In particular, patient-derived autologous cell therapy may not be functionally effective (21). Thus, HLA-matched allogeneic-cell therapies may be an alternative to autologous cell therapies. **Table 5** compares the advantages and disadvantages of HLA-matched cell therapy based on **Tables 1**–**4**. Most importantly, HLA-matched allogeneic cells can lower the induction of alloreactive memory T/B cells in recipients compared to mismatched allogeneic cells (47). In addition, the long-term survival of HLA-matched allogeneic cells reduces the number of repeated cell treatments (24,47,52-54). Although HLA-matched allogeneic cells have disadvantages in terms of convenience, they have the potential to be used effectively once national/international cell banking systems are well established, as their immunogenicity can be minimized (**Fig. 1**) (55).

Table 5. Benefits and defects of HLA-matched allogeneic cell therapy

Contents	Benefits of HLA-matching	Descripts
Convenience	Medium	· Mismatched allogeneic cells > matched allogeneic cells.
Major causes of immunogenicity in cell therapy	Y (minimization of defect)	· Allogeneic cells: HLA-alloantigens. · Genome-edited cells: foreign Ags produced by genome-editing system. · Xenogeneic Ags: FBS.
Production of alloreactive memory T cells	Y (minimization of defect)	· Mismatched allogeneic cells > matched allogeneic cells.
Prospects for repeat allogeneic cell therapy	Y (minimization of defect)	· Faster removal of allogeneic cells as the number of treatments increases (Tables 3 and 4). · Major causes of increased allograft rejection: HLA-mismatching and immune cells pre-sensitized to an alloantigens.
Verified cell therapy	Y	· Matched or mismatched allogeneic cells > autologous cells. · Cell banking is effective for the use of matched allogeneic cells (Fig. 2).
Long-term survival	Y	· Autologous cells > matched allogeneic cells > mismatched allogeneic cells. · Effective in reducing the number of repeat treatments: autologous and matched allogeneic cells.

Y, yes.

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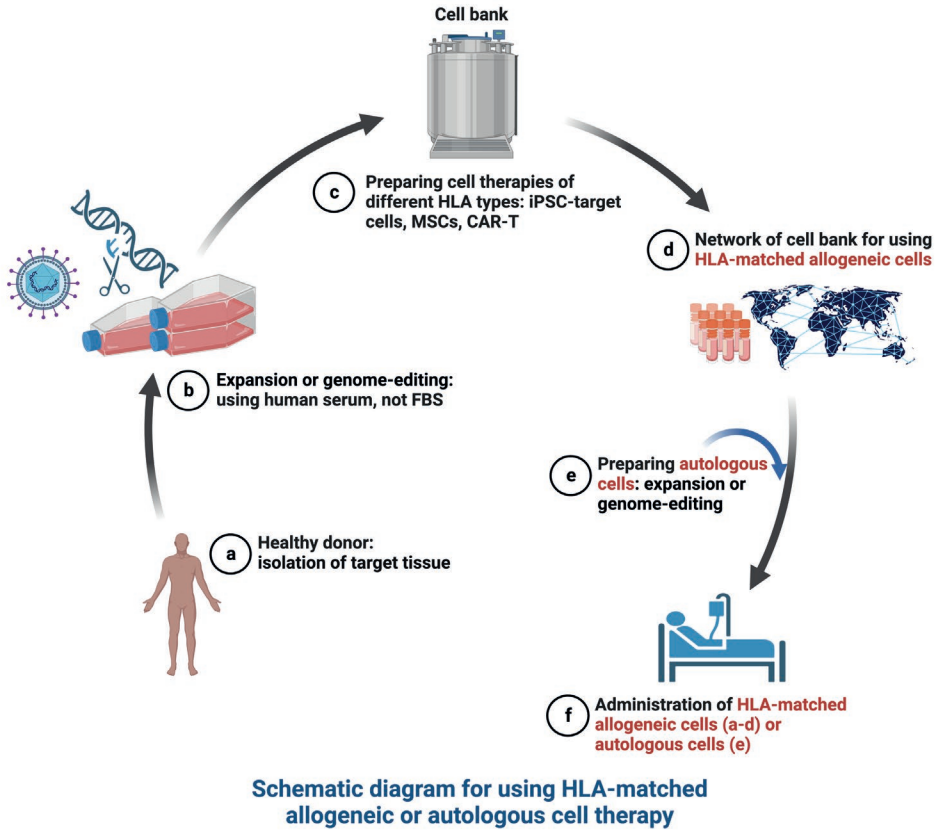

Figure 1. Overview of effective HLA matching in preparing allogeneic cell therapy. (a-c) Cultivation of allogeneic cell therapy (replacing FBS with human serum) and manipulation using genome editing system. (d) For better HLA-matched cell therapy, national/international cell banking systems may be effective (17,55). (e) Preparation of autologous cell therapy. (f) Treat patients with ready-made cell therapy. This figure was created using BioRender.com.

DIFFERENCES IN THE IMMUNOGENICITY OF ALLOGENEIC– AND GENOME-EDITED–THERAPEUTIC CELLS

Both allogeneic and genome-edited cell therapies cause immunogenicity in patients upon transplantation (**Tables 3** and **4**); however, there are differences in the consequences of their immunogenicity. **Table 6** presents the differences in immunogenicity according to the origin of the cell therapy and genetic manipulation based on **Tables 3–5**. Specifically, HLA-matched therapeutic cells with edited genomes are primarily associated with cytotoxic responses and

Table 6. Comparison of immunogenicity differences between allogeneic- and genome edited-therapeutic cells

Contents on immunogenicity	GE cell therapy using autologous cells	Cell therapy using allogeneic cells
Types of Ags	· Foreign-Ags induced by CRPSPR/Cas9 and inserted gene/vector.	· HLA-alloantigens.
Types of Ag-specific T cells	· Develop both CD4 T and CD8 T cells to Cas9 Ag via cross-presentation. · Cas9 also induces the production of Tregs (36).	· Development of alloreactive CD8 T cells to predominantly against mismatched HLA-A,B,C.
Impact of treatment of repeated cell therapy	· Immune rejection is faster for identical genome-edited cells, but HLA Ags are not the targeted.	· Faster rejection dependent on number of cell therapy: HLA-mismatched cells > HLA-matched cells. · The use of HLA-matched allogeneic cells is effective in reducing the number of treatments via long-term survival.
Comparison of immunogenicity levels	· GE-mismatched-allogeneic cell > GE-matched-allogeneic cell > GE-autologous cell.	· Mismatched allogeneic cells > matched allogeneic cells > autologous cells.

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the accumulation of memory cells in response to foreign Ags rather than the rejection of alloantigens (14,36,38,53,56,57). In other words, the effect of immunogenicity due to foreign Ags is limited to the cell populations associated with these Ags. In contrast, cell therapy using allogeneic cells sensitizes memory T/B cells to HLA-alloantigens, leading to their accumulation (36,58,59). Thus, immunogenicity problems caused by HLA alloantigens may be associated with a much broader range of adverse effects than those caused by foreign Ags.

HOW TO AVOID HLA-ALLOANTIGEN SENSITIZATION 1: B2MKO CELL THERAPY

Recently, attempts have been made to eliminate class I MHC as a major cause of allogeneic rejection. B2MKO iPSCs/iMSCs lacking class I HLA presentation were able to evade rejection by alloreactive CD8 T cells compared to wild-type controls (60). However, cells lacking class I HLA may have been targeted by NK cells (61,62), thus increasing their susceptibility to NK cell lysis ($p<0.001$) (60). NK cell lysis in B2MKO cell therapy could be suppressed by knock-in of HLA-E or HLA-G at the B2M locus, which would not induce allogeneic responses (62,63). However, B2MKO cell therapy may be fundamentally vulnerable because it is difficult for the recipient's immune system to function in the event of tumorigenesis or exposure to infection. Defects in B2M gene expression have been reported as the basic mechanism of drug resistance in patients with lung cancer (64,65). Thus, B2MKO cell therapy may be considered for the application of a safety system, such as a suicide system introduced into CAR-T cells, to control the occurrence of serious adverse effects (66). Three types of suicide systems have been reported:1) HSV-1 thymidine kinase suicide genes with immunogenicity (67,68), 2) human origin of the iCaspase 9 suicide system (69,70), and 3) uridine monophosphate synthetase-knockout cell line system for auxotrophy to uridine; suicide switches without transduction may avoid immunogenicity issues (71).

HOW TO AVOID HLA-ALLOANTIGEN SENSITIZATION 2: UNIVERSAL DONOR CELL THERAPY

CIITA is the master transcription factor for class II MHC genes, and it has been reported that ablation of class II MHC can alleviate rejection by CD4 T cells (72). Universal donor cells have been proposed that remove both class I and II MHC, the greatest source of immunogenicity in allogeneic transplantation, and include MSCs (73), iPSCs (74,75), and CAR-T (66,76). Wang et al. (74) demonstrated in a monkey xenogeneic model that human universal iPSCs with dual KO of B2M and *CIITA* can further reduce infiltration of T/B lymphocytes than single KO. Dexamethasone was also able to increase the expression of prostaglandin E-2 (PGE-2), indoleamine-2,3-dioxygenase (IDO), and HLA-G in MSCs, which is interesting because it may contribute to improved function of the cells as well as reduced NK lysis susceptibility (77). However, safeguards such as suicide systems may also be required to control these cells, which are outside the host's immune system.

DISCUSSION

Because of their immunomodulatory and progenitor cell capabilities, MSCs are attractive therapeutic agents for treating various diseases (**Tables 1** and **2**). Notably, in human kidney

transplantation, autologous MSCs replaced tacrolimus to reduce the adverse effects of CNI, and HLA-matched allogeneic MSCs suppressed rejection at lower concentrations of tacrolimus (**Table 2**). However, HLA-mismatched cell therapy, used for convenience and standardization, causes rejection owing to the activation of alloantigen-specific T/B cells preexisting in the recipient's immune system (**Tables 3** and **4**). Fortunately, there are few reports of recipient rejection of these cell therapies directly causing serious adverse effects (78,79). However, these results may lead to the repeated use of cell therapy from allogeneic sources without a deeper understanding of the trade-offs between adverse effects and therapeutic benefits. A second repeat transplantation of allogeneic MSCs into horses or monkeys has been associated with the development of adverse clinical effects thought to be due to adaptive immunity compared to controls (35,44,47). These results are supported by reports showing that HLA-mismatched allogeneic cells cause an increase in alloreactive memory CD8 T cells, an increase in Ag-specific Abs, decreased survival, and faster immune rejection (44) (**Tables 3** and **4**). Thus, these results suggest that reducing alloantigen-sensitized T/B cells through an HLA-matching design is the most important factor in improving engraftment and reducing the repeated use of any cell therapy that uses allogeneic cells (**Fig. 1**) (14,35,53).

The removal of class I MHC from the surface of cell therapy is being attempted as another way to avoid rejection due to the use of HLA-mismatched cell therapy. The neoantigen peptides bind to a class-I MHC molecule consisting of an α chain and a β chain of B2M, which is presented on the cell surface and recognized by the TCR of CD8 T cells (**Supplementary Fig. 1**) (65). Thus, B2MKO cell therapy was able to function while avoiding rejection by alloreactive T cells, compared to wild-type controls (60). However, since the B2MKO system avoids Ag recognition by CD8 T cells, it may be difficult to deal with the development of adverse effects, such as tumorigenicity or infection of genome-edited cells (64,65). Thus, this problem may be solved by applying a suicide system to prevent the adverse effects caused by the excessive immune response of CAR-T cells (66,67,69-71).

Allogeneic CAR-T cells are the most successful cell therapies approved by the FDA, but they are not free from rejection due to immunogenicity (66). However, universal–CAR-T, –MSCs, and –iPSC cell therapies in which both B2M and *CIITA* are knocked out have been shown to effectively evade immune rejection (66,73-76). Even more encouraging is that a number of universal cell therapies are being evaluated as functionally normal or well differentiated (66,74-76,80). These results show that universal cell therapy has the potential to be an effective treatment that overcomes the disadvantages of autologous or HLA-matched cells. Additionally, these results may provide hope for MSCs, which have failed to achieve efficacy in several clinical trials due to tissue source, donor heterogeneity, and heterogeneous manufacturing (81). However, the control of these cells outside the host's immune system may require safeguards such as artificial suicide systems. In addition, HLA-KO cell therapies are expected to suppress acute rejection, but immunogenicity to foreign Ags, which is expected to be relatively weak, remains (17,32,36,48).

CONCLUSION

The use of autologous or HLA-matched allogeneic cells in cell therapy may have disadvantages in terms of convenience but may reduce repeated use due to the long-term survival of therapeutic cells. In addition, the use of these cell therapies is expected to

increase curative opportunities and success rates in patients who are candidates for allogeneic transplantation (24,47,52-54). These results are attributed to the fact that although HLA-mismatched allogeneic cells such as MSCs and iPSCs have immunomodulatory effects, they are ineffective in suppressing the activity of Ag-specific memory cells in the recipient's immune system (**Tables 3** and **4**) (29). To prevent the rejection of immunogenic-cell therapy, the introduction of Ag-specific Tregs effectively suppresses the recipient's immune response to Cas9 and alloantigens (36,82,83). However, to produce Ag-specific Tregs, there are obstacles, such as high-level technology that has not yet been established, cost, and time consumption (84). Thus, B2MKO- and universal-cell therapies, with proven safety, as well as validated HLA-matched cells, has the potential to be an effective way to reduce immunogenicity. However, the number of times a patient should receive a validated HLA-matched-, B2MKO-, or universal-cell therapy compared with autologous treatment to minimize adverse effects remains to be evaluated. In addition, the use of xenogeneic-free serum benefits the long-term survival of therapeutic cells by reducing unnecessary activity of the recipient's immune system (8,35,51). This suggests that immunogenic cell therapy may have an opportunity cost in terms of the limited number of treatments between adverse effects and therapeutic benefit, so efforts to eliminate the causes of immunogenicity need to be sustained.

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SUPPLEMENTARY MATERIAL

Supplementary Figure 1

Recognition pathway of recipient T cells against HLA-alloantigen. (A) A direct pathway is the recognition of HLA-alloantigens presented on the surface of donor cells by recipient T cells. This pathway is primarily associated with acute allograft rejection (S1,S2). (B) In the indirect pathway, HLA-alloantigens from donor cells are recognized by recipient T cells by presentation of recipient APCs. This pathway is involved in chronic allograft rejection (S1,S2). This figure was created using BioRender.com.

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REFERENCES

1. Saler M, Caliogna L, Botta L, Benazzo F, Riva F, Gastaldi G. hASC and DFAT, multipotent stem cells for regenerative medicine: a comparison of their potential differentiation *in vitro*. *Int J Mol Sci* 2017;18:2699.
PUBMED | CROSSREF
2. Weiss AR, Dahlke MH. Immunomodulation by mesenchymal stem cells (MSCs): mechanisms of action of living, apoptotic, and dead MSCs. *Front Immunol* 2019;10:1191.
PUBMED | CROSSREF
3. Lynggaard CD, Grønhøj C, Christensen R, Fischer-Nielsen A, Melchiors J, Specht L, Andersen E, Mortensen J, Oturai P, Barfod GH, et al. Intraglandular off-the-shelf allogeneic mesenchymal stem cell treatment in patients with radiation-induced xerostomia: a safety study (MESRIX-II). *Stem Cells Transl Med* 2022;11:478-489.
PUBMED | CROSSREF

4. Chen CF, Hu CC, Wu CT, Wu HH, Chang CS, Hung YP, Tsai CC, Chang Y. Treatment of knee osteoarthritis with intra-articular injection of allogeneic adipose-derived stem cells (ADSCs) ELIXCYTE®: a phase I/II, randomized, active-control, single-blind, multiple-center clinical trial. *Stem Cell Res Ther* 2021;12:562.
PUBMED | CROSSREF
5. Meucci MC, Reinders ME, Groeneweg KE, Bezstarosti S, Ajmone Marsan N, Bax JJ, De Fijter JW, Delgado V. Cardiovascular effects of autologous bone marrow-derived mesenchymal stromal cell therapy with early tacrolimus withdrawal in renal transplant recipients: an analysis of the randomized triton study. *J Am Heart Assoc* 2021;10:e023300.
PUBMED | CROSSREF
6. Mo Y, Kim Y, Bang JY, Jung J, Lee CG, Elias JA, Kang HR. Mesenchymal stem cells attenuate asthmatic inflammation and airway remodeling by modulating macrophages/monocytes in the il-13-overexpressing mouse model. *Immune Netw* 2022;22:e40.
PUBMED | CROSSREF
7. Wang Y, Tian M, Wang F, Heng BC, Zhou J, Cai Z, Liu H. Understanding the immunological mechanisms of mesenchymal stem cells in allogeneic transplantation: From the aspect of major histocompatibility complex class i. *Stem Cells Dev* 2019;28:1141-1150.
PUBMED | CROSSREF
8. Chang SH, Park CG. Allogeneic ADSCs induce CD8 T cell-mediated cytotoxicity and faster cell death after exposure to xenogeneic serum or proinflammatory cytokines. *Exp Mol Med* 2019;51:1-10.
PUBMED | CROSSREF
9. Mukonoweshuro B, Brown CJ, Fisher J, Ingham E. Immunogenicity of undifferentiated and differentiated allogeneic mouse mesenchymal stem cells. *J Tissue Eng* 2014;5:2041731414534255.
PUBMED | CROSSREF
10. Li C, Chen S, Zhou Y, Zhao Y, Liu P, Cai J. Application of induced pluripotent stem cell transplants: autologous or allogeneic? *Life Sci* 2018;212:145-149.
PUBMED | CROSSREF
11. Karahan GE, Claas FH, Heidt S. Pre-existing alloreactive t and b cells and their possible relevance for pre-transplant risk estimation in kidney transplant recipients. *Front Med (Lausanne)* 2020;7:340.
PUBMED | CROSSREF
12. Lefaucheur C, Loupy A. Antibody-mediated rejection of solid-organ allografts. *N Engl J Med* 2018;379:2580-2582.
PUBMED
13. Pulecio J, Verma N, Mejía-Ramírez E, Huangfu D, Raya A. Crispr/cas9-based engineering of the epigenome. *Cell Stem Cell* 2017;21:431-447.
PUBMED | CROSSREF
14. Gorecka J, Kostiuk V, Fereydooni A, Gonzalez L, Luo J, Dash B, Isaji T, Ono S, Liu S, Lee SR, et al. The potential and limitations of induced pluripotent stem cells to achieve wound healing. *Stem Cell Res Ther* 2019;10:87.
PUBMED | CROSSREF
15. Pei WD, Zhang Y, Yin TL, Yu Y. Epigenome editing by CRISPR/Cas9 in clinical settings: possibilities and challenges. *Brief Funct Genomics* 2020;19:215-228.
PUBMED | CROSSREF
16. Scheiner ZS, Talib S, Feigal EG. The potential for immunogenicity of autologous induced pluripotent stem cell-derived therapies. *J Biol Chem* 2014;289:4571-4577.
PUBMED | CROSSREF
17. Morizane A, Kikuchi T, Hayashi T, Mizuma H, Takara S, Doi H, Mawatari A, Glasser MF, Shiina T, Ishigaki H, et al. MHC matching improves engraftment of iPSC-derived neurons in non-human primates. *Nat Commun* 2017;8:385.
PUBMED | CROSSREF
18. Embgenbroich M, Burgdorf S. Current concepts of antigen cross-presentation. *Front Immunol* 2018;9:1643.
PUBMED | CROSSREF
19. Arzi B, Clark KC, Sundaram A, Spriet M, Verstraete FJ, Walker NJ, Loscar MR, Fazel N, Murphy WJ, Vapniarsky N, et al. Therapeutic efficacy of fresh, allogeneic mesenchymal stem cells for severe refractory feline chronic gingivostomatitis. *Stem Cells Transl Med* 2017;6:1710-1722.
PUBMED | CROSSREF
20. Arzi B, Mills-Ko E, Verstraete FJ, Kol A, Walker NJ, Badgley MR, Fazel N, Murphy WJ, Vapniarsky N, Borjesson DL. Therapeutic efficacy of fresh, autologous mesenchymal stem cells for severe refractory gingivostomatitis in cats. *Stem Cells Transl Med* 2016;5:75-86.
PUBMED | CROSSREF

21. Kamen DL, Wallace C, Li Z, Wyatt M, Paulos C, Wei C, Wang H, Wolf BJ, Nietert PJ, Gilkeson G. Safety, immunological effects and clinical response in a phase I trial of umbilical cord mesenchymal stromal cells in patients with treatment refractory SLE. *Lupus Sci Med* 2022;9:e000704.
PUBMED | CROSSREF

22. Jolly M, Garris CP, Mikolaitis RA, Jhingran PM, Dennis G, Wallace DJ, Clarke A, Dooley MA, Parke A, Strand V, et al. Development and validation of the Lupus Impact Tracker: a patient-completed tool for clinical practice to assess and monitor the impact of systemic lupus erythematosus. *Arthritis Care Res (Hoboken)* 2014;66:1542-1550.
PUBMED | CROSSREF

23. Hernigou P, Guissou I, Homma Y, Poignard A, Chevallier N, Rouard H, Flouzat Lachaniette CH. Percutaneous injection of bone marrow mesenchymal stem cells for ankle non-unions decreases complications in patients with diabetes. *Int Orthop* 2015;39:1639-1643.
PUBMED | CROSSREF

24. Oliveira RL, Chagastelles PC, Sesterheim P, Pranke P. In vivo immunogenic response to allogeneic mesenchymal stem cells and the role of preactivated mesenchymal stem cells cotransplanted with allogeneic islets. *Stem Cells Int* 2017;2017:9824698.
PUBMED | CROSSREF

25. Coemans M, Süsal C, Döhler B, Anglicheau D, Giral M, Bestard O, Legendre C, Emonds MP, Kuypers D, Molenberghs G, et al. Analyses of the short- and long-term graft survival after kidney transplantation in Europe between 1986 and 2015. *Kidney Int* 2018;94:964-973.
PUBMED | CROSSREF

26. Pan GH, Chen Z, Xu L, Zhu JH, Xiang P, Ma JJ, Peng YW, Li GH, Chen XY, Fang JL, et al. Low-dose tacrolimus combined with donor-derived mesenchymal stem cells after renal transplantation: a prospective, non-randomized study. *Oncotarget* 2016;7:12089-12101.
PUBMED | CROSSREF

27. Dreyer GJ, Groeneweg KE, Heidt S, Roelen DL, van Pel M, Roelofs H, Huurman VA, Bajema IM, Moes DJ, Fibbe WE, et al. Human leukocyte antigen selected allogeneic mesenchymal stromal cell therapy in renal transplantation: the Neptune study, a phase I single-center study. *Am J Transplant* 2020;20:2905-2915.
PUBMED | CROSSREF

28. Reinders ME, Groeneweg KE, Hendriks SH, Bank JR, Dreyer GJ, de Vries AP, van Pel M, Roelofs H, Huurman VA, Meij P, et al. Autologous bone marrow-derived mesenchymal stromal cell therapy with early tacrolimus withdrawal: the randomized prospective, single-center, open-label TRITON study. *Am J Transplant* 2021;21:3055-3065.
PUBMED | CROSSREF

29. Večerić-Haler Ž, Sever M, Kojc N, Halloran PF, Boštjančič E, Mlinšek G, Oblak M, Poženel P, Švajger U, Hartman K, et al. Autologous mesenchymal stem cells for treatment of chronic active antibody-mediated kidney graft rejection: report of the phase i/ii clinical trial case series. *Transpl Int* 2022;35:10772.
PUBMED | CROSSREF

30. Kol A, Wood JA, Carrade Holt DD, Gillette JA, Bohannon-Worsley LK, Puchalski SM, Walker NJ, Clark KC, Watson JL, Borjesson DL. Multiple intravenous injections of allogeneic equine mesenchymal stem cells do not induce a systemic inflammatory response but do alter lymphocyte subsets in healthy horses. *Stem Cell Res Ther* 2015;6:73.
PUBMED | CROSSREF

31. Owens SD, Kol A, Walker NJ, Borjesson DL. Allogeneic mesenchymal stem cell treatment induces specific alloantibodies in horses. *Stem Cells Int* 2016;2016:5830103.
PUBMED | CROSSREF

32. Kawamura T, Miyagawa S, Fukushima S, Maeda A, Kashiyama N, Kawamura A, Miki K, Okita K, Yoshida Y, Shiina T, et al. Cardiomyocytes derived from MHC-homozygous induced pluripotent stem cells exhibit reduced allogeneic immunogenicity in MHC-matched non-human primates. *Stem Cell Reports* 2016;6:312-320.
PUBMED | CROSSREF

33. Nakagawa M, Taniguchi Y, Senda S, Takizawa N, Ichisaka T, Asano K, Morizane A, Doi D, Takahashi J, Nishizawa M, et al. A novel efficient feeder-free culture system for the derivation of human induced pluripotent stem cells. *Sci Rep* 2014;4:3594.
PUBMED | CROSSREF

34. Chang SH, Kim HJ, Park CG. Allogeneic ADSCs induce the production of alloreactive memory-CD8 T cells through HLA-ABC antigens. *Cells* 2020;9:1246.
PUBMED | CROSSREF

35. Joswig AJ, Mitchell A, Cummings KJ, Levine GJ, Gregory CA, Smith R 3rd, Watts AE. Repeated intra-articular injection of allogeneic mesenchymal stem cells causes an adverse response compared to autologous cells in the equine model. *Stem Cell Res Ther* 2017;8:42.
PUBMED | CROSSREF

36. Wagner DL, Amini L, Wendering DJ, Burkhardt LM, Akyüz L, Reinke P, Volk HD, Schmueck-Henneresse M. High prevalence of *Streptococcus pyogenes* Cas9-reactive T cells within the adult human population. *Nat Med* 2019;25:242-248.
PUBMED | CROSSREF

37. Arruda VR, Favaro P, Finn JD. Strategies to modulate immune responses: a new frontier for gene therapy. *Mol Ther* 2009;17:1492-1503.
PUBMED | CROSSREF

38. Deuse T, Hu X, Agbor-Enoh S, Koch M, Spitzer MH, Gravina A, Alawi M, Marishta A, Peters B, Kosaloglu-Yalcin Z, et al. De novo mutations in mitochondrial DNA of iPSCs produce immunogenic neoepitopes in mice and humans. *Nat Biotechnol* 2019;37:1137-1144.
PUBMED | CROSSREF

39. He Y, Wu J, Dressman DC, Iacobuzio-Donahue C, Markowitz SD, Velculescu VE, Diaz LA Jr, Kinzler KW, Vogelstein B, Papadopoulos N. Heteroplasmic mitochondrial DNA mutations in normal and tumour cells. *Nature* 2010;464:610-614.
PUBMED | CROSSREF

40. Yakes FM, Van Houten B. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proc Natl Acad Sci U S A* 1997;94:514-519.
PUBMED | CROSSREF

41. Badr Eslam R, Croce K, Mangione FM, Musmann R, Leopold JA, Mitchell RN, Waxman AB. Persistence and proliferation of human mesenchymal stromal cells in the right ventricular myocardium after intracoronary injection in a large animal model of pulmonary hypertension. *Cytotherapy* 2017;19:668-679.
PUBMED | CROSSREF

42. Hare JM, Fishman JE, Gerstenblith G, DiFede Velazquez DL, Zambrano JP, Suncion VY, Tracy M, Ghersin E, Johnston PV, Brinker JA, et al. Comparison of allogeneic vs autologous bone marrow–derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. *JAMA* 2012;308:2369-2379.
PUBMED | CROSSREF

43. Pigott JH, Ishihara A, Wellman ML, Russell DS, Bertone AL. Investigation of the immune response to autologous, allogeneic, and xenogeneic mesenchymal stem cells after intra-articular injection in horses. *Vet Immunol Immunopathol* 2013;156:99-106.
PUBMED | CROSSREF

44. Isakova IA, Lanclos C, Bruhn J, Kuroda MJ, Baker KC, Krishnappa V, Phinney DG. Allo-reactivity of mesenchymal stem cells in rhesus macaques is dose and haplotype dependent and limits durable cell engraftment *in vivo*. *PLoS One* 2014;9:e87238.
PUBMED | CROSSREF

45. Colbath AC, Dow SW, Hopkins LS, Phillips JN, McIlwraith CW, Goodrich LR. Allogeneic vs. autologous intra-articular mesenchymal stem cell injection within normal horses: clinical and cytological comparisons suggest safety. *Equine Vet J* 2020;52:144-151.
PUBMED | CROSSREF

46. Hwang JW, Lee NK, Yang JH, Son HJ, Bang SI, Chang JW, Na DL. A comparison of immune responses exerted following syngeneic, allogeneic, and xenogeneic transplantation of mesenchymal stem cells into the mouse brain. *Int J Mol Sci* 2020;21:3052.
PUBMED | CROSSREF

47. Rowland AL, Miller D, Berglund A, Schnabel LV, Levine GJ, Antczak DF, Watts AE. Cross-matching of allogeneic mesenchymal stromal cells eliminates recipient immune targeting. *Stem Cells Transl Med* 2021;10:694-710.
PUBMED | CROSSREF

48. Hoornaert CJ, Le Blon D, Quarta A, Daans J, Goossens H, Berneman Z, Ponsaerts P. Concise review: innate and adaptive immune recognition of allogeneic and xenogeneic cell transplants in the central nervous system. *Stem Cells Transl Med* 2017;6:1434-1441.
PUBMED | CROSSREF

49. Merlot AM, Kalinowski DS, Richardson DR. Unraveling the mysteries of serum albumin-more than just a serum protein. *Front Physiol* 2014;5:299.
PUBMED | CROSSREF

50. Tiruppathi C, Finnegan A, Malik AB. Isolation and characterization of a cell surface albumin-binding protein from vascular endothelial cells. *Proc Natl Acad Sci U S A* 1996;93:250-254.
PUBMED | CROSSREF

51. Haque N, Kasim NH, Rahman MT. Optimization of pre-transplantation conditions to enhance the efficacy of mesenchymal stem cells. *Int J Biol Sci* 2015;11:324-334.
PUBMED | CROSSREF

52. Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. *Nat Biotechnol* 2014;32:252-260.
PUBMED | CROSSREF

53. Stoddard-Bennett T, Pera RR. Stem cell therapy for Parkinson's disease: safety and modeling. *Neural Regen Res* 2020;15:36-40.
PUBMED | CROSSREF

54. Verboket R, Leiblein M, Seebach C, Nau C, Janko M, Bellen M, Bönig H, Henrich D, Marzi I. Autologous cell-based therapy for treatment of large bone defects: from bench to bedside. *Eur J Trauma Emerg Surg* 2018;44:649-665.
PUBMED | CROSSREF

55. Taylor CJ, Peacock S, Chaudhry AN, Bradley JA, Bolton EM. Generating an iPSC bank for HLA-matched tissue transplantation based on known donor and recipient HLA types. *Cell Stem Cell* 2012;11:147-152.
PUBMED | CROSSREF

56. Lu Y, Xue J, Deng T, Zhou X, Yu K, Deng L, Huang M, Yi X, Liang M, Wang Y, et al. Safety and feasibility of CRISPR-edited T cells in patients with refractory non-small-cell lung cancer. *Nat Med* 2020;26:732-740.
PUBMED | CROSSREF

57. Charlesworth CT, Deshpande PS, Dever DP, Camarena J, Lemgart VT, Cromer MK, Vakulskas CA, Collingwood MA, Zhang L, Bode NM, et al. Identification of preexisting adaptive immunity to Cas9 proteins in humans. *Nat Med* 2019;25:249-254.
PUBMED | CROSSREF

58. Xian B, Huang B. The immune response of stem cells in subretinal transplantation. *Stem Cell Res Ther* 2015;6:161.
PUBMED | CROSSREF

59. Dudek AM, Porteus MH. Answered and unanswered questions in early-stage viral vector transduction biology and innate primary cell toxicity for ex-vivo gene editing. *Front Immunol* 2021;12:660302.
PUBMED | CROSSREF

60. Zha S, Tay JC, Zhu S, Li Z, Du Z, Wang S. Generation of mesenchymal stromal cells with low immunogenicity from human PBMC-derived beta2 microglobulin knockout induced pluripotent stem cells. *Cell Transplant* 2020;29:963689720965529.
PUBMED | CROSSREF

61. Morvan MG, Lanier LL. NK cells and cancer: you can teach innate cells new tricks. *Nat Rev Cancer* 2016;16:7-19.
PUBMED | CROSSREF

62. Shin MH, Kim J, Lim SA, Kim J, Kim SJ, Lee KM. NK cell-based immunotherapies in cancer. *Immune Netw* 2020;20:e14.
PUBMED | CROSSREF

63. Gornalusse GG, Hirata RK, Funk SE, Riolobos L, Lopes VS, Manske G, Prunkard D, Colunga AG, Hanafi LA, Clegg DO, et al. HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells. *Nat Biotechnol* 2017;35:765-772.
PUBMED | CROSSREF

64. Wang C, Wang Z, Yao T, Zhou J, Wang Z. The immune-related role of beta-2-microglobulin in melanoma. *Front Oncol* 2022;12:944722.
PUBMED | CROSSREF

65. Zhao Y, Cao Y, Chen Y, Wu L, Hang H, Jiang C, Zhou X. B2M gene expression shapes the immune landscape of lung adenocarcinoma and determines the response to immunotherapy. *Immunology* 2021;164:507-523.
PUBMED | CROSSREF

66. Wagner DL, Fritsche E, Pulsipher MA, Ahmed N, Hamieh M, Hegde M, Ruella M, Savoldo B, Shah NN, Turtle CJ, et al. Immunogenicity of CAR T cells in cancer therapy. *Nat Rev Clin Oncol* 2021;18:379-393.
PUBMED | CROSSREF

67. Jensen MC, Popplewell L, Cooper LJ, DiGiusto D, Kalos M, Ostberg JR, Forman SJ. Antitransgene rejection responses contribute to attenuated persistence of adoptively transferred CD20/CD19-specific chimeric antigen receptor redirected T cells in humans. *Biol Blood Marrow Transplant* 2010;16:1245-1256.
PUBMED | CROSSREF

68. Berger C, Flowers ME, Warren EH, Riddell SR. Analysis of transgene-specific immune responses that limit the *in vivo* persistence of adoptively transferred HSV-TK-modified donor T cells after allogeneic hematopoietic cell transplantation. *Blood* 2006;107:2294-2302.
PUBMED | CROSSREF

69. Zhou X, Dotti G, Krance RA, Martinez CA, Naik S, Kamble RT, Durett AG, Dakhova O, Savoldo B, Di Stasi A, et al. Inducible caspase-9 suicide gene controls adverse effects from alloreplete T cells after haploidentical stem cell transplantation. *Blood* 2015;125:4103-4113.
PUBMED | CROSSREF

70. Di Stasi A, Tey SK, Dotti G, Fujita Y, Kennedy-Nasser A, Martinez C, Straathof K, Liu E, Durett AG, Grilley B, et al. Inducible apoptosis as a safety switch for adoptive cell therapy. *N Engl J Med* 2011;365:1673-1683.
PUBMED | CROSSREF

71. Wiebking V, Patterson JO, Martin R, Chanda MK, Lee CM, Srifa W, Bao G, Porteus MH. Metabolic engineering generates a transgene-free safety switch for cell therapy. *Nat Biotechnol* 2020;38:1441-1450.
PUBMED | CROSSREF

72. Kagoya Y, Guo T, Yeung B, Saso K, Anczurowski M, Wang CH, Murata K, Sugata K, Saijo H, Matsunaga Y, et al. Genetic ablation of HLA class I, class II, and the T-cell receptor enables allogeneic T cells to be used for adoptive T-cell therapy. *Cancer Immunol Res* 2020;8:926-936.
PUBMED | CROSSREF

73. Halm D, Leibig N, Martens J, Stark GB, Groß T, Zimmermann S, Finkenzeller G, Lampert F. Direct comparison of the immunogenicity of major histocompatibility complex-I and -II deficient mesenchymal stem cells *in vivo*. *Biol Chem* 2021;402:693-702.
PUBMED | CROSSREF

74. Wang X, Lu M, Tian X, Ren Y, Li Y, Xiang M, Chen S. Diminished expression of major histocompatibility complex facilitates the use of human induced pluripotent stem cells in monkey. *Stem Cell Res Ther* 2020;11:334.
PUBMED | CROSSREF

75. Mattapally S, Pawlik KM, Fast VG, Zumaquero E, Lund FE, Randall TD, Townes TM, Zhang J. Human leukocyte antigen class I and II knockout human induced pluripotent stem cell-derived cells: universal donor for cell therapy. *J Am Heart Assoc* 2018;7:e010239.
PUBMED | CROSSREF

76. Lee J, Sheen JH, Lim O, Lee Y, Ryu J, Shin D, Kim YY, Kim M. Abrogation of HLA surface expression using CRISPR/Cas9 genome editing: a step toward universal T cell therapy. *Sci Rep* 2020;10:17753.
PUBMED | CROSSREF

77. Rawat S, Dadhwal V, Mohanty S. Dexamethasone priming enhances stemness and immunomodulatory property of tissue-specific human mesenchymal stem cells. *BMC Dev Biol* 2021;21:16.
PUBMED | CROSSREF

78. Kot M, Baj-Krzyworzeka M, Szatanek R, Musiał-Wysocka A, Suda-Szczurek M, Majka M. The importance of HLA assessment in "off-the-shelf" allogeneic mesenchymal stem cells based-therapies. *Int J Mol Sci* 2019;20:5680.
PUBMED | CROSSREF

79. Shah K, Shah N, Ghassemi F, Ly C, George T, Lutz C, Sumer H. Alloreactivity of allogeneic mesenchymal stem/stromal cells and other cellular therapies: a concise review. *Stem Cells Int* 2022;2022:9589600.
PUBMED | CROSSREF

80. Mo F, Watanabe N, McKenna MK, Hicks MJ, Srinivasan M, Gomes-Silva D, Atilla E, Smith T, Ataca Atilla P, Ma R, et al. Engineered off-the-shelf therapeutic T cells resist host immune rejection. *Nat Biotechnol* 2021;39:56-63.
PUBMED | CROSSREF

81. Srinivasan A, Sathiyanathan P, Yin L, Liu TM, Lam A, Ravikumar M, Smith RA, Loh HP, Zhang Y, Ling L, et al. Strategies to enhance immunomodulatory properties and reduce heterogeneity in mesenchymal stromal cells during ex vivo expansion. *Cytotherapy* 2022;24:456-472.
PUBMED | CROSSREF

82. Juneja T, Kazmi M, Mellace M, Saidi RF. Utilization of Treg cells in solid organ transplantation. *Front Immunol* 2022;13:746889.
PUBMED | CROSSREF

83. Proics E, David M, Mojibian M, Speck M, Lounnas-Mourey N, Govehovitch A, Baghdadi W, Desnouveaux J, Bastian H, Freschi L, et al. Preclinical assessment of antigen-specific chimeric antigen receptor regulatory T cells for use in solid organ transplantation. *Gene Ther* 2023;30:309-322.
PUBMED | CROSSREF

84. Atif M, Conti F, Gorochov G, Oo YH, Miyara M. Regulatory T cells in solid organ transplantation. *Clin Transl Immunology* 2020;9:e01099.
PUBMED | CROSSREF