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Clinical trials of mesenchymal stem cell transplantation in patients with type 1 diabetes and systemic lupus erythematosus: is it time for larger studies?

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Abstract

Type 1 Diabetes (T1D) and Systemic Lupus Erythematosus (SLE) are two autoimmune diseases for which there is no definitive cure. Mesenchymal stem cells (MSC) transplantation is being tested as a therapeutic option in clinical trials for these two diseases. MSCs possess three important characteristics which could be exploited in cell-based approaches for autoimmune conditions: 1) they are potent immunomodulators, exerting suppressive functions on immune effector cells and orchestrating the action of other regulatory cells; 2) they can stimulate tissue repair and regeneration mechanisms; 3) they have shown a good safety profile in clinical trials, including a limited risk of tumour formation. Multiple clinical trials of MSC transplantation in patients with these diseases are ongoing. Here we review the results reported so far and highlight key emerging findings. These trials confirm the safety profile of this type of transplantation. In a cohort of T1D patients, the transplantation of autologous bone marrow-derived MSCs was associated with preservation of beta cell function over a 1-year follow-up. In a cohort of T1D patients who received autologous bone marrow-derived mononuclear cells along with umbilical cord-derived MSCs transplantation, beta cell function increased during the 1-year follow-up. In both studies, control patients experienced a decline in beta cell function. Non-randomized studies tested the transplantation of bone marrow- and umbilical cord-derived MSCs in patients affected by treatment-refractory SLE: the disease activity index improved, and immunologic parameters suggested partial remission from autoimmunity. The outcomes of these trials indicate that MSC transplantation is a safe procedure, and they suggest that MSCs may have efficacy in controlling the effects of the autoimmune processes. These findings should encourage larger and long-term randomized controlled studies of MSC transplantation in autoimmune disease to confirm safety and better assess efficacy.

Introduction

Both Type 1 Diabetes (T1D) and Systemic Lupus Erythematosus (SLE) are chronic diseases characterized by immune dysregulation. In T1D, patients suffer from a progressive destruction of their beta cell mass, eventually leading to loss of insulin secretion[1]. SLE has a range of clinical manifestations, from cutaneous rash and arthritis to a severe multi-organ dysfunction[2]. To date, there is no definitive cure for these autoimmune diseases. In T1D patients, exogenous insulin therapy is required life-long and while it is a life-saving intervention most patients fail to achieve satisfactory metabolic control. Moreover, patients remain exposed to the risk of developing both short and long-term complications, some of which can be fatal[3]. A subset

of T1D patients cannot be managed effectively with exogenous insulin, and present dangerous swings in glycaemia ('brittle' T1D). Clinical trials in patients with recent onset T1D have explored a series of immunotherapeutic strategies to halt beta cell destruction and hopefully preserve the residual beta cell mass, but so far there has been limited success and when effects have been observed these are limited in time[4]. This suggests that chronic but safe therapies may be needed to control islet autoimmunity, unless self-tolerance to islet cell antigens can be restored.

Immunosuppressive therapy is the gold standard for SLE, but it can cause severe drug toxicity and may pave the way to aggressive infections, malignancies and cardiovascular diseases[5]. Moreover, some patients affected by Lupus nephritis are refractory to conventional immunosuppressive treatments, namely cyclophosphamide, glucocorticoids and mycophenolate mofetil[6].

Therefore, scientists have been looking for alternative therapies: among others, mesenchymal stem cells (MSCs) have catalyzed a great interest in the last decades; MSCs transplantation has been tested extensively in animal models of autoimmune diseases[3,7,8]. Specifically, the immunomodulatory and tissue repair properties of MSCs are a strong rationale for a therapeutic application of MSCs in these diseases. In this review, we will discuss the most relevant results emerged in recent clinical trials of MSCs transplantation in T1D and SLE.

Mesenchymal stem cells

Mesenchymal stem cells are multipotent and ubiquitous precursors, as they have been found in a large variety of tissues including bone marrow, adipose tissue, umbilical cord and umbilical cord blood[9]. Phenotypic criteria to define MSCs have been proposed by the International Society for Cellular Therapy in 2006[10] and include i) adherence to plastic surfaces; ii) expression of CD73, CD90, CD105 surface markers; iii) negativity for MHC-II, CD19, CD11b, CD79α, CD34, CD45, CD14; iv) capacity to differentiate into chondrocytes, osteocytes and adipocytes. There is evidence that MSCs may be induced to differentiate also into other lineages, including neurons, hepatocytes, cardiocytes and beta cells, although further studies are required to confirm such results[3].

MSCs possess three important properties that have made them the workhorses for stem cell-based therapies: 1) they are potent immunomodulators, exerting suppressive functions on immune effector cells and orchestrating the action of other regulatory cells; 2) they can stimulate tissue repair and regeneration mechanisms; 3) they have shown a good safety profile in clinical trials, including a limited risk of tumour formation[11]. In addition, they show reduced immunogenicity, possibly due to a low expression of MHC-I and to the lack of expression of costimulatory molecules.

MSCs as immunomodulators

MSCs can exert potent immunomodulatory functions. Several mechanisms of action have been described. MSCs interact with a large variety of immune cells, including DC, NK cells, B cells and T cells[12]. Specifically, MSCs are thought to inhibit DC differentiation and maturation, suppress the proliferation of CD4+ and CD8+ T cells and impair the cytotoxic activity of CTL[3]. Immunomodulatory mechanisms also include the induction and expansion of T-regulatory (T-reg) cells, as well as their ability to balance Th subsets[13]. Concerning the research in T1D, studies conducted in the NOD mouse model showed that MSCs induce IL-10 secreting FoxP3+ T-reg cells[14]. The generation of functional T-reg cells has also been observed in SLE patients treated with MSCs[6,15]. Moreover, it has been proposed that MSCs could expand antigen-specific T-reg cells *in vivo* and could stimulate long-lasting tolerance[9].

The MSCs interference on the T helper polarization is of great interest, but the effects are not completely understood. MSCs seem to shift the cytokine profile from pro-inflammatory to anti-inflammatory in the murine pancreatic microenvironment (i.e. polarizing the response from Th1 to Th2 in T1D animal models)[3]; on the other hand, SLE patients showed clinical improvement when Th response shifted from Th2 to Th1[6].

Moreover, MSCs immunomodulation is dependent on the environment and should not be considered solely immunosuppressive: as an example, MSCs display a pro-inflammatory activity when homed into a low-inflamed microenvironment[9,13].

MSCs to promote tissue repair

MSCs have a prominent secretive activity, and great efforts have been made to understand the biological influence of this activity. Interestingly, MSCs display the ability to home into inflamed

tissues, which results from the interaction of different subsets of receptors with their ligands (e.g. CXCR4 with its ligands CCL12 and SDF-1, and VLA-4 with VCAM-1)[9]. Although thorough studies need to be performed in order to fully understand *in vivo* intercellular interactions, a number of molecules have been found to be secreted by MSCs at their homing site: IL-6, IL-8, TGF-beta, nitric oxide, indoleamine 2,3-dioxygenase, TIMP-2, VEGF, HGF, GM-CSF, bFGF, IGFBP3, IGFBP4, IGFBP7[9,16]. Such molecules promote tissue repair and act as chemo-attractants recruiting macrophages and endothelial cells at the site of injury or inflammation. In addition, it has been proposed that MSCs signaling through cell contact and microvesicles could participate in immunoregulation[17]. Scientists have wondered for a long time whether MSCs transdifferentiate into tissue-specific cells or help repair the target tissues. Nevertheless, the possibility that they could have a minor role in orchestrating local biological functions should not be underestimated. Studies in rodent models of T1D seem to validate the hypothesis that MSCs boost endogenous tissue regeneration, as mice transplanted with human MSCs displayed higher serum insulin than the control group, with no human insulin detected[7].

Safety and tumorigenicity of MSCs

A recurrent hurdle to stem-cell based therapies is the risk to give rise to neoplastic transformation. Some animal studies have shown that *in vitro* manipulated MSCs are able to become tumorigenic once reinjected into the subject[18]. However, MSCs appear to be safer than other subsets of stem cells[19] and most recent clinical trials have shown no progression to tumorigenesis[6,15,20-22]. A meta-analysis published in March 2016[23] reported a neoplasm prevalence of 0.3 % (n=7) among 2,372 MSC-treated patients who were followed on average for 2.2 years, with a mean age of 57 years. The annual cancer rate was 0.14% in this study group, a lower score than the annual incidence of cancer in the U.S according to the National Cancer Institute (0.78% in the 50-64-year-old population in 2011). Therefore, MSCs transplantation does not seem to confer an increased risk of tumor development in a timeframe of 2.2 years post transplantation. Such results suggest that MSC-based therapies are safe, at least in the time window of the follow-up of current clinical trials.

Clinical trials of msc transplantation in t1d and sle patients

Several clinical trials are testing MSCs transplantation in T1D[3,19] and in SLE patients. Table 1 and 2 present summaries of the MSC-based clinical trials for T1D and SLE registered in the *ClinicalTrials.gov* database (accessible at https://clinicaltrials.gov). Only few trials have been completed so far; here we will discuss the findings reported.

MSCs for Type 1 Diabetes

As mentioned before, T1D is a chronic autoimmune disease in which progressive loss of the beta cell mass leads to severe complications such as hyperglycaemia and ketoacidosis. Several genes confer an increased risk T1D and enigmatic environmental factors are believed to trigger disease development. A cellular-mediated immune response towards one or more beta cell autoantigens is thought to initiate the process leading to diabetes symptoms. Islet autoantibodies appear in the blood and can be detected as markers of β-cell autoimmunity years before clinical presentation[1]. Eventually, T1D manifests as a clinically overt disease when beta cell function and insulin secretion has become severely impaired. The relationship between beta cell function and beta cell mass at the time of diagnosis is poorly understood, but the old concept that 90% of the beta cell mass is lost at diagnosis is being challenged by emerging findings[24] showing that residual beta cell mass is much greater in many patients. The major hurdles for an effective treatment of T1D are: to halt the immune destruction of β-cells, preserve β-cell function and mass, and regenerate or replace beta cells[1]. Due to their properties, MSCs could help achieve these goals. Clinical trials have been performed to test the effect of MSCs transplantation at different stages of T1D (Table 1).

An open label pilot trial[25] enrolled recently diagnosed T1D patients from Sweden, age 18-40 years, within 3 weeks from diagnosis. Twenty patients were randomized to autologous bone marrow-derived MSC (BM-MSC) transplantation or to the control group which only received insulin therapy (Figure 1). The primary endpoint was safety, and the treatment was reported to be safe. The MSC therapy was associated with preservation of stimulated C-peptide secretion in most of the treated patients at one year. The C-peptide area under the curve ($AUC_{C\text{-}pep}$) and peak C-peptide were measured after a mixed meal tolerance test (MMTT) at 10 weeks and at 1 year after transplantation.

Table 1. Clinical trials of MSCs in type 1 diabetes. (data from https://clinicaltrials.gov)

Clinical Trial ID	Director and Country	Study Start Date	Estimated Completion Date	Phase	No	Study Design	Primary Outcome Measures	Route	Cell Source	Results
NCT01374854	Jianming Tan – China Completed	January 2009	December 2014	Phase 1 Phase 2	42	Allocation: randomized Masking: open label	C-peptide AUC during OGTT	ipa	UC-MSCs AutoBM-MNC	MSCs transplantation is safe and is associated with improvement of metabolic parameters in patients with established T1D
NCT01068951	Per-Ola Carlsson – Sweden Completed	June 2010	September 2013	Phase 1 Phase 2	20	Allocation: randomized Masking: open label	C-peptide AUC and peak C-peptide (MMTT)	iv	AutoBM-MSCs	MSC treatment is safe and moderately preserves β-cell function
NCT02745808‡	Jianwu Dai – China	September 2015	April 2017	Phase 1	30	Allocation: randomized Masking: single blind (subject)	Safety and Tolerability	ic	UC-MSCs	Not available
NCT01686139†	Itzhak Siev-Ner – Israel	March 2016	December 2017	Phase 1	12	Allocation: non -randomized Masking: open label	Frequency and severity of Adverse Events	is	AlloBM-MSCs	Not available
NCT00690066	Mesoblast International Sàrl – USA	June 2008	December 2011	Phase 2	63	Allocation: randomized Masking: double blind	C-peptide AUC response (MMTT)	iv	Ex-vivo cultured Human adult MSCs (Prochymal®) (tissue origin not specified)	Not available
NCT01219465	Wang, Yangang – China	September 2010	December 2012	Phase 1 Phase 2	50	Allocation: non-randomized Masking: open label	C-peptide release test	iv	UC-MSCs	Not available
NCT02057211	Per-Ola Carlsson – Sweden	February 2014	May 2017	Phase 2	50	Allocation: randomized Masking: double blind	C-peptide AUC (MMTT)	iv	Autologous MSC transplantation (tissue origin not specified)	Not available
NCT01143168	Cellonis Biotechnology Co. Ltd., Others – China	August 2010	December 2011	Phase 1	24	Allocation: non-randomized Masking: open label	Exogenous insulin requirement, HbA1c, FBG, postmeal blood glucose, C-peptide levels	ipa and iv	UC-MSCs AutoBM-MNCs	Not available

Table 1 *(Continued)*. Clinical trials of MSCs in type 1 diabetes. (data from https://clinicaltrials.gov)

Clinical Trial ID	Director and Country	Study Start Date	Estimated Completion Date	Phase	No	Study Design	Primary Outcome Measures	Route	Cell Source	Results
NCT00646724	Jianming Tan – China	January 2008	January 2014	Phase 1 Phase 2	30	Allocation: non-randomized Masking: open label	Exogenous insulin requirement, HbA1c, Glucose and C-peptide levels	co-pi	Autologous MSCs (tissue origin not specified)	Not available
NCT02763423	Dalong Zhu – China	January 2009	December 2019	Phase 2	30	Allocation: non-randomized Masking: open label	Exogenous insulin requirement in severe T1D patients with ketoacidosis	iv	UC-MSCs	Not available
NCT01157403	Chen Bing – China	July 2010	August 2014	Phase 2 Phase 3	80	Allocation: non-randomized Masking: double blind	C-peptide release test	iv	AutoBM-MSCs	Not available
NCT01322789	Carlos E Couri – Brazil	September 2008	December 2015	Phase 1 Phase 2	10	Allocation: non-randomized Masking: open label	C-peptide AUC (MMTT)	iv	AutoBM-MSCs	Not available
NCT01496339	Charile Xiang – China	January 2012	May 2014	Phase 1 Phase 2	50	Allocation: non-randomized Masking: open label	HbA1c	iv or ipa	MenSCs	Not available
NCT02579148‡	Jianwu Dai – China	September 2015	April 2017	Phase 1	0	Allocation: randomized	Masking: single blind (subject) Improvements in IIEF scores (erectile function in T1D patients)	ic	UC-MSCs	Not available
NCT01967186	Kaija Salmela – Finland	April 2007	July 2016	Undis-closed	36	Allocation: randomized Masking: open label	C-peptide (MMTT), Percentage of patients in each study group reaching a systemic C-peptide derived from the MMTT above 0.1 nmol/L basal (fasting) and 90-min 0.3 nmol/L 75 (+/-5) and 365 days (+/-14) after kidney transplantation	co-pi-k	Autologous MSCs (tissue origin not specified)	Not available

Table 2. Clinical trials of MSCs in systemic lupus erythematosus. (data from https://clinicaltrials.gov)

Clinical Trial ID	Director and Country	Study Start Date	Estimated Completion Date	Phase	No	Study Design	Primary Outcome Measures	Route	Cell Source	Results
NCT00698191	Lingyun Sun – China Completed	March 2007	December 2012	Phase 1 Phase 2	16	Allocation: non-randomized Masking: open label	Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), Lupus serology (ANA, dsDNA, C3, C4), Renal function (GFR, BUN, urinalysis)	iv	AlloBM-MSCs	Amelioration of disease activity, serologic parameters and renal function
NCT01741857	Lingyun Sun – China Completed	January 2012	December 2013	Phase 1 Phase 2	40	Allocation: non-randomized Masking: open label	British Isles Lupus Assessment Group score (BILAG)	iv	UC-MSCs	Amelioration of disease activity, serologic parameters and systemic manifestations of the disease
NCT02633163	Gary S. Gilkeson, Diane L. Kamen – USA	July 2016	June 2021	Phase 2	81	Allocation: randomized Masking: double blind	Systemic Lupus Erythematosus Responder Index (SRI)	iv	UC-MSCs	Not available
NCT00659217	Jianming Tan – China	May 2008	May 2010	Phase 1 Phase 2	20	Allocation: non-randomized Masking: open label	Percentage of patients achieving and maintaining remission	iv	AutoBM-MSCs	Not available
NCT01539902	DanQi Deng – China	February 2012	May 2013	Phase 2	25	Allocation: randomized Masking: double blind	Efficacy (improvement of renal function, proteinuria, urinary RBCs) and Safety	iv	UC-MSCs	Not available

KEY: ‡these studies aim to improve erectile dysfunction in type 1 diabetes patients; †this study is focused on treatment of diabetic foot ulcers.

iv: intravenous infusion; ipa: pancreatic artery; ic: intracavernous injection; is: third distal part of shin; co-pi: Co-transplantation of pancreatic islets and MSCs; co-pi-k: Co-transplantation of pancreatic islets, kidney and MSCs.

Acronyms: MSCs: Mesenchymal Stem Cells; UC-MSCs: Umbilical Cord-derived Mesenchymal Stem Cells; AutoBM-MNCs: Autologous Bone Marrow-derived Mononuclear Cells; AlloBM-MSCs: Allogenic Bone Marrow Derived Mesenchymal Stem Cells; AutoBM-MSCs: Autologous Bone Marrow-derived Mesenchymal Stromal Cells; MenSCs: Human Menstrual Blood-derived Mesenchymal Stem Cells.

AUC: Area under the curve; OGTT: Oral Glucose Tolerance Test; MMTT: Mixed Meal Tolerance Test; HbA1c: Hemoglobin A1c, Glycated hemoglobin; FBG: Fasting blood glucose; IIEF: International Index of Erectile Function; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; GFR: Glomerular Filtration Rate; BUN: Blood Urea Nitrogen; RBCs: Red Blood cells.

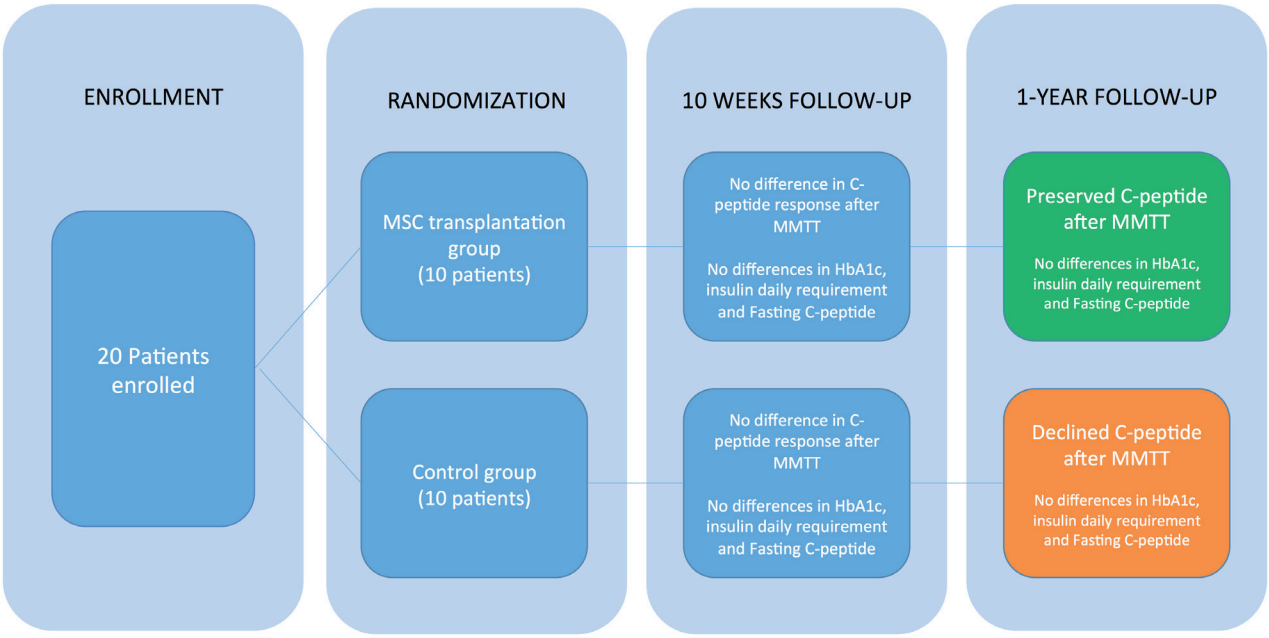


Figure 1. Carlsson et al (Diabetes 2015)[25] study chart.

Remarkably, the investigators observed that the average C-peptide release did not decrease in MSC recipients, whereas they observed a decline in C-peptide levels in control patients (mean decrease of 13% in the $AUC_{C\text{-}pep}$) during the 1-year follow-up. Both the control and the treated groups required insulin therapy and there were no statistically significant differences in insulin requirements and HbA1c levels between the two groups. There were no differences in the frequency of GAD65 and IA2 antibodies throughout the study.

Another trial was conducted in China[26]. The study enrolled 42 patients, aged 18-40 years, who had diabetes for an average of 8.12 years (range 2-16 years), who would be expected to have much more severe beta cell loss compared to newly diagnosed patients. Patients were randomized to receive cell transplantation or standard diabetes care. The treated patients received co-transplantation of allogeneic umbilical cord-derived MSCs and autologous bone marrow mononuclear cells (Figure 2). The rationale for this study was that such cell transplantation could stimulate the recovery of the beta cell mass, or even contribute to the beta cell mass with *de-novo* differentiation. Endpoints of this Phase I/II trial were safety and efficacy assessed by stimulated C-peptide, insulin requirements and HbA1c levels. The therapy was reported safe and resulted in a moderate improvement of the beta cell function and of metabolic parameters. At the one-year endpoint, the stimulated $AUC_{C\text{-}pep}$ during an oral glucose tolerance test was increased by 105.7% in cell transplant recipients compared to baseline; in contrast, the control patients experienced a 7.7% decline. Moreover, the HbA1c decreased by 12.6% in the treated group whereas it increased by 1.2% in the control group. Fasting blood glucose levels decreased significantly in transplant recipients (24.4% decrease at 1 year after treatment), whereas it remained substantially unchanged in the control group. A change in the cytokine profile was observed in transplanted patients, including increased levels of IL-10, decreased levels of IFN-gamma and lower ATP production by CD4+ T cells. This pattern suggests that cell therapy exerted immunomodulatory effects. It is noteworthy that such improvements were achieved in patients with established T1D with fasting C-peptide <0.1 pmol/ml at entry. Considering that such patients would likely have a severely reduced beta cell mass after many years since diagnosis, the improvement reported would suggest some effect of the therapy on beta cell mass, perhaps through differentiation of the transplanted cells into new beta cells and through expansion of the residual beta cells. Given the design of the trial, these remain open questions and the individual actions of BM-MNCs and UC-MSCs could not be determined.

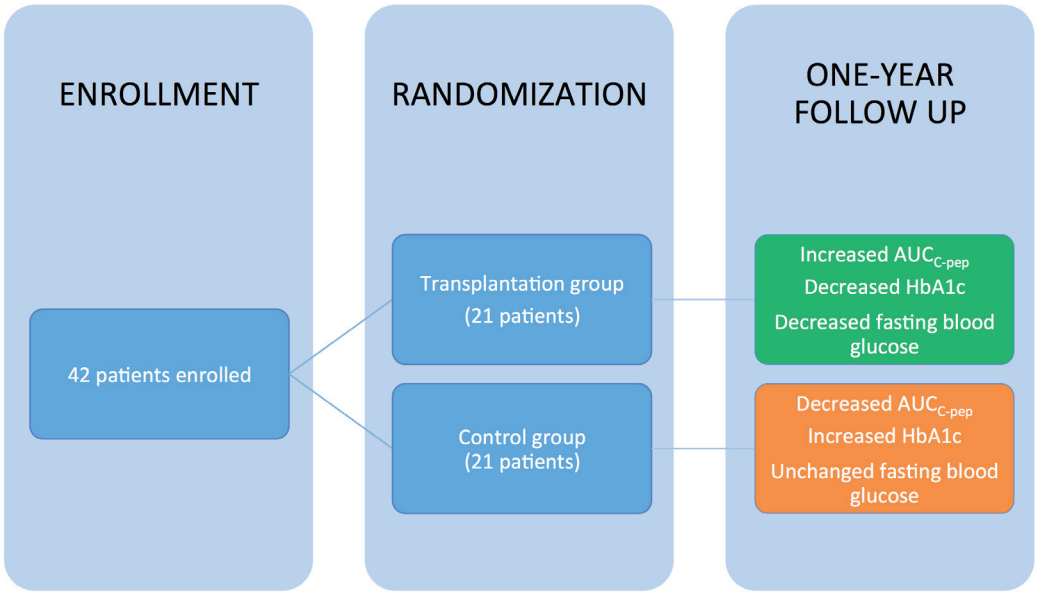


Figure 2. Cai et al (Diabetes Care 2016)[26] study chart.

The positive outcome of these trials encourages larger studies of MSC transplantation involving both newly diagnosed and long-standing T1D patients.

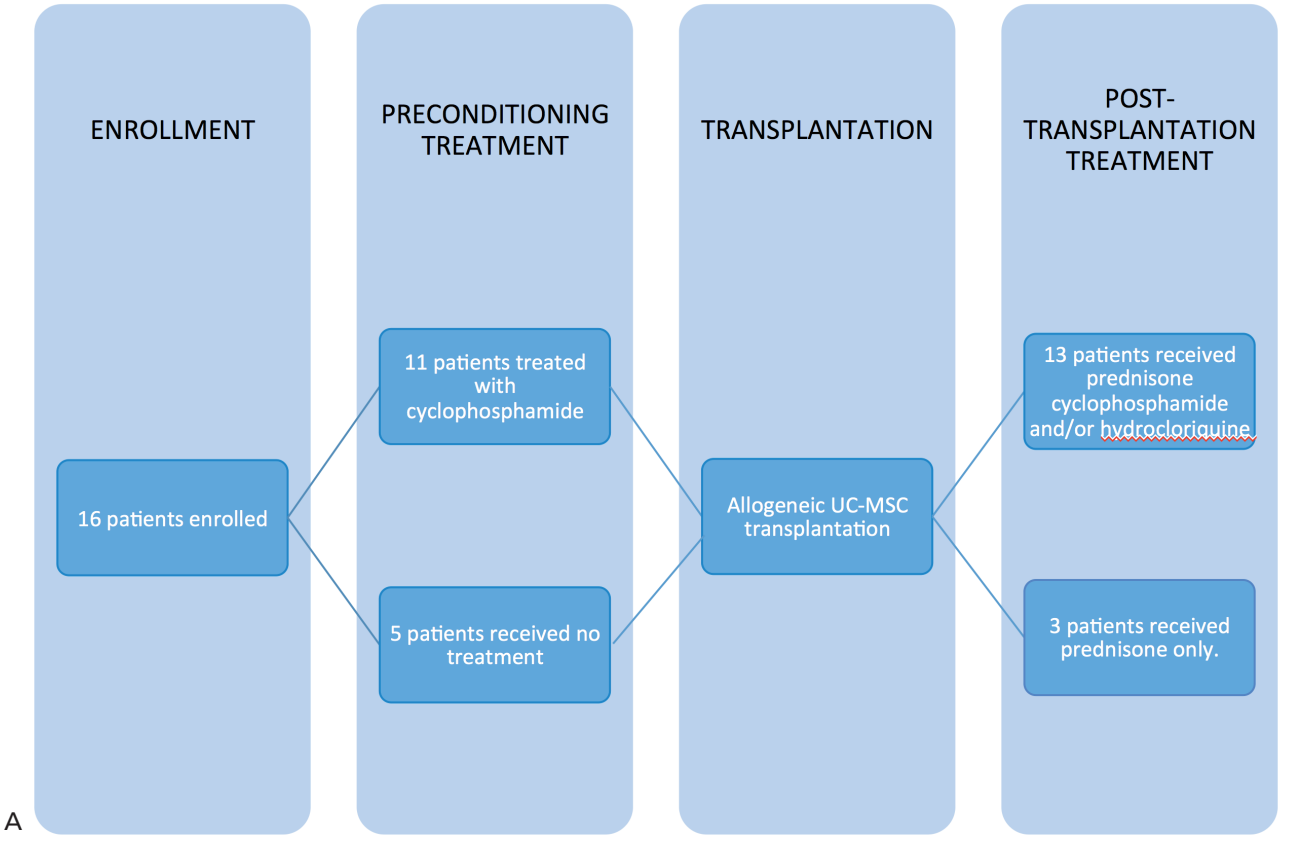
MSCs for Systemic Lupus Erythematosus

Systemic Lupus Erythematosus (SLE) is a multifactorial disease with a strong autoimmune component: genetic susceptibility and various environmental factors participate in the development of a disease which has several clinical manifestations. Genome-wide association studies showed that many genetic loci predispose to SLE. Specifically, defects in apoptotic clearance are believed to be of paramount importance: a lack of functionality of phagocytes leads to persistent exposure of apoptotic antigens and, eventually, to the capture of nuclear antigen fragments by antigen presenting cells, presentation to T and B cells and activation of autoimmune responses. Among environmental factors, UV light is deemed to be a major trigger of SLE. Other factors include cigarette smoking, infections, vitamin D deficiency, exogenous oestrogen and various biological agents[2].

Immunosuppression is the current gold standard treatment for SLE. Nevertheless, severe side effects and drug-resistance associated with aggressive forms of this disease contribute to high morbidity and mortality in SLE patients.

MSC therapy has been tested for the treatment of SLE, with a focus on patients who do not respond to conventional drug treatment (Table 2). In recent years Lingyun Sun and colleagues have administered MSCs of different origin in a series of single arm open label clinical trials[6,15,21,22] without a control group, which limits the interpretation of the results. They first attempted to transplant allogeneic bone marrow-derived MSCs in 4 patients (age 16-23) and reported no malignancies, infections, pulmonary or cardiovascular insufficiency, or metabolic disturbances[15]. As a secondary outcome, patients showed a net amelioration of the SLE disease activity index (SLEDAI), an improvement in kidney function during the 12-18 months of follow-up and an increase in the complement protein C3 level at 1-month post transplantation. The observation that BM-MSCs derived from SLE patients were impaired, possibly participating to the development of the disease, encouraged the group to explore allogeneic transplantation[15].

Subsequently, Sun and colleagues assessed the therapeutic effect of allogeneic umbilical cord-derived MSCs (UC-MSCs from Wharton's jelly) in severe and treatment-refractory SLE[6] (Figure 3). Sixteen patients were enrolled and underwent UC-MSC transplantation; 11 patients received a preconditioning treatment with cyclophosphamide, whereas the remaining patients did not receive preconditioning due to poor medical conditions or myelosuppression. After allogeneic UC-MSCs transplantation, all patients received prednisone and this drug was tapered off during the month following transplantation.



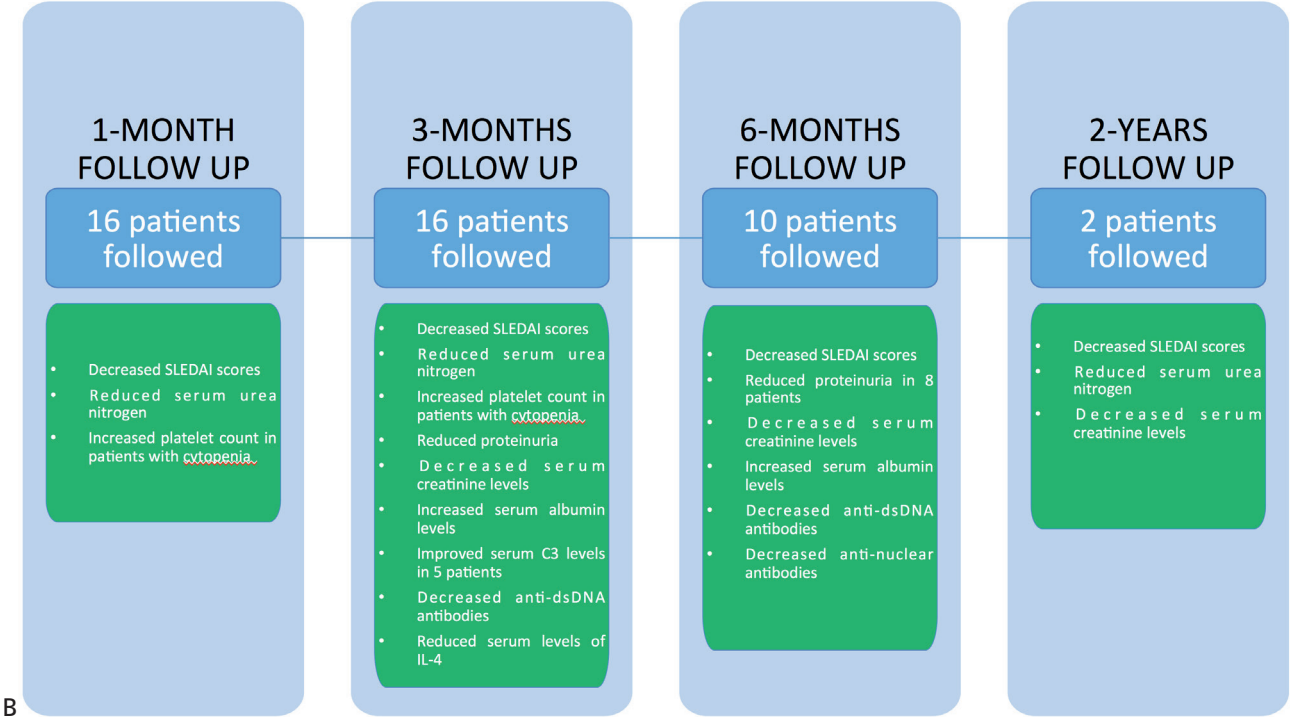


Figure 3. ***A,*** Sun et al (Arthritis Rheum 2010)6 study chart. ***B,*** Sun et al (Arthritis Rheum 2010)[6] study chart, part 2.

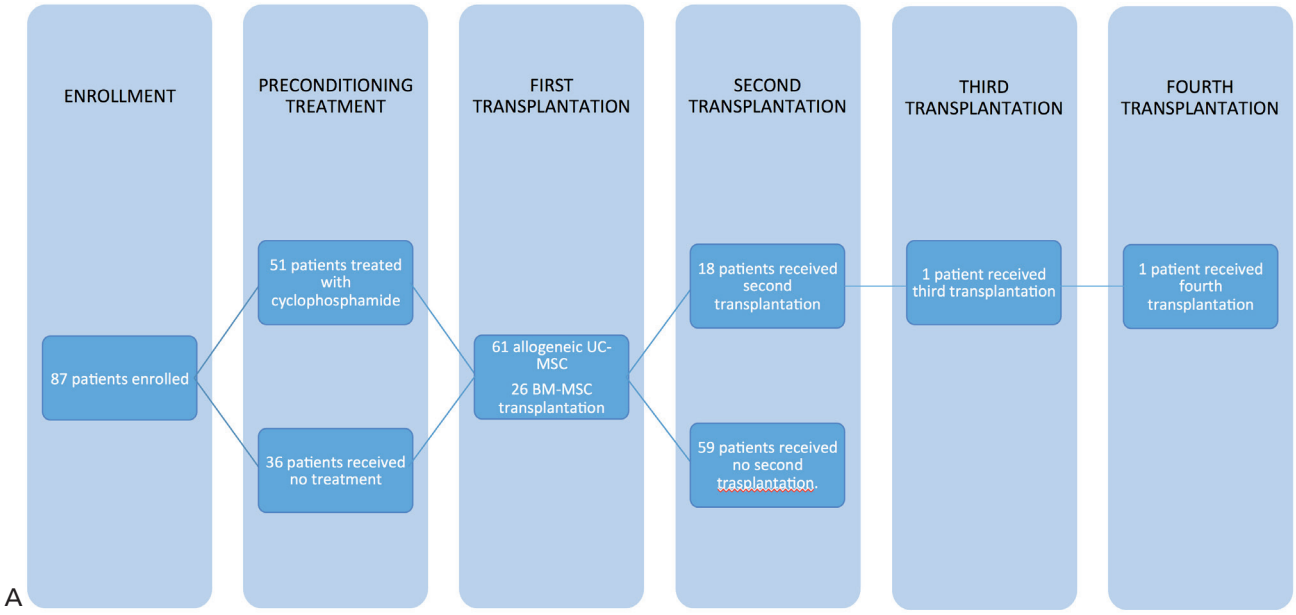
Thirteen patients also received cyclophosphamide and/or hydroxychloroquine post-transplant. All patients were followed-up at 1 and 3 months, 10 patients were followed-up for more than 6 months and 2 were followed for more than 2 years. The average SLEDAI score was 18.4 before UC-MSC transplantation. At 1 month after transplantation the SLEDAI scores decreased to an average of 10.8, and kept decreasing during the following months, averaging 7.9 at 3 months. For the patients who were followed up more than 6 months the SLEDAI kept decreasing. The two patients who were followed >2 years maintained SLEDAI scores below 4, suggesting a long-term positive effect. Proteinuria improved and became negative at 1 year, serum albumin levels reached levels close to normal at 6 months, complement protein C3 increased, anti-dsDNA antibody and anti-nucleus antibodies (ANA) decreased significantly. The balance between Th1 and Th2 response seemed to be restored, as IL-4 levels dropped. Neurological complications did not recur and hypertension was maintained within satisfactory values in these patients. Of significant importance is the increase of the percentage of CD4+ FoxP3+ T-reg cells, which strengthens the hypothesis that administering MSCs is a useful immunomodulatory approach for the treatment of autoimmune diseases. Augmented concentrations of TFG-beta were observed at 3 and 6 months, while no relevant changes were observed in the concentration of IL-10. Notably, no differences were detected during the follow-up between the cyclophosphamide pre-conditioned and the unconditioned cohorts, suggesting that the treatment effect derived mainly from MSC transplantation. This study did not report serious side effects connected to MSC transplantation.

In their following study Sun et al[22] administered allogeneic UC-MSCs and/or allogeneic BM-MSCs in a larger cohort of patients, achieving a more than satisfying scale of remission (Figure 4). Eighty-seven patients with a disease resistant to conventional immunosuppressive drugs (i.e. cyclophosphamide, mycophenolate mofetil, azathioprine, leflunomide) were recruited; 51 patients (59%) received a preconditioning treatment of cyclophosphamide, whereas 36 patients (41%) did not. After the first infusion, 18 (21%) patients showed no response to therapy or had a relapse. This group of patients underwent subsequent infusions of MSCs, with no CYC pre-treatment. 16 patients received two transplants, one patient received three transplants and one received four (Figure 4A). Apparently, no specific criteria were followed in the administration of either only UC-MSCs or UC-MSCs in combination with BM-MSCs.

After transplantation, 28% of recipients achieved clinical remission at 1 year (23/83), 31% at 2 years (12/39), 42% at 3 years (5/12), and 50% at 4 years (3/6). The overall rate of relapse was 23% (20/87). The rate of survival was 94% (82/87) as 6% (5 patients) died after complications of SLE. These complications were considered to be unrelated to the MSCs transplantation, and were reported as: gastroenteritis and heart failure (3 months post-transplantation), n=1; disseminated lung infection and uncontrolled Lupus Nephritis (6 months post-transplantation), n=1; lupus relapse with pulmonary hypertension and heart failure (8 months post-transplantation), n=1; pulmonary embolism (9 months post-transplantation), n=1; uncontrolled progressive disease and acute heart failure (1 week post-transplantation), n=1. With a mean follow-up of 27-months, this clinical trial suggests that MSC transplantation is overall safe in SLE patients. There are reasons to believe that MSC transplantation also has efficacy in controlling symptoms of SLE (Figure 3B, 4B). Nevertheless, a recent study by the same group suggests that repeated infusions of MSCs are necessary to avoid recurrence of SLE[20].

Proposed scheme for clinical trials

Randomized and double-blind controlled studies are expected in the near future, but may present ethical issues in the setting of autologous transplantation. The procedure of cellular isolation is invasive and may affect the clinical course of the disease in some patients. In a double-blind study aimed at testing the efficacy of autologous BM-MSC transplantation, bone marrow cells could be aspirated in all patients, but would be transplanted only in the MSC transplant group. In such case, we propose to design a clinical trial as shown in Figure 5. In the proposed scheme, participants are initially randomized either to the MSC treatment group, or to the Control group. All participants undergo cellular aspiration and MSC isolation. The samples from the Control group are cryopreserved, while the ones from the Treatment group are cultured for transplantation. Subsequently, the treatment group receives cell transplantation, whilst the control



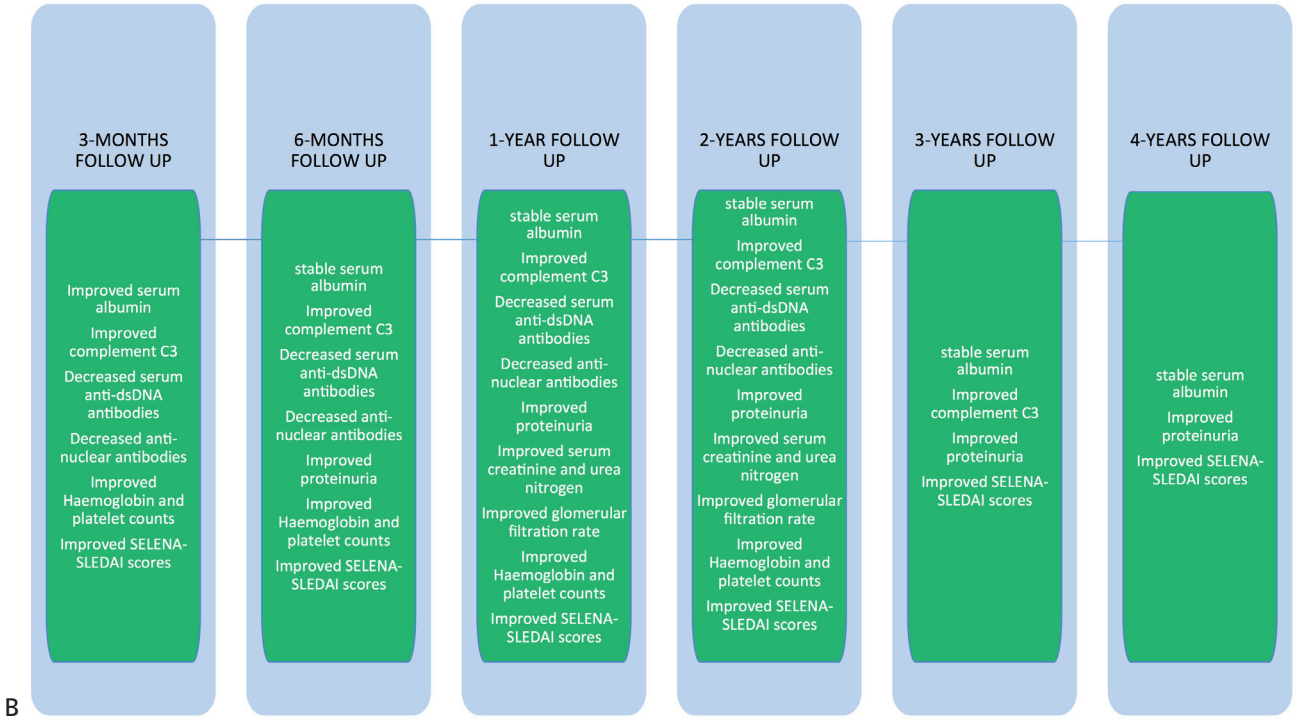


Figure 4. ***A,*** Wang et al (Cell Transplant 2013)[22] study chart. ***B,*** Wang et al (Cell Transplant 2013)[22] study chart, part 2.

group receives placebo. If the treatment is deemed safe and shows efficacy after a long-term follow-up (1-2 years), the option of transplantation can be extended to participants in the control group, who would receive cryopreserved MSC. By following this scheme, the efficacy of autologous BM-MSC transplantation would be assessed more clearly, and the potency of cryopreserved and non-cryopreserved cells could be compared.

Conclusions

The safety of MSCs transplantation was corroborated in clinical trials in T1D[25,26] and SLE patients[6,21,22]. In both conditions, various regimens involving MSCs showed some therapeutic efficacy. However, it must be noted that unlike the T1D trials, the studies in SLE patients did not include control groups[6,15,20,22]. None of these studies administered placebo[6,15,20-22,25,26]. Therefore, results

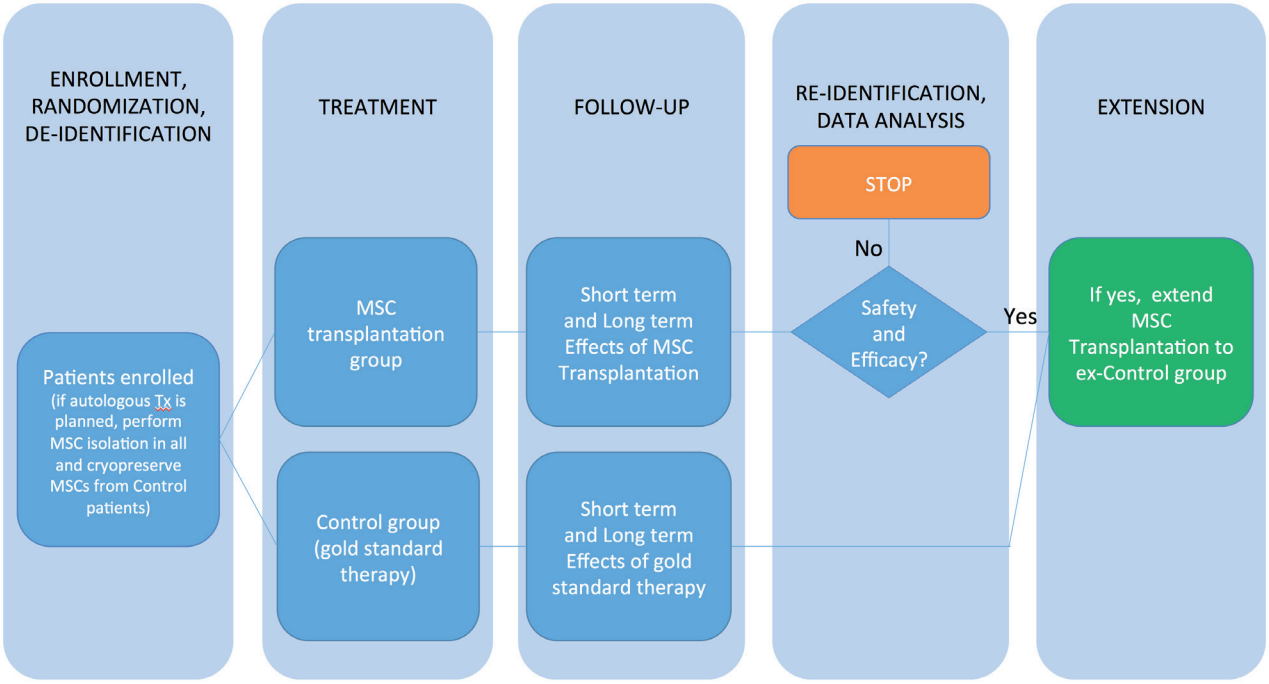


Figure 5. Proposed scheme for clinical trials.

should be ideally reproduced in randomized, placebo-controlled trials. Some of the approaches made use of cells from different sources, transplanted in the same recipients: allogeneic UC-MSCs and autologous BM-MNCs in T1D patients[26], allogeneic UC-MSCs and allogeneic BM-MSCs in SLE patients[22]. In those studies, the cell populations and biological mechanisms responsible for the observed effects were not clearly identified. Thus, critical questions about the therapeutic mechanisms remain, some of which may not be fully addressed in patients because of limitations in access to tissue. Overall, given the encouraging results, it is expected that controlled trials in the future will provide a more rigorous assessment of the efficacy of MSC transplantation in autoimmune diseases, which will guide further the development of clinical applications for MSC transplantation.

Conflict of Interests:

The Authors declare that they have no conflict of interests.

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