Chapter

# Clinical Use of Mesenchymal Stem Cells in Treatment of Systemic Lupus Erythematosus

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## Abstract

Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune inflammatory disorder with considerable clinical heterogeneity and a prevalence of 26 to 52 out of 100,000. In autoimmune diseases, such as SLE, the immune system loses its ability to distinguish between self and other. Treatment of SLE is challenging because of clinical heterogeneity and unpredictable disease flares. Currently available treatments, such as corticosteroids, cyclophosphamide (CYC), and other immunosuppressive or immunomodulating agents, can control most lupus flares but a definitive cure is rarely achieved. Moreover, standard therapies are associated with severe side effects, including susceptibility to infections, ovarian failure, and secondary malignancy. Alternative therapeutic options that are more efficacious with fewer side effects are needed to improve long-term outcome. Mesenchymal stem cells/multipotent stromal cells (MSCs), which secrete immunomodulatory factors that help restore immune balance, could hold promise for treating these diseases. Because MSCs do not express major histocompatibility complex II (MHC-II) or costimulatory molecules, they are also "immunologically privileged" and less likely to be rejected after transplant. Stem cells are defined as a class of undifferentiated cells in multicellular organisms that are pluripotent and self-replicating. MSCs are promising in regenerative medicine and cell-based therapies due to their abilities of their self-renewal and multilineage differentiation potential. Most importantly, MSCs have immunoregulatory effects on multiple immune system cells. While some studies report safety and efficacy of allogeneic bone marrow and/or umbilical cord MSC transplantation (MSCT) in patients with severe and drug-refractory systemic lupus erythematosus (SLE), others found no apparent additional effect over and above standard immunosuppression. The purpose of this chapter is to discuss immune modulation effects of MSCs and the efficacy of MSCs treatments in SLE.

**Keywords:** Mesenchymal stem cell, Cell therapy, Systemic Lupus Erythematosus, Clinical trials, Lupus nephritis

## 1. Introduction

Systemic Lupus Erythematosus (SLE) is a chronic multi system autoimmune inflammatory disease in which vascular inflammation cause devastating organ damage such as end-stage renal disease (ESRD). Sizeable patient populations;

12,600 end-stage kidney disease (ESKD) caused by SLE, are refractory for all current standard of care [1].

Clinical presentations of SLE, prototype autoimmune disease for interferon activation, are highly heterogeneous, ranging from mild systemic inflammation that affects skin or joints to severe organ damage (brain, kidney, lung etc.). Heterogeneity of clinical presentations requires diverse treatment protocols, addressing multiple immune abnormalities affecting variety of organs. The exact etiology of SLE is not completely understood. Pathogenesis of SLE comprises genetic, environmental, and hormonal factors which induces multiple immune cell lines and systems act abnormally which are mostly explained by autoimmune activation. All etiopathogenic immune pathways targeted with chemotherapy or biologics to date have failed to improve some portion of SLE patients. Heterogeneity of clinical presentations require diverse treatment protocols, addressing immune abnormalities.

There is an urgent clinical need for an effective treatment of chronic autoimmune diseases induced by abnormal activation of immune system that result in multiple organ damage in SLE and in others [1–3]. The current standard of care includes high dose corticosteroids, chemotheraphy with azathioprine, cyclophosphamide, mycophenolate mofetil, cyclosporin, and combination of all with biologics such as rituximab (Anti-CD20) or belimumab (anti-Blsy) [4, 5]. Current modalities that are available to treat SLE and SLE like diseases are immune suppressive and have toxic side effects. After treatments with corticosteroids and chemotherapy, patients become even more vulnerable to pathogens and develop sepsis and septic shock. In many patients, even combinations of all available medications are not effective in controlling the disease progression and development of end stage organ failure. Innovation of nontoxic cellular therapies that target both, the vascular wall and the immune responses within the local microenvironment, are needed.

In many patients, even combinations of all available medications are not effective in controlling the disease progression and development of end stage organ failure. Collectively, at least 10–15% of patients fail to respond to all existing treatments. Specifically, three groups of SLE patients with the greatest unmet need include:

1. 7–8% of patients who have severe nervous system involvement refractory to cytotoxic and immune suppressing medications [6];

2. 10–30% patients with severe nephritis who do not respond to cytotoxic and immune suppressing therapy or available biologic treatment (such as belimumab and rituximab) and become dependent on dialysis leading to death within 15 years [1]; and

3. 2–5% of patients develop thrombotic thrombocytopenic purpura who do not respond to combination of cytotoxic medications, immune suppressants, plasma exchange, and biologics, with mortality rate of 34–62% [7].

Disease burden of SLE and lupus nephritis in the US is estimated at 313,436 (100/100,000) and 63,256 (20/100,000), respectively [8–10]. Approximately 10 to 20 percent of patients with lupus nephritis progress to end-stage renal disease as they do not respond to commercially available treatments.

Unfortunately, there is still no uniformly effective treatment targeting both cellular and humoral autoimmunity for SLE. Therapies targeting components of cellular or humoral immune system fails to induce sustained remission in disease activity in multicenter clinical trials. To design a new treatment that can control the cellular

and innate immune activation and regenerate the damaged organs in active SLE, the understanding of the degree and exact kind of the immune dysregulation is necessary. Multiple immune cells and immune signaling pathways have been studied in etiopathogenesis of SLE and have been found to act abnormally. While a set of cells clonally expand and act abnormally, we see some of the cells that have homeostatic roles in controlling self-tolerance are diminished or dysfunctional in SLE.

## 2. Immune dysregulation that leads to SLE

Pathogenesis of SLE comprises genetic, environmental, and hormonal factors resulting in multi-system autoimmune inflammatory disease. **Systemic Lupus Erythematosus** [11] is suggested to be the prototype of several systemic inflammatory diseases that are induced by abnormal activation of the type I (−α, −β) [12] and II (−γ) interferon (IFN) [13] pathways. Interferon activation results in multiple immune cellular abnormalities, including; dendritic cells (DC), natural killer (NK) cells, cytotoxic T cells, T regulatory cells (Tregs), and autoreactive B cells [14].

SLE is characterized by irregularities in innate cellular and humoral immunity functions [15]. Abnormal T-cells and B-cells recognize self-antigens resulting in immune hyperactivity and autoantibody production that ends up in a multisystem inflammatory disease.

Immune dysregulation in SLE has been described by not one but multiple cell lineages such as CD4+ and CD8+ T-cells, dendritic cells (DC), Natural Killer (NK) cells, B-cell overproduction of autoantibodies, and T regulatory (Treg) cell dysfunction. CD8+ T cells and NK cells have decreased cytotoxic activity. There is a general inability of TGF- β production, which in return accounts for sustained T and B cell hyperactivity and reduced Tregs activity and numbers. There is a disproportional balance between the activated and tolerogenic DCs during SLE activity that limits the expansion of Tregs [16]. The remaining small amount of Tregs that are still existing during the inflammatory activity of lupus are not sufficient to overcome the strong T-cell activation [17, 18].

In both human patients with SLE and in lupus prone mice models, CD4 + CD25 + Foxp3+ Tregs are reported to be decreased during disease activity. CD4+ T helper cell subset (Th17 cells) are increased in SLE in response to IL-17 activation [19, 20]. Blockage of IL-17 has also been suggested as a new treatment option [21, 22].

Restoration of T-cell functions are important for disease control. On the other hand, lupus-like autoimmunity can result simply due to B-cell hyperactivity, with either minimal or no contribution from T-lymphocytes. B cell hyperactivity results with production variety of IgG and IgM autoantibodies directed against nuclear components such as double stranded (ds) DNA and/or single stranded (ss) DNA. Both anti-ssDNA and anti-dsDNA are involved in disease pathogenesis and clinical progress [23, 24].

The type I interferon system appears to play a critical role in SLE etiopathology [11, 25–27]. All the cellular and humoral immune abnormalities seem to activate type I interferons, which in return charge the immune cells further and result in loss of tolerance. Type I interferons control dendritic cell maturation into antigen presenting cells which contribute to B-cell hyperactivity and induce a Th1 response and sustain T-cell activation [28, 29]. Type I interferons are not controlled well and are in excess amount partially due to deficiency of Treg activities in SLE [30–33].

Another major etiopathogenic immune pathway is explained by multiple complement pathway abnormalities. Complement deficiency can be seen up to 5% of all lupus patients [34]. In addition, 50% of SLE patients with deficiencies or dysfunction of the early classical complement pathway develop a lupus-like disease.

## 3. MSC treatment in SLE

While there is systemic inflammation and autoimmunity ongoing, patients with SLE have less active immune cells that defend against pathogens and tumors [35, 36]. Cytotoxic CD8+ T cells and T regulatory (Treg) cells that play fundamental role in immune defense are depleted during SLE activity [37].

Currently available treatments of SLE (Systemic Lupus Erythematosus) target one cell (CD20+ B cells) or one pathway at a time leaving the others to continue to function abnormally and their immunosuppressant side effects to diminish patients' ability to fight infections. After these treatments, patients become immune compromised and vulnerable to pathogens and develop sepsis and septic shock. In many patients, even combinations of all are not effective in controlling disease progression sometimes developing end stage organ failure.

MSCs are multipotent stromal cells than have the potential to differentiate into multiple mesenchymal lineages [38–43]. Core standardized definition of the 'multipotent mesenchymal stromal cell' as a plastic-adherent cell type bearing various stromal surface makers, but lacking hematopoietic markers, capable of at least osteogenic, chondrogenic and adipogenic differentiation was proposed by a consensus group [44]. The name was later modified and was changed to 'mesenchymal stromal cell'. No unique marker exists to define MSCs still and clinical studies will certainly involve different heterogeneous MSCs that can be isolated from different adult and fetal tissues such bone marrow (BM), umbilical cord (UC) and adipose tissue (AT). MSCs are so far defined with the presence of their characteristic cell surface markers such as CD105, CD90, CD73, CD106, CD146, CD166, CD271 and the absence of hematopoietic progenitor cells markers such as CD45, CD34 and CD14. They are uniquely immune privileged and can escape rejection reactions from hosts since they do not express class II MHC, such as HLA-DR and co-stimulatory molecules such as CD80, CD86 and CD40 [43, 45, 46]. Therefore, they are easily used as adoptive transfer cell treatment without any prior immune ablation therapies.

Besides their differentiation potentials, MSCs have potent immune regulatory effects. MSCs mediate immune system either by secreting soluble factors or directly interacting with a variety of immune effector cells. MSCs uniquely gain different properties and immunoregulatory effects depending on the inflammatory milieu and disease setting. MSCs secrete numerous cytokines, chemokines, and hormones to exert paracrine effects on adjacent immune cells to modulate their proliferation, differentiation, migration, and adhesion functions under injury conditions.

It has been suggested that with their potent immune regulatory effects MSCs are future of cell therapy in refractory lupus. However, the studies thus far published do not agree on the kind, amount and frequency of MSC treatments or showed consistent efficacy. MSCs have not been FDA approved for any disease indication, mostly due to challenges in potency. MSCs have been used as therapeutics in hundreds of clinical trials, including SLE, with no adverse reactions reported.

## 4. Immune modulating effects of MSCs that may help suppressing auto inflammatory activity during SLE

MSCs produce a collection of immune modulating molecules, which can locally (paracrine) or systemically (endocrine) effect inflammation. The actions of MSCs are dependent on the environmental signals they receive and are directed to control the excess inflammatory response. It is well studies that MSCs can switch the T cell

balance from a pro-inflammatory Th1 phenotype (secreting INF-γ and TNF-α) or Th17 phenotype (secreting IL-17) [47] to an anti-inflammatory to Th2 profile (secreting IL-4) (**Figure 1**) [48, 49].

More relevant MSC activities that may help in SLE treatment are 1) MSCs decrease IFN-γ production *in vitro* by T-cells [50] 2) MSCs are able to modulate the cytokine-production profile of (*in vivo*) differentiated Th17 cells, as well as the production of the IL-17 [51–53], 3) MSCs also influence the development and function of DCs [54, 55], 4) MSCs promote the generation of antigen-specific Tregs either directly or indirectly by modulating dendritic cells (DCs) [56], 5) MSCs modulate macrophages [57–60] 6) down-regulate the production of pro-inflammatory cytokines TNF-α, IL-1, IL-6 and IL-12p70 and increase the production of anti-inflammatory cytokine IL-10, 7) enhance the phagocytic activity which in return induce resolution of inflammation [61–63] (**Figure 1**).

MSCs can suppress proliferation of both CD4+ and CD8+ T lymphocytes in vitro in a dose-dependent, non-apoptotic-induced manner, and the immunosuppressive properties against T cells varies among different MSC sources. Transforming growth factor-β (TGF-β), prostaglandin E2 (PGE2), nitric oxide (NO), and indoleamine 2,3-dioxygenase (IDO) have been reported to be involved in the MSC-mediated T cell suppression. CD8+ T cells and their activation axis with Indolamine 2, 3-Dioxygenase (IDO) an important anti-inflammatory factor, is suggested to be required for successful suppression of SLE [64], and there is significant data showing the need to increase the Treg activity in SLE treatment (**Figure 1**) [51].

One key element of the possible effect of MSCs in SLE is that once MSCs enter the inflammatory environment particularly those SLE affected or injured organs;

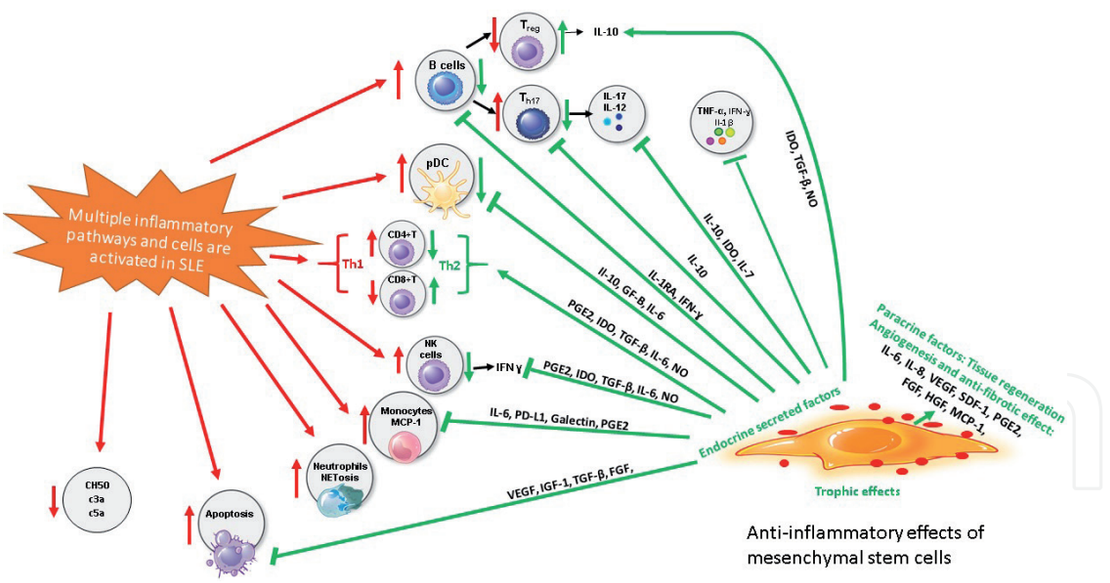


**Figure 1.**
*Suggested pathways of how anti-inflammatory effects of MSCs that control the loss of tolerance, cellular dysfunction and inflammation. During SLE active disease multiple immune cells that works in both innate and adaptive immune system are dysfunctional leading to loss of tolerance and sever inflammation. MSCs, can sense the inflammatory microenvironment and act on attenuating inflammatory activity by secreting soluble factors, such as IDO, TGF-β, PGE-2. VEGF, BMP-7, TNF-α, IL-6, IL-7and IL-10, i.e. endocrine effect. MSC exert the immunomodulatory function by promoting a switch from pro-inflammatory to anti-inflammatory phenotype and cytokine secretion by T- cells, dendritic cells and NK cells. MSCs can inhibit the proliferation and activation of B effector cells and CD4 + T lymphocytes, while changing and strengthening the cytotoxic effects of CD8 + T cells and NK cells. MSCs anti-inflammatory effects is also explained by its effect on increase of the Tregs, while its potent effect in decreasing the IL-17 secreting Th17 cells. Red arrows are showing the SLE inflammation activation signaling for pathogenic cellular expansion or decrease, while green arrows and blunted lines are showing the opposing effects of MSCs on the abnormal cellular activation and anti-inflammatory effects. MSCs endocrine secreted factors by which they are suggested to act specific cellular expansion and activity are defined on the arrows.*

their immune-modulatory phenotype could become activated by IFN-γ, TNF-α and IL-1β in the microenvironment [65]. Furthermore, it has been shown that MSCs are chemotactically drawn toward a variety of wound healing cytokines in vitro, including IL-1 and TNF-α. These data suggest that MSCs or endogenous cells resembling MSCs, such as pericytes, are likely to migrate to and participate in the response to tissue injury [66–69].

When MSCs are exposed to the microenvironment of diseased tissue, they control/suppress inflammation inducing regeneration [56]. With their potent immune regulatory and regenerative effects in response to their microenvironment, and as no adverse reactions in clinical trials have been reported, MSCs are an attractive treatment in SLE. By increasing the potency of MSCs in SLE, it is anticipated that primed MSCs will lower the overall cost of care for SLE patients that are refractory for the current standard of care.

Effects of human MSCs on interferon regulated mediators, and the connections of these mediators with clinical outcomes in SLE have been suggested, but MSC treatments have not been efficacious across heterogeneous organ involvement of SLE to date.

MSCs have been used as therapeutics in hundreds of clinical trials, as of July 2020, there were a total of 1,138 registered clinical trials to clinicaltrials.gov including SLE. In the 18 published clinical trials with outcomes there were no serious adverse events reported [70]. However, MSCs have not been FDA approved for any disease indication yet, mostly due to challenges in potency. MSC treatment has been shown to be successful for a short time and there were relapses in SLE patients in 6–12 months [71, 72].

MSC sources used in clinical trials have different donor pools and are isolated from different tissues with variable immune regulatory function. Furthermore, large-scale MSC-based cell therapy remains restricted due to the cells' ability to expand, and then efficiently respond to inflammatory environment after several number of passages.

## 5. Recent SLE clinical trials using stem cells

Stem cell treatment to those SLE patients who have been refractory to all known therapies have been the last resort. Although the results of studies reported in early 2000 suggested that autologous stem cells treatment (ASCT) suggested the efficacy for remission induction of refractory SLE, mortality among those patients with longer disease duration was particularly high and mostly due to immune suppressive procedure (12%). Almost 30 percent patients relapsed after therapy and longer duration of immune suppressive therapies post ASCT was suggested [73, 74]. It was clearly shown that severe myeloablative therapies prior to ASCT's to SLE patients who already have immune compromised status the success rate has been poor. Therefore, other groups assessed the safety of intense immunosuppression and autologous hematopoietic stem cell support in patients with severe and treatment refractory SLE [75, 76]. Overall 5-year survival of those SLE patients was 84%, and probability of disease-free survival at 5 years following HSCT was 50% (**Table 1**).

While the initial stem cell clinical trials were being performed for treatment of SLE, first report of successful MSC treatment in a child with acute graft-versus-host disease (GvHD) using allogeneic MSCs was published in 2004 [89]. After two infusions of bone-marrow-derived MSCs obtained from his mother this child responded very well to the infusion treatment. Following the success of this pediatric case with GVHD, multiple preclinical animal studies and other human clinical trials for treatment of other autoimmune diseases started to take place. The initial

| Reference (First author, date) | Study type/ SLE, organ involvement | Number of patients studied, Age range | MSC source* | Type and amount (dose) | Prior treatment | Outcome criteria | Improvement (%) in 6 months | Improvement (%) in 12 m and above |
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| Jayne et al. (2004) [74] | Retrospective registry. SLE or nephritis | 53, (9–52 yo) | Peripheral blood (n = 44), bone marrow (n = 8), from both (n = 1) | Autologous stem cell treatment (ASCT) | Cyclophosphamide (84%), anti-thymocyte globulin (76%) and lymphoid irradiation (22%) | SLEDAI, brain MR scan, pulmonary function tests, echocardiogram, serum creatinine, ANA, anti-dsDNA, other anti-nuclear autoantibodies and C3, C4 | Remission rate (based on a reduction of the SLEDAI to <3) in 66%, one-third of whom later relapsed to some degree. | Mortality 12% at one year |
| Burt et al. (2006) [75] | Single arm trial. Severe fractory SLE | 50, Mean age (SD) 30(10.9) years | Peripheral blood | Autologous stem cell treatment (ASCT) | IV Cyc, 50 mg/kg daily, before transplantation (total dose 200 mg/kg) and intravenous equine ATG, 30 mg/kg daily, before transplantation (total dose 90 mg/kg). | Primary, survival, disease-free. Secondary end points included (SLEDAI), ANA and anti– (ds) DNA, C3 and C4, and changes in renal and pulmonary organ function assessed before treatment and at 6 months, 12 months, and then yearly for 5 years. | 2/50 patients died after mobilization 48 patients underwent HSCT. Treatment-related mortality was 2% (1/50). By intention to treat, treatment-related mortality was 4% (2/50). Renal function stabilized and improved SLEDAI, ANA, anti-ds DNA, complement, and carbon monoxide diffusion lung capacity adjusted for hemoglobin. | 5-year survival was 84% and probability of disease-free survival at 5 years following HSCT was 50%. |

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| Sun et al. (2010) [77] | Single arm SLE nephritis | 16 | UC MSC | Allogeneic | Cyclophosphamide iv for 2–4 days | Percent Tregs improved in 3 months | | Decreased SLEDAI and proteinuria in all patients in 28 months |
| Liang et al. (2010) [78] | Single arm SLE nephritis | 15 | | | | Percentage of Treg cells increased at 1 week and 3 and 6 months ($P < 0.05$) | | Decreased SLEDAI and proteinuria in all patients |
| Carrion F et al. (2010) [79] | SLE | 2 (19 yr., 25 yr) | BM-derived MSCs, | Autologous, $1 \times 10^6$/kg | | | Disease activity indexes and immunological parameters were assessed at baseline, 1, 2, 7 and 14 weeks | |
| Shi D et al. (2012) [80] | SLE associated diffuse alveolar hemorrhage. | 4 (32 ± 15 years) | UC-MSCT | Allogenic | $1 \times 10^6$/kg | hemoglobin, platelet level, oxygen saturation, and serological factors. High-resolution CT (HRCT) scans of the chest were performed to evaluate pulmonary manifestation | Clinical changes before and after transplantation | |

| Reference (First author, date) | Study type/ SLE, organ involvement | Number of patients studied, Age range | MSC source* | Type and amount (dose) | Prior treatment | Outcome criteria | Improvement (%) in 6 months | Improvement (%) in 12 m and above |
|---|---|---|---|---|---|---|---|---|
| Wang et al. (2012) [81] | Unblinded, randomized, 2-arm/12 months | 58 | BM/UC MSC single vs. 2× every 7 days | | CYC 10 mg/kg per day, day 4, 3, and 2 | | | Complete remission 1× 53% 2× 29% |
| X Li et al. (2013) [82] | SLE refractory cytopenia | 35(16–62 years) | BM/UC MSC. | Allogenic $1 \times 10^6$/kg | 1 = Pretreatment group: (15/35) Cyc 0.4–1.8 gm IV for 2–4 days 2 = No Cyc Pretreatment (20/35) | CBC's Hb and Platelet, Th17, Treg, SLEDAI | 57% patients with leukopenia and 68% patients with thrombocytopenia showed hematological improvement. | 75% of SLE remained stable after 12 months |
| Wang et al. (2013) [83] | Severe and refractory SLE | 87(12–56 years) | BM/ UC-MSC | Allogenic $1 \times 10^6$/kg | Pretreatment 59% Cyc 10 mg/kg/day IV on day −4, −3, −2. 36% No treatment | Primary: Survival, disease remission and relapse, transplantation-related adverse events. Secondary: SLEDAI and serology | Complete clinical remission 28% at 1 year Relapse rates 12% at 1 year. | Complete clinical remission rate was 31% at 2 years (12/39), 42% at 3 years (5/12), and 50% at 4 years (3/6). 4-year follow-up overall rate of survival was 94% (82/87). |
| | | | | One-time treatment | | | | |

| Reference (First author, date) | Study type/ SLE, organ involvement | Number of patients studied, Age range | MSC source* | Type and amount (dose) | Prior treatment | Outcome criteria | Improvement (%) in 6 months | Improvement (%) in 12 m and above |
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| Fei Gu et al. (2014) [72] | Open Label and single center Active and refractory Lupus Nephritis | 81(12–55 years) | BM or UC-derived MSC | Allogenic $1 \times 10^6$/kg | No IV Cyc pretreatment. vs. Pretransplant medication: Pred/ Cyclophosphamide(monthly)/ MMF | Primary outcome: Renal remission (complete/ partial) as well as renal flares. The secondary outcome included renal activity score | The mean leukocyte counts still stayed normal for 5 patients completing 24-month follow-up | For 24 SLE patients with anemia, normalized remained stable at 12- and 24-month visits |
| Wang et al. (2014) [71] | Severe and refractory SLE | 40, (17–54 years) | UC-MSC | Allogenic | No IV Cyc pretreatment. 26/40 pts. received Cyc as a basal treatment. | Safety, Major clinical response (MCR), Partial clinical response (PCR) and relapse. SLEDAI, BILAG and renal functional indices | Disease relapse at 9 months 12.5%, at 12 months 16.7% of follow-up. | Survival rate was 92.5% in 12 months. |
| | | | | $1 \times 10^6$/kg at 0 and 7 days | | | | |
| | | | | | | | 32.5% achieved MCR and 27.5% achieved PCR, during 12 months. | |

| Reference (First author, date) | Study type/ SLE, organ involvement | Number of patients studied, Age range | MSC source* | Type and amount (dose) | Prior treatment | Outcome criteria | Improvement (%) in 6 months | Improvement (%) in 12 m and above |
|---|---|---|---|---|---|---|---|---|
| Deng et al. (2017) [84] | Randomized, double blind, placebo controlled SLE nephritis | 18 patients Randomized. 12 patients h UC-MSC group and 6 patients placebo group. Mean age in both groups 29 years. | UC-MSC | Allogenic | 11/18 pts. received methylprednisolone and CYP induction therapy, and the 12th to 18th patients enrolled received IV. methylprednisolone only and intravenous CYP | 24 h urine protein, serum albumin, serum creatinine, SLEDAI and BILAG scores, C3, C4, anti-dsDNA and ANA | Remission occurred in 75% in the hUC-MSC group and 83% in the placebo group. | Stopped in less than 12 months due to lack of efficacy |
| | | | | $20 \times 10^6$/ patient one time | | | | |
| Chen C et al. (2017) [85] | Active SLE refractory to conventional treatment | 10 | UC-MSCT | $1 \times 10^6$/kg | | Soluble human leukocyte antigen G was measured 24 h and 1 mo after infusion | Negative correlation between s HLA-G levels and SLEDAI score. | |
| Wang et al. (2018) [86] | Open-label phase II Severe and drug refractory SLE | 81(12–62 years) | BM or UC-MSC | Allogeneic $1 \times 10^6$/kg (Multiple infusions of MSCs were permitted) | 39/81 received IV Cyc (10 mg/kg/day) in days −4, −3, −2; 42/81- no IV Cyc. | 5-year overall survival. Complete and partial clinical remission. | | 5-year overall survival rate was 84%. |
| | | | | | Patients receiving repeat MSCT, no IV Cyc used. | | | |

| Reference (First author, date) | Study type/ SLE, organ involvement | Number of patients studied, Age range | MSC source* | Type and amount (dose) | Prior treatment | Outcome criteria | Improvement (%) in 6 months | Improvement (%) in 12 m and above |
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| J Barbado et al. (2018) [87] | Active SLE with proteinuria (1,000 mg in 24 h) and class IV proliferative nephritis | 3(40–45years) | BM-MSC | Allogenic $1.5 \times 10^6$/kg | Patients were pretreated with variety of chemotherapy before enrollment to the study | The 24-h proteinuria level, glomerular hematuria, leukocyturia, serum creatinine, and the glomerular filtration rate was measured just before treatment (0), and at 1, 3, 6, and 9 months after treatment. | 100% of patients showed decreased level of proteinuria SLEDAI scores revealed early, durable, and substantial remissions | Follow up stopped after 9 months |
| Yuan X et al. (2019) [88] | SLE refractory to conventional therapies | 21 | UC-MSCT | Allogenic $1 \times 10^6$/kg | | To study the mechanisms of immunoregulatory mechanism in SLE patients. | Number of peripheral tolerogenic $CD1c^+$ dendritic cells and levels of serum FLT3L are significantly decreased in SLE patients esp. with lupus nephritis compared with healthy controls. Following transplant, significant upregulation of peripheral blood $CD1c^+$ dendritic cells and serum FLT3L was seen. | |

| Reference (First author, date) | Study type/ SLE, organ involvement | Number of patients studied, Age range | MSC source* | Type and amount (dose) | Prior treatment | Outcome criteria | Improvement (%) in 6 months | Improvement (%) in 12 m and above |
|---|---|---|---|---|---|---|---|---|
| Wen L et al. (2019) [88] | Retrospective cohort study SLE pts. with active disease (SLEDAI score > =8 | 69 | BM-/ UC-MSCs | Allogenic $1 \times 10^6$/kg | | SLE symptoms and SLEDAI scores were assessed at baseline and during follow up to determine low disease activity and clinical remission at 1, 3, 6 and 12 months. To identify predictors of clinical response to allogenic BM or UC MSC treatment | | Severe SLE pts. undergo sustained clinical remission with reduced disease maintained over a 1 year follow up. Older age, no arthralgia/ arthritis at baseline, and no prior CYC or HCQ treatment had better first year outcomes after allogenic BM-UC-MSC transplantation. |

*UC, umbilical cord; bone marrow (BM) or adipogenic (AD) tissue derived MSCs; CYC, cyclophosphamide; HCQ, Hydroxychloroquine.*

**Table 1.**
*Human clinical trials that used mesenchymal stem cells (MSCs) for treatment of systemic lupus erythematosus.*

approach to MSC treatment took hematopoietic stem cell replacement therapies (HSCT) as examples, and protocols that mimicked HSCT were investigated. One similarity was to use autologous cells rather than allogeneic stem cells and the other similarity was to use myeloablation therapies with chemotherapy agents before the MSC treatment.

While autologous MSC treatment trials showed efficacy in increasing the amount of immune regulatory cells that play an important role in SLE, the clinical disease activity scores were not changed [79]. Same center that published the failure in 2 patients treated with autologous MSCs also performed a study using allogeneic MSCs in 15 patients and showed efficacy [78]. Because sources of allogeneic MSCs are more available and carry less concern of being defective due to disease state or genetic background [90], the following SLE clinical trials used mostly allogeneic MSC sources from variable tissues.

Initial reports of allogeneic MSC trials came from a group of investigators from China. Sun et al. reported a study performed between April 2007 to July 2009 on 16 patients with active SLE nephritis who were enrolled and underwent allogeneic umbilical cord (UC) driven MSC treatment. Study showed efficacy of allogeneic UC MSCs in SLE and suggested that clinical remission was correlating to the increase in peripheral Treg cells and an improved balance between Th1- and Th2- cytokines [77]. Cellular significance was correlating with the decreased amount of proteinuria and decreased SLEDAI (Systemic Lupus Erythematosus Disease Activity Index) scores. Patients in this trial received IV cyclophosphamide treatment for 2–4 days prior to UC MSC treatment.

Same group continued to treat resistant SLE patients and enrolled eighty-seven patients with persistently active SLE who were refractory to standard treatment or had severe organ involvement. While some patients received allogeneic bone marrow some received umbilical cord derived MSCs intravenously ($1 \times 10^6$ cells/kg of body weight). Three of them were given a second UC-MSC treatment (8, 3, 4 months after the first BM MSC treatment and one was given UC-MSCT additional three times (11, 19, 20 months after the first BM MSC treatment). During the 4-year follow-up the overall rate of survival was 94% (82/87). Complete clinical remission rate was 28% at 1 year (23/83). The overall rate of relapse was 23% (20/87). Only five patients (6%) died after MSC treatment from non-treatment-related events in the 4-year follow-up. Allogeneic MSC were suggested to result in the induction of clinical remission and improvement in organ dysfunction in drug resistant severe SLE patients [83].

Debate of allogeneic versus autologous stem cell treatment continued while initial phase I and II trials were ongoing with MSCs. Sui et al. [91] compared the research of autologous or allogeneic HSC/MSC in SLE. They analyzed the data of Wang et al. [83] i.e. allogeneic group and that of Jayne et al. [74] and Burt et al. [75], i.e. autologous group. In conclusion, they found that the rate of complete clinical remission was similar in these clinical trials (approximately 50%). However, there was higher overall survival rate, lower overall rate of relapse and no transplantation-related mortality in the allogeneic group. Because these 3 studies were not randomized, and it was not possible to compare them with each other exactly due to the heterogeneous disease manifestation at baseline. Authors suggested the importance of randomized clinical trials consisting of a large sample and long term follow up of these patients to further investigate the efficacy and safety of autologous/allogeneic stem cell transplantation [91].

**X Li et al.** [82, 92] further assessed the roles of allogeneic (BM and UC) MSC treatment with in SLE patients with refractory cytopenia. Thirty-five SLE patients with refractory cytopenia were enrolled and hematological changes of pre- and post-transplantation were evaluated. Significant improvements in blood cell count

were found after MSC treatment for most patients, in parallel with the decline of disease activity. Clinical remission was again correlating with increased Treg cells and decreased Th17 cells. Results suggested that MSCs are successful in correcting refractory cytopenia in SLE patients which might be associated with reconstitution of Treg and Th17.

Use of chemotherapy together or before MSC treatment for induction was also assessed by variety of small clinical trials. Wang et al. [71] found no differences between the patient groups that received pretreatment with cyclophosphamide and untreated with cyclophosphamide. There was no difference in the rate of clinical remission after MSC treatments [71]. In addition there were significant number of patients that developed relapse in 6 months and additional MSC treatments were given to those patients with relapse.

**Fei Gu et al.** [72] assessed the role of allogeneic MSC treatment to induce renal remission in patients with active and refractory lupus nephritis (LN). They conducted an open-label and single-center clinical trial conducted from 2007 to 2010 in which 81 Chinese patients with active and refractory LN were enrolled. Allogeneic bone marrow- or umbilical cord-derived mesenchymal stem cells (MSCs) were administered intravenously at the dose of 1 million cells per kilogram of bodyweight. During the 12-month follow-up, the overall rate of survival was 95% (77/81). Totally, 60.5% (49/81) patients achieved renal remission during 12-month visit by MSCT. Eleven of 49 (22.4%) patients experienced renal flare by the end of 12 months after a previous remission. Renal activity evaluated by BILAG (British Isles Lupus Assessment Group) scores significantly declined after MSC treatment, in parallel with the obvious amelioration of renal function. Glomerular filtration rate (GFR) improved significantly 12 months after. Total disease activity evaluated by SLEDAI scores also decreased after treatment. Additionally, the doses of concomitant prednisone and immunosuppressive drugs were tapered. No transplantation-related adverse event was observed. They concluded that allogeneic MSC treatment resulted in renal remission for active LN patients within 12-month visit, confirming its use as a potential therapy for refractory LN.

**Woodworth et al.** [93] examined whether collective data from Wang et al. [71] provided sufficient evidence for the feasibility, safety, dose rationale, and potential efficacy of UC-MSCs to conduct a randomized controlled trial in treatment-refractory SLE nephritis. They observed that results, though confounded by variable baseline prednisone and immuno-suppressive treatment, appear to indicate near term response rates of approximately 50%, which are comparable to those seen with hematopoietic stem cell transplantation but with less morbidity and mortality. They also noticed that apparently, conditioning pre-MSC dosing is not required, although this aspect of the treatment had not been studied in a controlled manner [93].

Another group performed an interesting combination therapy with HSCs and MSCs for life threatening organ involvement involving SLE patient refractory to cyclophosphamide. After being pretreated with CYC, Fudarabine and antithymocyte globulin, the patient was transplanted with autologous CD34+ HSCs and MSCs by intravenous infusion. Hematopoietic regeneration was observed on day 12 thereafter. After HSC and MSC transplantation, the patient's clinical symptoms caused by SLE were remitted, and the SLEDAI score decreased. One more time CD4 + CD25 + FoxP3+ Treg cells were found to be increased in peripheral blood mononuclear cells (PBMCs) after transplantation. This study was important to show that combined transplantation of HSCs and MSCs may reset the adaptive immune system to re-establish self-tolerance in SLE. A 36-month follow-up showed that the clinical symptoms remained in remission for the index patient [94].

A randomized double blind placebo control trial was reported by **Deng et al.** [84] that assessed the efficacy of human umbilical cord-derived mesenchymal

stem cell (hUC-MSC) for the treatment of lupus nephritis (LN) among 18 patients with WHO class III or IV LN. Patients were randomly assigned to hUC-MSC (dose $2 \times 10^8$ cells) or placebo. All patients received standard immunosuppressive treatment, which consisted of intravenous methylprednisolone and cyclophosphamide, followed by maintenance oral prednisolone and mycophenolate mofetil. Initial 11 patients enrolled to the study received hUC-MSC concurrently with the intravenous methylprednisolone and CYP induction therapy, and for the 12th to 18th patients enrolled, the hUC-MSC were administered together with the intravenous methylprednisolone only and intravenous CYP was delayed to 4 weeks later. In result, similar proportion of patients on hUC-MSC and placebo achieved complete remission. Improvements in serum albumin, complement, renal function, SLEDAI and BILAG scores were similar in both groups. The trial was abandoned after 18 patients were enrolled when it had become obvious it would not demonstrate a positive treatment effect. They concluded that hUC-MSC has no apparent additional effect over and above standard immunosuppression [84].

A pilot study investigated the effect of MSCs on soluble human leukocyte antigen G (s HLA-G) levels 24 hours and 30 days after MSC injection (UC) and reported a negative correlation between the HLA-G levels and clinical SLE activity scores [85]. The levels of s HLA-G were lower in patients with renal involvement than without it.

An open label phase II trial the following year reported safety and long-term efficacy of UC MSCs in severe SLE. Wang et al. [86] reported a long-term follow-up study of allogeneic bone marrow and/or umbilical cord MSC transplantation (MSCT) in severe and drug-refractory systemic lupus erythematosus (SLE) patients. Eighty-one patients were enrolled, and the 5-year overall survival rate was 84% (68/81) after MSCT. At 5-year follow-up, 27% of patients (22/81) were in complete clinical remission and another 7% (6/81) were in partial clinical remission, with a 5-year disease remission rate of 34% (28/81). In total, 37 patients had achieved clinical remission and then 9 patients subsequently relapsed, with 5-year overall rate of relapse of 24% (9/37). SLEDAI scores, serum albumin, complement C3, peripheral white blood cell, and platelet numbers, as well as proteinuria levels, continued to improve during the follow-up. Their results demonstrated that allogeneic MSC treatment is safe and resulted in long-term clinical remission in SLE patients.

Barbado et al. [87] infused three SLE patients with MSCs who were diagnosed with class IV nephritis by kidney biopsies. MSCs were allogeneic MSCs from healthy donors. Total of ninety million cells were infused intravenously into each patient during high and very high activity disease. Patient 1 was treated with cyclophosphamide, azathioprine, methotrexate, mycophenolate and cyclosporine, patient 2 was treated with cyclophosphamide, mycophenolate, rituximab and patient 3 was treated with cyclophosphamide and mycophenolate before MSC treatment. Then, follow-up was performed after 9 months. Proteinuria levels improved significantly during the 1st month and then continued to be sustained in normal levels. Clinical outcome scores such as SLEDAI was perfect for 2 patients while the third SLE patient only had a partial response and the patient could reduce the dose of her current therapies down to 50–60%. Follow up stopped after 9 months SLEDAI scores revealed early, durable, and substantial remissions that were complete for two patients and partial for the third patient and that permitted medication doses to be reduced 50–90%.

In 2019 using slightly older patient population with severe SLE (SLEDAI score > =8), Wen et al. [88] also reported efficacy of allogeneic bone marrow and umbilical cord MSC treatment over one year of follow up in those patients that did not have any baseline arthritis or use of cyclophosphamide of hydroxychloroquine

in 2019. Same year Yuan et al. [95] attempted to explore the immunoregulatory mechanism of MSC treatments in SLE patients. They showed that number of peripheral tolerogenic $CD1c^+$ dendritic cells and levels of serum FLT3L are significantly decreased in severely affected SLE patients especially with lupus nephritis. UC-MSC treatment however tapered the FLT3L and inhibited the apoptosis of tolerogenic CD1c + DCs. It is suggested that MSCs carry FLT3L that binds the FLT3 on CD1c + DCs and enhance their ability to proliferate and stops them from being apoptotic [95]. CD1c + DCs in human peripheral blood and in lymphoid and non-lymphoid tissues. CD1c + DCs have been previously reported to play important immune regulatory work such as secreting cytokines when exposed to (poly I:C), LPS or others and regulate the activity of many immune cells such as T regulatory cells and interferon secreting cells [96, 97]. Interferon gamma-FLT3L-FLT3 axis is one of many mechanisms that MSCs are regulating and its implications in treatment of SLE has been recently recognized. Tregs were shown to respond well to allogeneic MSC treatment in several studies. Furthermore, Chen et al. previously have shown that serum HLA-G levels correlated with the levels of Tregs after treatment with allogeneic umbilical cell derived MSCs [85].

Latest report when this chapter was being prepared was by Zhou et al. Zhou et al. [81] did a meta-analysis aiming at assessing whether MSCs can become a new treatment for SLE with good efficacy and safety. Ten studies fulfilled the inclusion criteria and were eligible for this meta-analysis, which comprised 8 prospective or retrospective case series and four randomized controlled trails (RCTs) studies. In the RCT, the results indicated that the MSC group had lower proteinuria than the control group at 3 months and 6 months and the MSC group displayed a lower SLEDAI than the control group at 2 months and 6 months. Furthermore, the MSC group showed a lower rate of adverse events than the control group (OR = 0:26, 95% CI: 0.07, 0.89, P = 0:03). In the case series trials, the results indicated that the MSC group had lower proteinuria at 1 month, 2 months, 3 months, 4 months, 6 months, and 12 months. They concluded that MSCs might be a promising therapeutic agent for patients with SLE. However, they suggested that more studies with longer-term end points and larger sample sizes should be designed and conducted to identify additional and robust patient-centered outcomes in the future [81].

## 6. Summary/conclusions

The clinical outcome parameters and the kind and amount of MSCs used in the clinical trials we reviewed in this chapter are variable. Most important difference of MSCs used in the clinical trials is whether they are autologous, extracted from the patient's own tissue or allogeneic extracted from health donors. When we reviewed the clinical trials using autologous MSCs trials treating SLE we observed that autologous MSCs did not show much efficacy while allogeneic MSCs regardless of their origins seem to be showing consistently better efficacy in most trials (**Table 1**). The reason for lack of efficacy in autologous use of MSCs is most probably due to their intrinsic abnormalities, and their inability to function at their best capacity. Autologous MSCs may not be functioning due their previous exposure the inflammatory micro environment in SLE or due to their genetic predisposition [79].

Allogeneic mesenchymal stem cell treatment has been shown to be efficacious in the treatment of various systemic lupus erythematosus activity, mainly in refractory lupus nephritis. Allogeneic MSCs, at $1 \times 10^6$/kg seems to be efficacious but the results are not as homogeneous as expected from clinical trials and FDA approval for MSCs use in rheumatologic diseases have been challenging. Heterogeneous results could be due to the heterogenous disease manifestations among patients

enrolled to the clinical trials. In addition, although there are plenty of MSC trial reports that shows evidence for MSCs efficacy in SLE, randomized prospective controlled trials using MSCs are still missing.

In addition, the tissue source of donor MSCs shows remarkable variability, while some investigators believe in the superior anti-inflammatory effects of audiogenic MSCs other disagree and suggest umbilical cord MSCs immune modulatory efficacies. Since future MSC clinical trials and MSC therapies will be dependent on the availability of the donor tissue, technologic advancement to optimize the MSCs that can be easily obtained such as adipogenic tissue or peripheral blood must be prioritized.

Most MSC products used in clinical trials still lack a clear product definition, how they are selected, and application protocols. It is possible that the dose, route and frequency of the cell product protocol used in a clinical trial may not be universally applicable. Furthermore, due to the ever-thriving knowledge about MSCs functions we are yet to establish clear outcome criteria for testing MSC efficacy and safety.

Most MSC clinical trials have the inclusion criteria to enroll patients with severe disease activity and criteria of failure of currently available treatments. Therefore, there might be already irreversible and secondary tissue damage and MSCs may not be able to reverse this outcome when used in the late phase of the organ damage. If MSCs can be given in an earlier stage of disease their efficacy might be a lot better.

In summary, as you would see from the list of clinical trials and their outcomes (**Table 1**) discussed in this chapter the investigators that take roles in MSC clinical trials are not only struggling with the source of MSCs and optimization of efficacy they are also facing very complex regulatory issues. The variable sources of stem cells, cumbersome manufacturing processes are further complicating design of clinical trials. Further studies assessing the efficacy of MSC treatments needs to be performed.

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## References

[1] Maroz N and Segal MS. Lupus nephritis and end-stage kidney disease. *Am J Med Sci* 2013; 346: 319-323. 2013/02/02. DOI: 10.1097/MAJ.0b013e31827f4ee3.

[2] Mavragani CP and Moutsopoulos HM. Sjogren's syndrome: Old and new therapeutic targets. *J Autoimmun* 2020; 110: 102364. 2019/12/14. DOI: 10.1016/j.jaut.2019.102364.

[3] Ball LM, Egeler RM and Party EPW. Acute GvHD: pathogenesis and classification. *Bone Marrow Transplant* 2008; 41 Suppl 2: S58-64. 2008/07/24. DOI: 10.1038/bmt.2008.56.

[4] Bawazier LA. Current and Emerging Therapy on Lupus Nephritis. *Acta Med Indones* 2017; 49: 369-377. 2018/01/20.

[5] Raja TW, Veeramuthu D, Savarimuthu I, et al. Current Trends in the Treatment of Systemic Lupus Erythematosus. *Curr Pharm Des* 2020; 26: 2602-2609. 2020/02/19. DOI: 10.2174/1381612826666200211122633.

[6] Hanly JG, Kozora E, Beyea SD, et al. Review: Nervous System Disease in Systemic Lupus Erythematosus: Current Status and Future Directions. *Arthritis Rheumatol* 2019; 71: 33-42. 2018/06/22. DOI: 10.1002/art.40591.

[7] Adam Z, Sokwala A, Shah J, et al. A delay in diagnosis: thrombotic thrombocytopenia purpura occurring in systemic lupus erythematous. *Pan Afr Med J* 2019; 34: 103. 2020/01/15. DOI: 10.11604/pamj.2019.34.103.20524.

[8] Safiri S, Kolahi AA, Cross M, et al. Global, regional, and national burden of other musculoskeletal disorders 1990-2017: results from the Global Burden of Disease Study 2017. *Rheumatology (Oxford)* 2020 2020/08/26. DOI: 10.1093/rheumatology/keaa315.

[9] Tanaka Y, O'Neill S, Li M, et al. Systemic Lupus Erythematosus: Targeted literature review of the epidemiology, current treatment and disease burden in the Asia Pacific region. *Arthritis Care Res (Hoboken)* 2020 2020/08/26. DOI: 10.1002/acr.24431.

[10] McCormick N, Marra CA, Sadatsafavi M, et al. Socioeconomic status at diagnosis influences the incremental direct medical costs of systemic lupus erythematosus: A longitudinal population-based study. *Semin Arthritis Rheum* 2020; 50: 77-83. 2019/07/31. DOI: 10.1016/j.semarthrit.2019.06.010.

[11] Bezalel S, Guri KM, Elbirt D, et al. Type I interferon signature in systemic lupus erythematosus. *Isr Med Assoc J* 2014; 16: 246-249. 2014/05/20.

[12] Dema B and Charles N. Advances in mechanisms of systemic lupus erythematosus. *Discov Med* 2014; 17: 247-255. 2014/06/03.

[13] Torell F, Eketjall S, Idborg H, et al. Cytokine Profiles in Autoantibody Defined Subgroups of Systemic Lupus Erythematosus. *J Proteome Res* 2019; 18: 1208-1217. 2019/02/12. DOI: 10.1021/acs.jproteome.8b00811.

[14] Hayashi T. Therapeutic strategies for SLE involving cytokines: mechanism-oriented therapies especially IFN-gamma targeting gene therapy. *J Biomed Biotechnol* 2010; 2010 2010/09/10. DOI: 10.1155/2010/461641.

[15] O'Neill S and Cervera R. Systemic lupus erythematosus. *Best Pract Res Clin Rheumatol* 2010; 24: 841-855. 2011/06/15. DOI: 10.1016/j.berh.2010.10.006.

[16] Trucci VM, Salum FG, Figueiredo MA, et al. Interrelationship

of dendritic cells, type 1 interferon system, regulatory T cells and toll-like receptors and their role in lichen planus and lupus erythematosus -- a literature review. *Arch Oral Biol* 2013; 58: 1532-1540. 2013/07/13. DOI: 10.1016/j.archoralbio.2013.06.016.

[17] Larkin J, 3rd, Ahmed CM, Wilson TD, et al. Regulation of interferon gamma signaling by suppressors of cytokine signaling and regulatory T cells. *Front Immunol* 2013; 4: 469. 2014/01/07. DOI: 10.3389/fimmu.2013.00469.

[18] Talaat RM, Mohamed SF, Bassyouni IH, et al. Th1/Th2/Th17/Treg cytokine imbalance in systemic lupus erythematosus (SLE) patients: Correlation with disease activity. *Cytokine* 2015; 72: 146-153. 2015/02/04. DOI: 10.1016/j.cyto.2014.12.027.

[19] Liu Y, Liao J, Zhao M, et al. Increased expression of TLR2 in CD4(+) T cells from SLE patients enhances immune reactivity and promotes IL-17 expression through histone modifications. *Eur J Immunol* 2015; 45: 2683-2693. 2015/06/17. DOI: 10.1002/eji.201445219.

[20] Mizui M, Koga T, Lieberman LA, et al. IL-2 protects lupus-prone mice from multiple end-organ damage by limiting CD4-CD8- IL-17-producing T cells. *J Immunol* 2014; 193: 2168-2177. 2014/07/27. DOI: 10.4049/jimmunol.1400977.

[21] Rafael-Vidal C, Perez N, Altabas I, et al. Blocking IL-17: A Promising Strategy in the Treatment of Systemic Rheumatic Diseases. *Int J Mol Sci* 2020; 21 2020/10/01. DOI: 10.3390/ijms21197100.

[22] Mohammadi S, Sedighi S and Memarian A. IL-17 is Aberrantly Overexpressed Among Under-treatment Systemic Lupus Erythematosus Patients. *Iran J Pathol* 2019; 14: 236-242. 2019/10/05. DOI: 10.30699/ijp.2019.94878.1934.

[23] Li QZ, Zhou J, Lian Y, et al. Interferon signature gene expression is correlated with autoantibody profiles in patients with incomplete lupus syndromes. *Clin Exp Immunol*; 159: 281-291. 2009/12/09. DOI: CEI4057 [pii] 10.1111/j.1365-2249.2009.04057.x.

[24] Hahn BH, Anderson M, Le E, et al. Anti-DNA Ig peptides promote Treg cell activity in systemic lupus erythematosus patients. *Arthritis Rheum* 2008; 58: 2488-2497. 2008/08/01. DOI: 10.1002/art.23609.

[25] Lee PY and Reeves WH. Type I interferon as a target of treatment in SLE. *Endocr Metab Immune Disord Drug Targets* 2006; 6: 323-330. 2007/01/12.

[26] Zhuang H, Kosboth M, Lee P, et al. Lupus-like disease and high interferon levels corresponding to trisomy of the type I interferon cluster on chromosome 9p. *Arthritis Rheum* 2006; 54: 1573-1579. 2006/04/29. DOI: 10.1002/art.21800.

[27] Mangini AJ, Lafyatis R and Van Seventer JM. Type I interferons inhibition of inflammatory T helper cell responses in systemic lupus erythematosus. *Ann N Y Acad Sci* 2007; 1108: 11-23. 2007/09/26. DOI: 10.1196/annals.1422.002.

[28] Sozzani S, Bosisio D, Scarsi M, et al. Type I interferons in systemic autoimmunity. *Autoimmunity* 2010; 43: 196-203. 2010/03/20. DOI: 10.3109/08916930903510872.

[29] Ronnblom L. The importance of the type I interferon system in autoimmunity. *Clin Exp Rheumatol* 2016; 34: 21-24. 2016/09/03.

[30] Yan B, Ye S, Chen G, et al. Dysfunctional CD4+,CD25+ regulatory T cells in untreated active systemic lupus erythematosus secondary to interferon-alpha-producing antigen-presenting cells. *Arthritis Rheum* 2008; 58: 801-812. 2008/03/04. DOI: 10.1002/art.23268.

[31] Ferreira RC, Castro Dopico X, Oliveira JJ, et al. Chronic Immune Activation in Systemic Lupus Erythematosus and the Autoimmune PTPN22 Trp(620) Risk Allele Drive the Expansion of FOXP3(+) Regulatory T Cells and PD-1 Expression. *Front Immunol* 2019; 10: 2606. 2019/11/30. DOI: 10.3389/fimmu.2019.02606.

[32] Kailashiya V, Singh U, Rana R, et al. Regulatory T Cells and Their Association with Serum Markers and Symptoms in Systemic Lupus Erythematosus and Rheumatoid Arthritis. *Immunol Invest* 2019; 48: 64-78. 2018/10/17. DOI: 10.1080/08820139.2018.1527852.

[33] Dall'Era M, Pauli ML, Remedios K, et al. Adoptive Treg Cell Therapy in a Patient With Systemic Lupus Erythematosus. *Arthritis Rheumatol* 2019; 71: 431-440. 2018/10/03. DOI: 10.1002/art.40737.

[34] Ptacek T, Li X, Kelley JM, et al. Copy number variants in genetic susceptibility and severity of systemic lupus erythematosus. *Cytogenet Genome Res* 2008; 123: 142-147. 2008/01/01. DOI: 000184701 [pii]10.1159/000184701.

[35] Levine AB and Erkan D. Clinical assessment and management of cytopenias in lupus patients. Curr Rheumatol Rep 2011; 13: 291-299. DOI: 10.1007/s11926-011-0179-5.

[36] Tsokos GC, Lo MS, Costa Reis P, et al. New insights into the immunopathogenesis of systemic lupus erythematosus. *Nat Rev Rheumatol* 2016; 12: 716-730. 2016/11/23. DOI: 10.1038/nrrheum.2016.186.

[37] Zhang L, Bertucci AM, Ramsey-Goldman R, et al. Regulatory T cell (Treg) subsets return in patients with refractory lupus following stem cell transplantation, and TGF-beta-producing CD8+ Treg cells are associated with immunological remission of lupus. *J Immunol* 2009; 183: 6346-6358. 2009/10/21. DOI: 10.4049/jimmunol.0901773.

[38] Jaiswal N, Haynesworth SE, Caplan AI, et al. Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. *J Cell Biochem* 1997; 64: 295-312. 1997/02/01. DOI: 10.1002/(SICI)1097-4644(199702)64:2<295::AID-JCB12>3.0.CO;2-I [pii].

[39] Lennon DP, Schluchter MD and Caplan AI. The effect of extended first passage culture on the proliferation and differentiation of human marrow-derived mesenchymal stem cells. *Stem Cells Transl Med* 2012; 1: 279-288. 2012/12/01. DOI: 10.5966/sctm.2011-0011.

[40] Somoza RA, Welter JF, Correa D, et al. Chondrogenic differentiation of mesenchymal stem cells: challenges and unfulfilled expectations. *Tissue Eng Part B Rev* 2014; 20: 596-608. 2014/04/23. DOI: 10.1089/ten.TEB.2013.0771.

[41] Lee Z, Dennis J, Alsberg E, et al. Imaging stem cell differentiation for cell-based tissue repair. *Methods Enzymol* 2012; 506: 247-263. 2012/02/22. DOI: 10.1016/B978-0-12-391856-7.00037-8.

[42] Li R, Liang L, Dou Y, et al. Mechanical stretch inhibits mesenchymal stem cell adipogenic differentiation through TGFbeta1/Smad2 signaling. J Biomech 2015; 48: 3665-3671. DOI: 10.1016/j.jbiomech.2015.08.013.

[43] Bourin P, Bunnell BA, Casteilla L, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the

International Society for Cellular Therapy (ISCT). *Cytotherapy* 2013; 15: 641-648. 2013/04/11. DOI: 10.1016/j.jcyt.2013.02.006.

[44] Tyndall A and Uccelli A. Multipotent mesenchymal stromal cells for autoimmune diseases: teaching new dogs old tricks. *Bone Marrow Transplant* 2009; 43: 821-828. 2009/03/25. DOI: 10.1038/bmt.2009.63.

[45] Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; 8: 315-317. 2006/08/23. DOI: 10.1080/14653240600855905.

[46] Stacey GN, Andrews PW, Barbaric I, et al. Stem cell culture conditions and stability: a joint workshop of the PluriMes Consortium and Pluripotent Stem Cell Platform. *Regen Med* 2019; 14: 243-255. 2019/04/03. DOI: 10.2217/rme-2019-0001.

[47] Duffy MM, McNicholas BA, Monaghan DA, et al. Mesenchymal stem cells and a vitamin D receptor agonist additively suppress T helper 17 cells and the related inflammatory response in the kidney. *Am J Physiol Renal Physiol* 2014; 307: F1412-1426. 2014/10/24. DOI: 10.1152/ajprenal.00024.2014.

[48] Darlington PJ, Boivin MN, Renoux C, et al. Reciprocal Th1 and Th17 regulation by mesenchymal stem cells: Implication for multiple sclerosis. *Ann Neurol* 2010/07/28. DOI: 10.1002/ana.22065.

[49] Leyendecker A, Jr., Pinheiro CCG, Amano MT, et al. The Use of Human Mesenchymal Stem Cells as Therapeutic Agents for the in vivo Treatment of Immune-Related Diseases: A Systematic Review. *Front Immunol* 2018; 9: 2056. 2018/09/27. DOI: 10.3389/fimmu.2018.02056.

[50] Bai L, Lennon DP, Eaton V, et al. Human bone marrow-derived mesenchymal stem cells induce Th2-polarized immune response and promote endogenous repair in animal models of multiple sclerosis. *Glia* 2009; 57: 1192-1203. 2009/02/05. DOI: 10.1002/glia.20841.

[51] Wang D, Huang S, Yuan X, et al. The regulation of the Treg/Th17 balance by mesenchymal stem cells in human systemic lupus erythematosus. *Cell Mol Immunol* 2017; 14: 423-431. 2015/10/06. DOI: 10.1038/cmi.2015.89.

[52] Ghannam S, Pene J, Moquet-Torcy G, et al. Mesenchymal stem cells inhibit human Th17 cell differentiation and function and induce a T regulatory cell phenotype. *J Immunol* 2010; 185: 302-312. 2010/06/01. DOI: 10.4049/jimmunol.0902007.

[53] Pianta S, Bonassi Signoroni P, Muradore I, et al. Amniotic membrane mesenchymal cells-derived factors skew T cell polarization toward Treg and downregulate Th1 and Th17 cells subsets. *Stem Cell Rev Rep* 2015; 11: 394-407. 2014/10/29. DOI: 10.1007/s12015-014-9558-4.

[54] Zeng SL, Wang LH, Li P, et al. Mesenchymal stem cells abrogate experimental asthma by altering dendritic cell function. Mol Med Rep 2015; 12: 2511-2520. DOI: 10.3892/mmr.2015.3706.

[55] Liang J and Sun L. Mesenchymal stem cells transplantation for systemic lupus erythematosus. *Int J Rheum Dis* 2015; 18: 164-171. 2015/01/23. DOI: 10.1111/1756-185X.12531.

[56] De Miguel MP, Fuentes-Julian S, Blazquez-Martinez A, et al. Immunosuppressive properties of mesenchymal stem cells: advances and applications. *Curr Mol Med* 2012; 12: 574-591. 2012/04/21.

[57] Gao C, Wang X, Lu J, et al. Mesenchymal stem cells transfected with sFgl2 inhibit the acute rejection of heart transplantation in mice by regulating macrophage activation. *Stem Cell Res Ther* 2020; 11: 241. 2020/06/20. DOI: 10.1186/s13287-020-01752-1.

[58] Cho DI, Kim MR, Jeong HY, et al. Mesenchymal stem cells reciprocally regulate the M1/M2 balance in mouse bone marrow-derived macrophages. *Exp Mol Med* 2014; 46: e70. 2014/01/11. DOI: 10.1038/emm.2013.135.

[59] Geng Y, Zhang L, Fu B, et al. Mesenchymal stem cells ameliorate rhabdomyolysis-induced acute kidney injury via the activation of M2 macrophages. *Stem Cell Res Ther* 2014; 5: 80. 2014/06/26. DOI: 10.1186/scrt469.

[60] Song T, Eirin A, Zhu X, et al. Mesenchymal Stem Cell-Derived Extracellular Vesicles Induce Regulatory T Cells to Ameliorate Chronic Kidney Injury. *Hypertension* 2020; 75: 1223-1232. 2020/04/01. DOI: 10.1161/HYPERTENSIONAHA.119.14546.

[61] Poltavtsev AM, Poltavtseva RA, Yushina MN, et al. Cytokine Production in Mixed Cultures of Mesenchymal Stromal Cells from Wharton's Jelly and Peripheral Blood Lymphocytes. *Bull Exp Biol Med* 2017; 163: 169-175. 2017/06/06. DOI: 10.1007/s10517-017-3759-1.

[62] Chen HW, Chen HY, Wang LT, et al. Mesenchymal stem cells tune the development of monocyte-derived dendritic cells toward a myeloid-derived suppressive phenotype through growth-regulated oncogene chemokines. *J Immunol* 2013; 190: 5065-5077. 2013/04/17. DOI: 10.4049/jimmunol.1202775.

[63] Cui R, Rekasi H, Hepner-Schefczyk M, et al. Human mesenchymal stromal/stem cells acquire immunostimulatory capacity upon cross-talk with natural killer cells and might improve the NK cell function of immunocompromised patients. *Stem Cell Res Ther* 2016; 7: 88. 2016/07/09. DOI: 10.1186/s13287-016-0353-9.

[64] Wang D, Feng X, Lu L, et al. A CD8 T cell/indoleamine 2,3-dioxygenase axis is required for mesenchymal stem cell suppression of human systemic lupus erythematosus. *Arthritis Rheumatol* 2014; 66: 2234-2245. 2014/04/24. DOI: 10.1002/art.38674.

[65] Cassano JM, Schnabel LV, Goodale MB, et al. The immunomodulatory function of equine MSCs is enhanced by priming through an inflammatory microenvironment or TLR3 ligand. Vet Immunol Immunopathol 2018; 195: 33-39. DOI: 10.1016/j.vetimm.2017.10.003.

[66] da Silva Meirelles L, Caplan AI and Nardi NB. In search of the in vivo identity of mesenchymal stem cells. *Stem Cells* 2008; 26: 2287-2299. 2008/06/21. DOI: 10.1634/stemcells.2007-1122.

[67] da Silva Meirelles L, Sand TT, Harman RJ, et al. MSC frequency correlates with blood vessel density in equine adipose tissue. *Tissue Eng Part A* 2009; 15: 221-229. 2008/10/14. DOI: 10.1089/ten.tea.2008.0103.

[68] Spitzer TL, Rojas A, Zelenko Z, et al. Perivascular human endometrial mesenchymal stem cells express pathways relevant to self-renewal, lineage specification, and functional phenotype. *Biol Reprod* 2012; 86: 58. 2011/11/15. DOI: 10.1095/biolreprod.111.095885.

[69] Caplan AI. New era of cell-based orthopedic therapies. *Tissue Eng Part B Rev* 2009; 15: 195-200. 2009/02/21. DOI: 10.1089/ten.TEB.2008.0515.

[70] Rodriguez-Fuentes DE, Fernandez-Garza LE, Samia-Meza JA, et al. Mesenchymal Stem Cells Current

Clinical Applications: A Systematic Review. *Arch Med Res* 2020 2020/09/27. DOI: 10.1016/j.arcmed.2020.08.006.

[71] Wang D, Li J, Zhang Y, et al. Umbilical cord mesenchymal stem cell transplantation in active and refractory systemic lupus erythematosus: a multicenter clinical study. *Arthritis Res Ther* 2014; 16: R79. 2014/03/26. DOI: 10.1186/ar4520.

[72] Gu F, Wang D, Zhang H, et al. Allogeneic mesenchymal stem cell transplantation for lupus nephritis patients refractory to conventional therapy. *Clin Rheumatol* 2014; 33: 1611-1619. 2014/08/15. DOI: 10.1007/s10067-014-2754-4.

[73] Jayne D and Tyndall A. Autologous stem cell transplantation for systemic lupus erythematosus. *Lupus* 2004; 13: 359-365. 2004/07/03. DOI: 10.1191/0961203304lu1027oa.

[74] Jayne D, Passweg J, Marmont A, et al. Autologous stem cell transplantation for systemic lupus erythematosus. *Lupus* 2004; 13: 168-176. 2004/05/04. DOI: 10.1191/0961203304lu525oa.

[75] Burt RK, Traynor A, Statkute L, et al. Nonmyeloablative hematopoietic stem cell transplantation for systemic lupus erythematosus. *JAMA* 2006; 295: 527-535. 2006/02/02. DOI: 10.1001/jama.295.5.527.

[76] Burt RK, Verda L, Oyama Y, et al. Non-myeloablative stem cell transplantation for autoimmune diseases. *Springer Semin Immunopathol* 2004; 26: 57-69. 2004/11/19. DOI: 10.1007/s00281-004-0162-6.

[77] Sun L, Wang D, Liang J, et al. Umbilical cord mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus. *Arthritis Rheum* 2010; 62: 2467-2475. 2010/05/28. DOI: 10.1002/art.27548.

[78] Liang J, Zhang H, Hua B, et al. Allogenic mesenchymal stem cells transplantation in refractory systemic lupus erythematosus: a pilot clinical study. *Ann Rheum Dis* 2010; 69: 1423-1429. 2010/07/24. DOI: 10.1136/ard.2009.123463.

[79] Carrion F, Nova E, Ruiz C, et al. Autologous mesenchymal stem cell treatment increased T regulatory cells with no effect on disease activity in two systemic lupus erythematosus patients. *Lupus* 2010; 19: 317-322. 2009/11/19. DOI: 10.1177/0961203309348983.

[80] Shi D, Wang D, Li X, Zhang H, Che N, Lu Z, et al. Allogeneic transplantation of umbilical cord-derived mesenchymal stem cells for diffuse alveolar hemorrhage in systemic lupus erythematosus. Clin Rheumatol. 2012;31(5):841-846. doi: 10.1007/s10067-012-1943-2.

[81] Zhou T, Li HY, Liao C, et al. Clinical Efficacy and Safety of Mesenchymal Stem Cells for Systemic Lupus Erythematosus. *Stem Cells Int* 2020; 2020: 6518508. 2020/04/24. DOI: 10.1155/2020/6518508.

[82] Li X, Wang D, Liang J, Zhang H, Sun L. Mesenchymal SCT ameliorates refractory cytopenia in patients with systemic lupus erythematosus. Bone Marrow Transplant. 2013;48(4):544-550. doi: 10.1038/bmt.2012.184.

[83] Wang D, Zhang H, Liang J, et al. Allogeneic mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus: 4 years of experience. *Cell Transplant* 2013; 22: 2267-2277. 2014/01/07. DOI: 10.3727/096368911X582769c.

[84] Deng D, Zhang P, Guo Y, et al. A randomised double-blind, placebo-controlled trial of allogeneic umbilical cord-derived mesenchymal stem cell for lupus nephritis. *Ann Rheum Dis* 2017; 76: 1436-1439. 2017/05/10. DOI: 10.1136/annrheumdis-2017-211073.

[85] Chen C, Liang J, Yao G, et al. Mesenchymal stem cells upregulate Treg cells via sHLA-G in SLE patients. *Int Immunopharmacol* 2017; 44: 234-241. 2017/01/28. DOI: 10.1016/j.intimp.2017.01.024.

[86] Wang D, Zhang H, Liang J, et al. A Long-Term Follow-Up Study of Allogeneic Mesenchymal Stem/Stromal Cell Transplantation in Patients with Drug-Resistant Systemic Lupus Erythematosus. *Stem Cell Reports* 2018; 10: 933-941. 2018/02/27. DOI: 10.1016/j.stemcr.2018.01.029.

[87] Barbado J, Tabera S, Sanchez A, et al. Therapeutic potential of allogeneic mesenchymal stromal cells transplantation for lupus nephritis. *Lupus* 2018; 27: 2161-2165. 2018/10/07. DOI: 10.1177/0961203318804922.

[88] Wen L, Labopin M, Badoglio M, et al. Prognostic Factors for Clinical Response in Systemic Lupus Erythematosus Patients Treated by Allogeneic Mesenchymal Stem Cells. *Stem Cells Int* 2019; 2019: 7061408. 2019/06/14. DOI: 10.1155/2019/7061408.

[89] Le Blanc K, Rasmusson I, Sundberg B, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 2004; 363: 1439-1441. 2004/05/04. DOI: 10.1016/S0140-6736(04)16104-7.

[90] Nie Y, Lau CS, Lie AK, et al. Defective phenotype of mesenchymal stem cells in patients with systemic lupus erythematosus. *Lupus* 2010/02/02. DOI: 0961203310361482 [pii]10.1177/0961203310361482.

[91] Sui W, Hou X, Che W, et al. Hematopoietic and mesenchymal stem cell transplantation for severe and refractory systemic lupus erythematosus. *Clin Immunol* 2013; 148: 186-197. 2013/06/19. DOI: 10.1016/j.clim.2013.05.014.

[92] Belimumab. No tangible efficacy but a risk of immunosuppression. Prescrire Int 2013; 22: 149.

[93] Woodworth TG and Furst DE. Safety and feasibility of umbilical cord mesenchymal stem cells in treatment-refractory systemic lupus erythematosus nephritis: time for a double-blind placebo-controlled trial to determine efficacy. *Arthritis Res Ther* 2014; 16: 113. 2014/08/29. DOI: 10.1186/ar4677.

[94] Wang Q, Qian S, Li J, et al. Combined transplantation of autologous hematopoietic stem cells and allogenic mesenchymal stem cells increases T regulatory cells in systemic lupus erythematosus with refractory lupus nephritis and leukopenia. *Lupus* 2015; 24: 1221-1226. 2015/04/29. DOI: 10.1177/0961203315583541.

[95] Yuan X, Qin X, Wang D, et al. Mesenchymal stem cell therapy induces FLT3L and CD1c(+) dendritic cells in systemic lupus erythematosus patients. *Nat Commun* 2019; 10: 2498. 2019/06/09. DOI: 10.1038/s41467-019-10491-8.

[96] Liu J and Cao X. Regulatory dendritic cells in autoimmunity: A comprehensive review. *J Autoimmun* 2015; 63: 1-12. 2015/08/10. DOI: 10.1016/j.jaut.2015.07.011.

[97] Bamboat ZM, Stableford JA, Plitas G, et al. Human liver dendritic cells promote T cell hyporesponsiveness. *J Immunol* 2009; 182: 1901-1911. 2009/02/10. DOI: 10.4049/jimmunol.0803404.