Stem cell transplantation and mesenchymal cells to treat autoimmune diseases

Alan Tyndall 1, Jacob M. van Laar 2

Summary

Since the start of the international stem cell transplantation project in 1997, over 2000 patients have received a haematopoietic stem cell transplant (HSCT), mostly autologous, as treatment for a severe autoimmune disease, the majority being multiple sclerosis (MS), systemic sclerosis (SSc) and Crohn’s disease. There was an overall 85% 5-year survival and 43% progression-free survival. Around 30% of patients in all disease subgroups had a complete response, often durable despite full immune reconstitution. In many cases, e.g. systemic sclerosis, morphological improvement such as reduction of skin collagen and normalization of microvasculature was documented, beyond any predicted known effects of intense immunosuppression alone. It is hoped that the results of the three running large prospective randomized controlled trials will allow modification of the protocols to reduce the high transplant-related mortality which relates to regimen intensity, age of patient, and comorbidity. Mesenchymal stromal cells (MSC), often incorrectly called stem cells, have been the intense focus of in vitro studies and animal models of rheumatic and other diseases over more than a decade. Despite multiple plausible mechanisms of action and a plethora of positive in vivo animal studies, few randomised controlled clinical trials have demonstrated meaningful clinical benefit in any condition so far. This could be due to confusion in cell product terminology, complexity of clinical study design and execution or agreement on meaningful outcome measures. Within the rheumatic diseases, SLE and rheumatoid arthritis (RA) have received most attention. Uncontrolled multiple trial data from over 300 SLE patients have been published from one centre suggesting a positive outcome; one single centre comparative study in 172 RA was positive. In addition, small numbers of patients with Crohn’s disease, multiple sclerosis, primary Sjögren’s disease, polymyositis/dermatomyositis and type II diabetes mellitus have received MSC therapeutically. The possible reasons for this apparent mismatch between expectation and clinical reality will be discussed.
Hematopoietic stem cell transplantation and autoimmunity

The first use of immune ablation followed by autologous haematopoietic stem cell rescue for treating a severe autoimmune disease (systemic sclerosis) was published in 1996 [1]. This was the result of suggestive evidence from coincidental case reports and supportive animal model data [2]. This was followed by a plethora of single case reports and small case series such that by 2011, the combined European group for blood and marrow transplantation (EBMT) and European league against rheumatism (EULAR) database contained data on 469 patients with MS, 266 with SSc, 95 with SLE and 59 with inflammatory bowel disease (IBD) [3]. Among all patients, the 5-year survival was 85% and the progression-free survival 43%, although the rates varied widely according to the type of autoimmune disease [4].

Earlier enthusiasm for autologous HSCT in treating various severe arthritides was tempered by the subsequent introduction of effective biologic therapies. From an early stage, it was recognised that an initial high treatment related morbidity and mortality (TRM) [6] would be expected, hopefully to be offset by durable drug free remissions or improvement. This indeed turned out to be the case in around one third of registry-studied cases. Increasing experience generated guidelines and recommendations for selecting the ideal patients for autologous HSCT [3,7]. These were essentially those with severe, poor prognosis AD unresponsive to conventional therapy but with sufficient reserves of vital functions to support the transplant regimen and guarantee a meaningful quality of life if all inflammatory disease were arrested. It was also recognised that only through prospective controlled trials would the final value of autologous HSCT be established. At the time of writing (June 2015), only 5 such studies are known (table I).

Prolonged immunosuppression or "resetting" of autoimmunity?

In some of those studied (SLE, MS, and SSc patients), remission was sustained despite full immune reconstitution. In SLE, the authors demonstrated that humoral responses to recall antigens (tetanus, polio, measles, and mumps) were ablated following autologous HSCT, as would be expected, but in addition, eradication of autoantibodies such as anti-double stranded DNA was also achieved coincident with clinical remission in five cases [8]. Following immune reconstitution, a comparison between the patients’ and normal subjects’ T-cell receptor Vβ repertoires

<table>
<thead>
<tr>
<th>Trial name and type</th>
<th>ASTIS (scleroderma)</th>
<th>SCOT (scleroderma)</th>
<th>ASTIC (Crohn’s disease)</th>
<th>ASSIST (scleroderma)</th>
<th>Multiple sclerosis (secondary progressive and relapsing remitting, phase II randomised)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principle investigator</td>
<td>J. van Laar UK</td>
<td>K. Sullivan USA</td>
<td>C. Hawkey UK</td>
<td>R. Burt USA</td>
<td>G.L. Mancardi Italy</td>
</tr>
<tr>
<td>Patients</td>
<td>156</td>
<td>75</td>
<td>45</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>Transplant regimen</td>
<td>Cyclophosphamide 200 mg/kg ATG (rabbit), 7.5 mg/kg CD 34 selection</td>
<td>Cyclophosphamide 120 mg/kg ATG (equine): 90 mg/kg TBI 800 cGy CD34 selection</td>
<td>Cyclophosphamide 200 mg/kg ATG: 7.5 mg/kg Unselected graft</td>
<td>CYC: 200 mg/kg ATG: 6.5 mg/kg Unselected graft</td>
<td>Carmustine Cytosine arabinoside Etoposide Melphalan ATG</td>
</tr>
<tr>
<td>Control arm</td>
<td>Monthly CYC 750 mg/m² IVI 12 months</td>
<td>Monthly CYC 750 mg/m² IVI 12 months</td>
<td>Mobilisation then delayed transplant 12 months</td>
<td>Monthly CYC 1 g/m² 6 months</td>
<td>Mitoxanthrone 20 mg/month for 6 months</td>
</tr>
<tr>
<td>Primary end point</td>
<td>Event (organ failure or death) free survival (EFS)</td>
<td>Composite end point (death, end organ failure) at 54 months</td>
<td>Proportion of patients in sustained remission at 12 months</td>
<td>mRSS and/or lung function at 12 months</td>
<td>MRI disease activity</td>
</tr>
<tr>
<td>Current status</td>
<td>Published Significantly improved EFS 10% TRM</td>
<td>Recruitment completed, May 2011</td>
<td>34 out to 12 months Early transplant effective 1 TRM in early transplant arm</td>
<td>Published Early transplant effective 2 relapses Published HSCT superior to mitoxanthrone</td>
<td></td>
</tr>
</tbody>
</table>

Cyc: cyclophosphamide; ATG: antithymocyte globulin; TBI: total body irradiation; mRSS: modified Rodnan Skin Score; MRI: magnetic resonance imaging; HSCT: haematopoietic stem cell transplantation.

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showed a fully normal pattern. Importantly, no patient had relapsed in the 8-year follow-up reported. Similar findings were described in seven MS patients who remained in remission up to 3 years post-transplant despite regaining a normal T-cell repertoire [9]. These findings are particularly important, since at the onset of the project many considered autologous HSCT to be doomed to failure, given that the identical “autoaggressive” immune system was being given back to the patient. However, the initial choice of autologous over allogeneic HSCT was mainly based on the lower toxicity of autologous HSCT, mainly due to absence of graft-vs-host disease (GvHD). It is now appreciated that in many patients who achieved clinical remission, the autoaggressive immune system was “debulked” rather than fully ablated, allowing re-establishment of normal immune regulation, in part due to increased Treg numbers and activity [10]. Several groups have described reduced collagen deposition in skin [11] and normalization of microvasculature in SSc patients [12,13] following autologous HSCT. None of these observations are readily explained by either sustained immunosuppression or direct effects on fibroblasts and endothelial cells, and suggest a more profound modulation of the inflammatory niche by mechanisms yet to be fully elucidated. Currently, three large prospective randomized trials have been performed: Autologous stem cell transplantation international scleroderma (ASTIS), Scleroderma cyclophosphamide or transplant (SCOT), and Autologous stem cell transplantation international Gohn’s (ASTIC). ASTIS and SCOT are similar in patient selection, control arms, and endpoints, but the transplant protocols differ. SCOT employs a more intense regimen including TBI. Each has experienced its own toxicity issues, all previously known in HSCT medicine. Only time will tell which approach, if any, imparts a clinically useful and durable outcome; ASTIS is completed and published [14] (156 patients). The primary endpoint of superior event-free survival, events being defined as death or end-organ (renal, cardiac or pulmonary) failure was achieved long term in the transplanted arm. A treatment related mortality of 10% was seen in the HSCT arm. SCOT finished recruitment in May 2011 and ASTIC is completed and submitted for publication [15].

A smaller phase II randomized trial in SSc showed a positive outcome in the 10 transplanted patients compared with 9 control patients who received CYC 1 g/m² for 6 months [16]. The conditioning regimen was CYC 200 mg/kg plus five doses of rabbit ATG, each one accompanied by 1 g of intravenous methyl prednisolone. The primary endpoint at 12 months was based on a percentage improvement of the modified Rodnan skin score (mRSS) and/or pulmonary status. Although the low toxicity and positive outcome was gratifying, the low numbers of patients and short follow-up were not conclusive [17].

Another multicenter, phase II, randomized trial included 21 patients with secondary progressive or relapsing-remitting MS, with a documented increase in the last year on the Expanded Disability Status Scale, in spite of conventional therapy, and presence of one or more gadolinium-enhancing (Gd+) areas. Patients were randomized to receive intense immunosuppression (mobilization with cyclophosphamide and filgrastim, conditioning with carmustine, cytosine-arabinoside, etoposide, melphalan, and anti-thymocyte globulin) followed by HSCT or MTX 20 mg every month for 6 months. The primary endpoint was the cumulative number of new T2 lesions in the 4 years following randomization. The authors concluded that intense immunosuppression followed by AHSCT was significantly superior to MTX in reducing MRI activity in severe cases of MS [18].

**Treatment-related mortality**

A growing literature had suggested that uncontrolled systemic inflammation leads to premature atherosclerosis and cardiovascular deaths [19], as well as toxicity from chronic immunosuppression, especially glucocorticoids. Despite this, it is still a challenge for a rheumatologist, neurologist, or gastroenterologist to accept an immediate TRM of 5–10%, especially since long-term benefits have yet to be demonstrated. The hypothesis is that in a randomized prospective trial of HSCT vs conventional treatment, early toxicity from TRM would eventually be surpassed by later deaths and/or organ failure from disease progression in the control arm.

Apart from the well-known acute toxicity of HSCT (infection and bleeding during the aplastic period and late infection during the T-cell reconstitution phase), several other factors emerged during the programme. Some SSc patients experienced serious lung toxicity from TBI, while others suffered a scleroderma renal crisis during the conditioning phase, attributed to a combination of rapid fluid and electrolyte shifts and high-dose glucocorticoids given as prophylaxis for ATG-induced cytokine storm. In some children with JIA, a fatal macrophage activation syndrome occurred, thought to be infection triggered and due to the profound immunosuppression resulting from TBI and CD34 purging [20]. Such toxicity problems were mostly eliminated by lung shielding, concurrent angiotensin converting enzyme (ACE) inhibition, and reduced intensity of the regimen respectively. However, an inevitable TRM will always exist, which must be weighed against the potential long-term benefit, a calculation that requires efficacy data from the randomized trials.

Late complications include not just the well-known fungal and other opportunistic infections during the T-cell reconstitution phase (which may last up to 2 years or more) but also the emergence of second autoimmunity [21]. It is almost always antigen specific – e.g. platelet, erythrocyte, thyroid – and often, but not always, resolves as the Treg network is reconstituted. However, some patients have succumbed from this, e.g. acquired haemophilia A antibodies after HSCT for MS [22].

**Mesenchymal stromal cell treatment of autoimmune disease**

Autologous HSCT for severe AD has demonstrated remarkable clinical, laboratory, and morphological improvement in many
patients, but at a high price including a TRM up to 10% in some conditions. Retrospective analysis from established databases is inevitably incomplete, especially in those patients "lost to follow-up" and assumed to be still alive. The advent of biologic agents has reduced the need for more radical therapies such as HSCT in RA and MS, but for SSc and severe forms of Crohn's disease, it still remains an option. The results of the prospective RCTs will be critical in deciding the future of this treatment. Allogeneic HSCT for AD has been performed and a summary of 35 patients so treated showed up to 50% remission induction in some AD subgroups [23]. However, the lack of a clear advantage over autologous HSCT and the risk of GvHD relegates this to a second-line strategy at the moment.

The first successful therapeutic application of mesenchymal stromal cells (MSC) in humans was reported 15 years ago in the context of bone marrow graft enhancement [24], but translational interest accelerated dramatically after the publication of a single case report of successful acute graft versus host disease (GvHD) in the Lancet in 2004 [25]. The demonstration that MSC could inhibit the mixed lymphocyte reaction independent of HLA restriction in 2003 [26] was followed by thousands of publications suggesting antiproliferative, anti-inflammatory and immunomodulatory properties of MSC [27]. In parallel, dozens of mechanisms for the mode of action of MSC have been proposed, not always consistent [26,28]. Although variable experimental conditions and cell product definitions cloud the literature, it seems clear that no single factor is responsible for the observed biological effects of MSC. Most likely diverse and multiple factors are operative depending on the source of MSC and experimental conditions ranging from cell – cell contact factors to released molecules. MSC modulation of immune competent cells has drawn particular attention (figure 1). The most recent addition to this collection is micro-RNA [29,30]. MSC may be involved in more fundamental homeostatic "niche management" functions such as seen in osteoimmunology. In the presence of fibroblast growth factor, MSC will express RANK ligand as well as IL-1β dependent MHC class II expression, suggesting a role in injury healing [31].

The first in vivo model showing a positive effect of MSC was a murine skin graft model [32] followed rapidly by dozens of similar positive reports in models ranging from autoimmune disease (SLE, arthritis, multiple sclerosis), GvHD, sepsis, solid organ transplantation, ischemia (myocardial, limb, hepatic), radiation injury and degenerative disease such as osteoarthritis [33]. Again, disparate results were often neglected in reviews of the subject. One arthritis model exacerbated [34] and one murine SLE model showed increased autoimmune production in vitro [28].

Equally prolific were the case reports and small series suggesting varying degrees of success in a wide range of disorders. Interpretation of these data is complicated by the multiple MSC sources used, differing ex vivo manipulation of the MSC, treatment protocols and variable follow-up periods. Most reports end with the statement that MSC are feasible and safe and that

**Figure 1**

MSC interaction with immune competent cells

Interactions of MSCs and immune cells demonstrated in vitro. Each arrow represents functional interaction between the indicated cell types, as detailed in publications in peer-reviewed journals (reviewed elsewhere [19]). In many cases, bi-directional crosstalk exists, which influences the final outcome of the interaction, and thus the effects of MSC-based therapy on disease. Black arrows and text pertain to responses driven by MSCs, whereas the effects of immune cells on MSCs are indicated by blue arrows and boxes. In general, the effects of MSCs on cells of the immune system are anti-inflammatory. DCs: dendritic cells; MSCs: mesenchymal stem cells; NK: natural killer (cell); PGE2: prostaglandin E2; TLR4: toll-like receptor 4; TREG: regulatory T (cell). From: Tyndall A. *Nat Rev Rheumatol* 2014;10(2):117-24 [27]. Courtesy of Nature Publications

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randomized controlled studies are required to establish their clinical utility. However, very few such trials have been reported, and some of those that have been were negative. Parallel to these scientific activities, there has been a proliferation of private centers offering MSC as a panacea to almost all human ailments from autism to erectile dysfunction, as well as extensive use of MSC in cosmetic surgery. This has at times led to scandals and public enquiry such as the Stamina trial in Italy [35], all of which has muddied the waters for those individuals and companies sincerely attempting to establish the place if any of MSC in human disease treatment. This report focuses on the use of MSC in humans suffering from autoimmune diseases, taking into account the above comments.

**MSC in autoimmune and autoinflammatory rheumatic diseases**

**Systemic lupus erythematosus (SLE)**

Over 300 patients with “treatment refractory” SLE have been reported since 2010, most of whom being from a single center (table II). Initially, three reports appeared in the same year; one with two patients who failed to respond to autologous bone marrow derived MSC clinically despite an increase in circulating Treg cells [36] and two other reports, both from the same center, showing clinical improvement in 15 patients treated with allogeneic bone marrow derived MSC [37] and also in 16 similar patients who had received allogeneic umbilical derived MSC [38]. Further reports from the same center showed improvement in four SLE patients with pulmonary hemorrhage [39] using umbilical cord derived MSC and 35 with refractory cytopenias [40] using either allogeneic bone marrow or umbilical cord MSC. Between 2012 and 2014, the same group has published 4 further SLE case series; 87 clinically heterogeneous patients [41], 58 refractory nephritis patients [42], 40 mostly refractory nephritis patients [43] and a further 81 refractory nephritis patients [44]. Most responded with 12-month renal remission rates of around 60%.

In the earlier study [41], 87 “treatment-resistant” patients with SLE, who had a spectrum of clinical manifestations, were given either umbilical-cord-derived or bone-marrow-derived MSCs (1 × 10^6 cells per kg) by intravenous infusion once with Table II: Uncontrolled clinical trials using MSC in SLE

<table>
<thead>
<tr>
<th>Autoimmune rheumatic disease</th>
<th>Number of patients</th>
<th>Source of MSCs</th>
<th>Delivery route</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>2</td>
<td>Autologous bone marrow</td>
<td>Intravenous infusion</td>
<td>No clinical change</td>
<td>[36]</td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>15</td>
<td>Allogeneic bone marrow</td>
<td>Intravenous infusion</td>
<td>Improved SLEDAI scores and decreased proteinuria and anti-dsDNA antibody levels</td>
<td>[37]</td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>16</td>
<td>Allogeneic umbilical cords</td>
<td>Intravenous infusion</td>
<td>Improved SLEDAI scores and renal function</td>
<td>[38]</td>
</tr>
<tr>
<td>SLE-associated diffuse alveolar haemorrhage</td>
<td>4</td>
<td>Allogeneic umbilical cords</td>
<td>Intravenous infusion</td>
<td>Normalization of haemoglobin levels and oxygen saturation, increased serum albumin levels and improvements in pulmonary manifestation visualized using HRCT</td>
<td>[39]</td>
</tr>
<tr>
<td>SLE (treatment-refractory patients with heterogenous manifestations)</td>
<td>87</td>
<td>Allogeneic bone marrow or umbilical cords</td>
<td>Intravenous infusion</td>
<td>Improved SLEDAI score and serological markers in most patients</td>
<td>[42]</td>
</tr>
<tr>
<td>SLE-associated cytopenia</td>
<td>35</td>
<td>Allogeneic bone marrow or umbilical cords</td>
<td>Intravenous infusion</td>
<td>Blood cell counts and disease activity improved in most patients</td>
<td>[40]</td>
</tr>
<tr>
<td>SLE (mostly Tx refractory nephritis)</td>
<td>40</td>
<td>Allogeneic umbilical cords</td>
<td>Intravenous infusion</td>
<td>Mostly improved</td>
<td>[57]</td>
</tr>
<tr>
<td>SLE (mostly Tx refractory nephritis)</td>
<td>81</td>
<td>Allogeneic bone marrow or umbilical cords</td>
<td>Intravenous infusion</td>
<td>Most improved. Renal remission 60%</td>
<td>[44]</td>
</tr>
</tbody>
</table>

*Majority of patients in these studies from the same centre (The Affiliated Drum Tower hospital of Nanjing university medical school, Nanjing, China).
apparently excellent outcomes, including an overall survival rate of 94% (mean follow-up of 27 months), improvements in clinical and serological variables, and a clinical remission rate of 28% at 1-year follow-up. However, detailed examination of the text and tables included in this report revealed that no patient included had received cyclophosphamide (CYC) for a period longer than 2 months before MSC infusion and that patients were administered CYC (10 mg per kg per day) intravenously on days -4, -3, and -2 before allogeneic MSC treatment to "inhibit active lymphocyte responses". The exact relevance of the findings of this study therefore remains elusive, and further information from an extended patient follow-up period of this cohort is awaited.

In a more recent study of 40 treatments refractory, mostly lupus nephritis patients from the same centre, 35 patients had received over 6 months of CYC and 26 of these were still on nephritis patients from the same centre, 35 patients had received CYC at the time of MSC infusion [43]. All patients received 1 × 10^6 MSC/kg body weight on days 0 and 7, which is surprising since the same group had previously published that a double dosing had no advantage over single dose [42]. Some aspects of the study remain inconclusive such as definition of "refractory to treatment" and concomitant therapy. The authors’ conclusions that it is feasible and safe to administer MSC as therapy for refractory SLE patients seems reasonable, but their claim of proven efficacy and need for a second MSC infusion at 6 months to prevent relapse are not supported by the data presented. Given the large experience and patient availability, it seems timely for this group to perform an appropriately powered, randomised blinded prospective trial which, if supported by data from another independent group, may establish the role if any for MSC in the treatment of SLE. It is remarkable that up until now the bulk of data in this field is from one centre only.

**Other autoimmune rheumatic diseases**

**Rheumatoid arthritis**

A preliminary report on four rheumatoid arthritis (RA) patients receiving allogeneic or bone marrow derived MSC IVI was essentially negative, though no toxicity was observed. Two of the three had a European League against rheumatism (EULAR) moderate response at 6 months but experienced a relapse at 7 and 23 months, respectively. Two patients had no EULAR response to MSC transplant. No patient achieved the DAS28-defined remission in the follow-up period [45]. However, a second larger, non-randomised comparative trial in 172 RA patients with active RA who had inadequate responses to traditional medication was published in which 136 patients received 40 × 10^6 allogeneic umbilical cord derived MSC (UC-MSC) and 36 patients received only the cell-solvent without the cells [46]. The two treatment options were: disease-modifying antirheumatic drugs (DMARDs) plus medium without UC-MSC, or DMARDs plus UC-MSC group via intravenous injection. No serious adverse effects were observed during or after infusion. The treatment induced a significant remission of disease according to the American College of Rheumatology improvement criteria, the 28-joint disease activity score, and the Health Assessment Questionnaire. The therapeutic effects maintained for 3–6 months without continuous administration, correlating with the increased percentage of regulatory T cells of peripheral blood. In contrast, there were no such benefits observed in the control group of DMARDs plus medium without UC-MSC.

No patients showed acute serious side-effects either during or after UC-MSC infusion, and 4% showed mild adverse effects during the infusion, such as chill and/or fever (< 38.5 °C), which disappeared within 2 h without any treatment. No major abnormal findings in hematologic or serum chemical profiles were found in the study. It was concluded that treatment with DMARDs plus UC-MSC may provide safe, significant, and persistent clinical benefits for patients with active RA.

A single center series published in 2011 presented three RA patients who received autologous adipose derived expanded MSC. Clinical benefit was seen in all cases without significant toxicity. All patients received multiple injections of MSC, in two cases IVI and a third, intra-articular [47].

A further study presented at the ACR-congress in San Diego in 2013 showed in a randomized clinical trial concerning 53 refractory RA patients that i.v. allogeneic adipose-derived MSC was safe in a dose of 3 times 1–4 × 10^6 cells per kg when given at day 1,8 and 15 with rescue therapy allowed at months 3 and 6 (Alvaro-Gracia JM, et al. In: Abstract at the Annual Scientific Meeting ACR, 2013). Dose-limiting safety signals were not identified and only one of the 53 patients experienced a serious adverse event leading to discontinuation of the treatment (lacerar infarction). Most other adverse events were mild and transient.

**Systemic sclerosis**

The first report of a rheumatic disease treated with MSC was in 2008 was of a 41-year-old woman with 4-year history of diffuse cutaneous systemic sclerosis (SSc) complicated by acral ulcers. One dose IVI of allogeneic MSC (10^6/kg body weight) resulted in significant improvement of both skin thickening and ulcer. The ulcers relapsed later and responded to bosentan [48]. This was followed by a later report by the same group of four further patients with SSc manifesting various degrees of internal organ involvement [49]. The authors concluded cautiously that MSC improved the acral ulcers and to a lesser extent the skin thickening, but that given the heterogeneity of the clinical manifestations, larger studies were required.

Further reports and uncontrolled small series have also shown positive effects following the local injection of autologous adipose tissue derived MSC into face [50] and digits [51], autologous blood and bone marrow derived mononuclear cells [52]...
and IVI autologous bone marrow derived MSC [53] in SSc patients. No significant toxicity was reported.

**Primary Sjögren’s Syndrome**

One single centre open label study in 24 patients with severe primary Sjögren’s syndrome showed remarkable responses [54]. Sicca symptoms and salivary flow improved in most as did severe systemic involvement (13 patients) and autoantibody (SSA, SSB) levels. One dose of 1 million MSC/kg body weight umbilical cord derived MSC was used and toxicity was not seen. These results are extraordinary, given the otherwise dearth of effective disease modifying drugs for the challenging and miserable disease [55]. Further data, particularly in the form of prospective controlled trials are eagerly awaited.

**Dermatomyositis/polymyositis (DM/PM)**

Ten patients with therapy resistant DM/PM or severe systemic involvement received one dose of either allogeneic bone marrow derived MSC or umbilical cord derived MSC at a dose of 1 million/kg body weight IVI [56]. All responded clinically

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**TABLE III**

Phase I/II uncontrolled clinical trials using MSC in inflammatory rheumatic diseases

<table>
<thead>
<tr>
<th>Autoimmune rheumatic disease</th>
<th>Number of patients</th>
<th>Source of MSCs</th>
<th>Delivery route</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSc</td>
<td>5</td>
<td>Allogeneic bone marrow</td>
<td>Intravenous infusion</td>
<td>Some improvement, but with variable persistence, in skin- ulcer healing and MRSS</td>
<td>[48]</td>
</tr>
<tr>
<td>SSc (digital ischaemic ulcers)</td>
<td>2</td>
<td>Autologous peripheral blood and bone marrow</td>
<td>Local injection (CD34+ cells in the hands; mononuclear cells in the lower extremities)</td>
<td>Improvements in multiple ulcers, Raynaud condition, and physical function and disability outcomes</td>
<td>[52]</td>
</tr>
<tr>
<td>SSc (ischaemic limbs)</td>
<td>1</td>
<td>Autologous bone marrow</td>
<td>Intravenous pulses</td>
<td>Improved ischaemia: reduced areas of necrotic skin and revascularization</td>
<td>[53]</td>
</tr>
<tr>
<td>Scleroderma-microstomia</td>
<td>4</td>
<td>Autologous adipose derived</td>
<td>Local</td>
<td>Improved</td>
<td>[50]</td>
</tr>
<tr>
<td>Scleroderma fingers</td>
<td>12</td>
<td>Autologous adipose derived</td>
<td>Local</td>
<td>Most improved</td>
<td>[51]</td>
</tr>
<tr>
<td>Primary Sjögren’s syndrome</td>
<td>24a</td>
<td>Allogeneic umbilical cords</td>
<td>Intravenous infusion</td>
<td>Improved SSDAI scores in all patients; Salivary flow rates increased in patients with xerostomia; Decreased serum levels of anti-SSA/Ro and anti-SSB/La autoantibodies</td>
<td>[54]</td>
</tr>
<tr>
<td>Dermatomyositis/polymyositis</td>
<td>10a</td>
<td>Allogeneic bone marrow or umbilical cords</td>
<td>Intravenous infusion</td>
<td>Improved serological markers (creatine kinase levels), patient global assessment scores and muscle strength improvements in interstitial lung disease in some patients</td>
<td>[56]</td>
</tr>
<tr>
<td>Dermatomyositis</td>
<td>1</td>
<td>Autologous adipose derived</td>
<td>Intravenous infusion × 4</td>
<td>Improved strength</td>
<td>[47]</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>4a</td>
<td>Allogeneic bone marrow/sadipose derived MSC</td>
<td>IVI</td>
<td>No response</td>
<td>[45]</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>172 (136 MSC treated)</td>
<td>Allogeneic Umbilical cord</td>
<td>IVI</td>
<td>Positive ACR improvement, 28 joint count and HAQ</td>
<td>[46]</td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
<td>31</td>
<td>Allogeneic BM (35 young donors)</td>
<td>IVI × 4</td>
<td>77% ASAS “responders” Reduced MRI inflammatory burden</td>
<td>[57]</td>
</tr>
</tbody>
</table>

*a*Majority of patients in these studies from the same centre (The Affiliated Drum Tower hospital of Nanjing university medical school, Nanjing, China).
regarding strength and reduced CK, as did some with pulmonary interstitial disease and skin ulcers. Some required a second MSC infusion for relapse. A controlled prospective trial was recommended by the authors.

A single case report of a 35-year-old female showed significantly improved strength after 4 IVI injections of autologous expanded adipose derived MSC at 3 months follow-up [47].

**Ankylosing spondylitis**

A single centre open label study to evaluate the feasibility, safety, and efficacy of intravenous (i.v.) infusion of allogeneic mesenchymal stem cells (MSCs) in ankylosing spondylitis (AS) patients who are refractory to or cannot tolerate the side effects of nonsteroidal anti-inflammatory drugs (NSAIDs) was reported in 2014 [57]. Patients enrolled in this study received four i.v. infusions of MSCs on days 0, 7, 14, and 21. The MSC were of bone marrow origin from 35 healthy young volunteers. The percentage of ASAS20 responders (the primary endpoint) at the 4th week and the mean ASAS20 response duration (the secondary endpoint) were used to assess treatment response to MSC infusion and duration of the therapeutic effects. Magnetic resonance imaging (MRI) was performed to detect changes of bone marrow oedema in the spine. Thirty-one patients were included, and the percentage of ASAS20 responders reached 77.4% at the 4th week, and the mean ASAS20 response duration was 7.1 weeks. A significant reduction in the MRI inflammatory burden was observed. There were no significant toxicity issues.

These results should be confirmed by prospective double-blind controlled studies, given the complexity in assessing disease activity and MRI changes in this condition.

**Other autoimmune disorders (table IV)**

**Multiple sclerosis**

Five small studies with ten or less patients have been published suggesting some positive clinical responses following MSC derived from autologous or allogeneic bone marrow or allogeneic adipose tissue and umbilical cord [58–62]. This was not always reflected in MRI changes.

**Inflammatory bowel disease**

Two studies have shown improved fistula closure using intralesional autologous adipose derived MSC in 14 [63] and 10 patients [63,64] while two other small studies showed some improvement in mucosal disease in some of ten patients using autologous bone marrow derived MSC [65] and seven using allogeneic umbilical cord MSC [66].

One study using three IVI infusions of allogeneic placental MSC in ten patients with type II diabetes mellitus suggested improvement at 3 months.

**Conclusion**

Despite over 13,000 publications relating to MSC in PubMed in the past decade, so few include randomised prospective controlled clinical trials.

Perhaps the ease of availability of MSC from various sources and their apparent low acute toxicity has encouraged some groups to target uncontrolled case series and comparative studies rather than engage in the rigor of controlled clinical trials. Two positive prospective controlled trials have been published, one relating to living donor renal transplant in which autologous bone marrow derived expanded MSC replaced the IL-2 receptor blocking monoclonal antibody [67] and in knee OA [68]. Both are single centre trials.

# Table IV

**Uncontrolled clinical trials using MSC in other autoimmune diseases**

<table>
<thead>
<tr>
<th>Disease</th>
<th>No.</th>
<th>MSC product</th>
<th>Route</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>10</td>
<td>Allobone marrow</td>
<td>Intrathecal</td>
<td>Mixed</td>
<td>[58]</td>
</tr>
<tr>
<td>MS</td>
<td>1</td>
<td>Allo-umbilical</td>
<td>IVI</td>
<td>Improved</td>
<td>[59]</td>
</tr>
<tr>
<td>MS</td>
<td>3</td>
<td>Allo/auto-fat</td>
<td>Mixed IVI/intrathecal</td>
<td>Mixed clinic (not MRI)</td>
<td>[60]</td>
</tr>
<tr>
<td>MS</td>
<td>10</td>
<td>Autobone marrow</td>
<td>Intrathecal</td>
<td>Improved clinic (not MRI)</td>
<td>[61]</td>
</tr>
<tr>
<td>MS</td>
<td>10</td>
<td>Autobone marrow</td>
<td>IVI</td>
<td>Some visual improvement only</td>
<td>[62]</td>
</tr>
<tr>
<td>Crohns fistulae</td>
<td>14</td>
<td>Autologous fat</td>
<td>Intrafistula</td>
<td>71% closure</td>
<td>[63]</td>
</tr>
<tr>
<td>Crohns fistulae</td>
<td>10</td>
<td>Autologous bone marrow</td>
<td>IVI</td>
<td>Some improved</td>
<td>[65]</td>
</tr>
<tr>
<td>Crohns fistulae</td>
<td>10</td>
<td>Autologous fat</td>
<td>Intrafistula</td>
<td>100% closure (30% partial)</td>
<td>[64]</td>
</tr>
<tr>
<td>Crohns ulcerative colitis</td>
<td>4</td>
<td>Allo-umbilical cord</td>
<td>IVI (1 mill/kg)</td>
<td>Improved (no prior TNF alpha)</td>
<td>[66]</td>
</tr>
<tr>
<td>Diabetes mellitus Type II</td>
<td>10</td>
<td>Alloplacental</td>
<td>IVI × 3</td>
<td>All improved (within 3 months)</td>
<td>[73]</td>
</tr>
</tbody>
</table>
On the other hand, three negative prospective multicentre controlled trials have been published all relating to the inability of autologous expanded bone marrow derived MSC to improve left ventricular function in ischemic injury or chronic heart failure [69–71]. In addition, several prospective controlled trials in GvHD and Crohn’s disease which failed to achieve their primary end points have been reported at meetings but never published. Clearly, the field is complicated by complex and resource demanding regulatory issues regarding stem cell trials, which although entirely appropriate, places high demands on committed investigators and industry alike, reviewed in [27]. Further clouding the field is a lack of cell product definition [72] and selection, evidence-based dosing protocols and meaningful clinical outcome measures.

Disclosure of interest: the authors declare that they have no competing interest.

References


[70] Penn FC, Willerson JT, Pepine CJ, et al. Effect of transendocardial delivery of autologous...

