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Stem cells in the treatment of inflammatory arthritis

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Autologous haematopoietic stem cell transplantation in patients with rheumatoid arthritis (RA) resulted in a positive short-term outcome clinically with low treatment-related toxicity. However, early conditioning regimens were of low immunoblative intensity and most patients relapsed. Mechanistic studies suggest that residual lesional effector cells may have been responsible for the relapses. The introduction of biopharmaceuticals has, for the moment, reduced the need for further experimental studies.

Juvenile idiopathic arthritis patients, mostly of the systemic subgroup, have shown nearly 33% durable drug-free remission, but with significant toxicity, including fatal macrophage-activation syndrome early in the programme. Later modifications to the protocol have reduced this toxicity.

Mesenchymal stem cells (MSCs), derived from several sources including bone marrow and adipose tissue, are being tested as tissue-regenerative and immunomodulating agents in many autoimmune diseases and animal models of inflammatory arthritis have been positive. MSCs and other stromal cells derived from actively inflamed synovium and peripheral blood of RA patients do not always demonstrate a full range of differentiation potential compared with healthy MSCs, although their immunomodulatory capacity is unimpaired.

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Despite the implementation of new treatment strategies and introduction of biologicals, long-term drug-free remission remains an elusive goal in most patients with chronic inflammatory arthritis, notably those with rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA). A recent systematic review showed that the efficacy of a second biological agent, irrespective of the mode of action, after a first biological in RA patients is limited with ACR70 (ACR70, American College of Rheumatology criteria 70) responses ranging from 5% to 15% and disease activity score (DAS) remission from 9% to 15.4% [1]. These figures underscore the need for new treatment modalities. Although data on other chronic idiopathic inflammatory arthritides are less robust, it is generally accepted that JIA and psoriatic arthritis (PsA) also constitute an area of huge unmet need [2], which explains why there is scope for new (including cellular) therapies. The rationale of cellular therapies for chronic inflammatory arthritis (and other rheumatic autoimmune diseases) is based on the concept that immune dysregulation can be restored by *ex vivo* expansion and reinfusion of cells with immunoregulatory function or therapeutic ablation of autoaggressive lymphocytes allowing subsequent preferential *in vivo* homeostatic expansion of such cells. Technological advances in cell processing and improvements in the medical care of complex patients have facilitated studies in this field. This article focusses on haematopoietic stem cell transplantation (HSCT) and mesenchymal stromal cell (MSC) therapy as examples of different types of cellular therapies that are both aimed at tilting the balance towards improved immune regulation while being fundamentally different in approach.

Haematopoietic stem cell transplantation

HSCT is the short name of a complex therapeutic intervention, comprising mobilisation of haematopoietic progenitor cells using high-dose chemotherapy and granulocyte-colony stimulating factor (G-CSF) or harvest of bone marrow, followed by intensification with myelo- or lymphoablative doses of chemotherapy and/or lymphocyte-depleting antibodies and/or total body irradiation (TBI), and (re)infusion of the graft to reduce the duration of aplasia. RA and JIA were among the first diseases to be targeted with HSCT in the 1990s based on long-term remissions observed in RA patients who received an allogeneic bone marrow transplantation (BMT) for haematological conditions and in studies in experimental animal models of autoimmune disease. The latter showed a dose–effect relationship of TBI on arthritis, providing evidence that deletion of autoaggressive T lymphocytes was necessary for maximum effect [3]. More recent studies, however, showed that less intensive conditioning in combination with major histocompatibility complex (MHC)-mismatched BMT was equally effective both in terms of disease suppression and elimination of autoreactivity [4]. Nevertheless, the risks of treatment-related mortality and graft-versus-host disease (GvHD) related to myeloablative conditioning and allotransplanting were felt to be high to justify treatment of patients with a chronic disease; hence, autologous transplants were pursued after preclinical studies in experimental arthritis demonstrated their curative potential. It is thought that the conditioning regimen is the key component in the immunobliteration, but the potential immunomodulatory role of the autologous graft has never been properly investigated.

Rheumatoid arthritis

Early dose-finding studies showed that higher doses of intravenous cyclophosphamide for mobilisation of HSCs led to superior disease control when compared with lower doses [5]. Subsequent pilot studies in the UK and the Netherlands confirmed the remission-induction potential of high-dose cyclophosphamide, but relapses were universally seen in most patients within a follow-up period of 3 years [6,7]. The temporary improvements of disease activity translated into significant improvement of quality of life and arrest of joint destruction [8,9]. HSCT was well tolerated by most patients, and no unexpected adverse events were seen. More intensive conditioning with the use of anti-thymocyte globulin (ATG) and/or CD34-selection of autologous grafts did not seem to be more effective, although the total numbers of RA patients treated was low and controlled studies were not done [10].

The need for HSCT waned after the introduction of effective biopharmaceuticals and implementation of new treatment strategies aimed at remission induction in early disease. The transplant activity in RA has now almost come to a standstill according to a recently published analysis of the

European Group for Bone Marrow Transplantation (EBMT) data registry, which includes 89 RA cases, but only a few cases since 2006 [11]. All of these patients had active, destructive, disease-modifying anti-rheumatic drug (DMARD)-refractory disease with a median disease duration of 86 months. The registry analysis confirmed the data from the pilot studies and an earlier registry analysis on 70 RA patients, showing that HSCT was relatively well tolerated in RA patients with 100-day transplant-related mortality (TRM) of 1% and overall survival of 94% (95% confidence interval (CI): 87–100%), probably because vital organ function was preserved. The high incidence of relapses in the RA population was reflected in a relatively low 5-year progression-free survival of 18% (95% CI: 9–27%; Fig. 1). Of note, most RA patients had been treated with low-intensity conditioning, comprising high-dose cyclophosphamide (200 mg kg^{-1}) and/or $\text{CD}34^{+}$ -selected peripheral blood grafts. It must be emphasised that the level of detail of clinical information – for example, on clinical responses and toxicity – in registry analyses is limited, and so robust conclusions cannot be drawn. In an Australian randomised, controlled trial, RA patients treated with high-dose cyclophosphamide (200 mg kg^{-1}) were treated with either an unmanipulated graft or a $\text{CD}34^{+}$ -selected graft [12]. This study failed to show a benefit of $\text{CD}34$ -selection, although it was argued that this might be due to the absence of *in vivo* T-cell depletion. In a subsequent study, the authors showed that relapses could be treated effectively with rituximab [13]. This was in keeping with similar observations in the European studies showing that responsiveness to methotrexate and cyclosporine were restored post transplant.

Juvenile idiopathic arthritis

JIA is a heterogeneous disease, encompassing different subsets [14]. Two subsets have been targeted with SCT: polyarticular destructive disease and systemic disease. Data on HSCT for JIA are mainly derived from the EBMT Registry and studies in the Netherlands, Italy and the UK. Long-term follow-up data were recently analysed and published [11,15,16]. In the EBMT Registry analysis of 65 cases, 100-day TRM was 11% (95% CI: 6–22%), overall survival at 5 years was 82% (95% CI: 72–92%) and progression-free survival was 52% (95% CI: 38–66%; Fig. 1).

In the Dutch study (involving two tertiary referral centres), 23 patients with progressive refractory JIA were enrolled, one withdrew after bone marrow harvest but prior to BMT, 22 patients received treatment with rabbit ATG (20 mg kg^{-1}), cyclophosphamide (200 mg kg^{-1}) and low-dose TBI (4 Gy), followed by T-cell-depleted autologous BMT from 1997 to 2001. T-cell depletion was achieved with either immunosetting ($n = 17$) or $\text{CD}34^{+}$ -selection ($n = 5$), resulting in a median of 2.8 and 1.0×10^4 $\text{CD}3^{+}$ cells kg^{-1} in the graft. Eighteen patients had systemic JIA, four had polyarticular JIA, with a median disease duration of 70 months (range: 13–135). Five patients were refractory to tumour-necrosis factor (TNF)-blockade. DMARDs were stopped prior to conditioning; steroids were slowly tapered and discontinued. All patients received selective antimicrobial suppression, pentamidine and 17 patients received antiviral prophylaxis as well. Core-set variables were assessed using validated outcome criteria. Two patients (9%) died from macrophage activation syndrome (MAS), which was ascribed to the intensive T-cell depletion and triggered by *Staphylococcus epidermidis* bacteraemia and

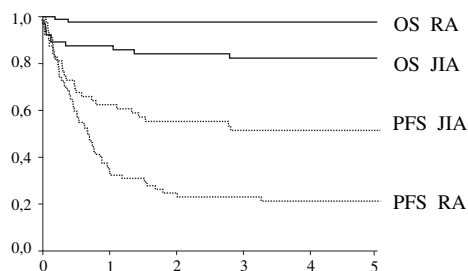


Fig. 1. Effects of HSCT on overall survival (OS) and progression-free survival (PFS) in patients with rheumatoid arthritis (RA) ($n = 89$) and juvenile idiopathic arthritis (JIA) ($n = 65$). PFS was defined as survival without evidence of relapse or progression of disease activity. OS was defined as time to death, irrespective of the cause. Data from the EBMT Paris Office.

Epstein–Barr virus (EBV) reactivation. The protocol was amended in 1999 to make it safer by introducing antiviral monitoring and treatment, improving pre-transplant systemic disease control, implementing a corticosteroid-tapering protocol and pre-emptive treatment with steroids and cyclosporine A when MAS was suspected. Two other patients died later from EBV and varicella zoster virus (VZV) infection, respectively, following reinstatement of immunosuppressive treatment. The median post-SCT follow-up of the surviving 18 children described in this article was 80 months (range: 52–104), 15 of whom had completed a follow-up of 5 years or more. The probability of survival at 5 years was 82%, and of disease-free survival was 36% (censored for relapse and death as events). Eight of the 20 evaluable patients reached complete clinical remission according to predefined criteria, seven were considered partial responders and five relapsed, of whom three had ongoing disease and two died (as explained above). One patient relapsed after 7 years. The effects of HSCT on the core variables are depicted in Fig. 1. The authors reported that progression of joint destruction by X-rays was arrested. A large number of infections were seen, including nine with VZV infection and five with cytomegalovirus (CMV) (four reactivation and one primary). In addition, all patients developed adverse reactions to ATG, responding to intravenous steroids.

Immune reconstitution studies showed normal haematological recovery, but slow reconstitution of particularly naive CD4⁺ CD45RA⁺ cells. The authors explained that while the duration of lymphopaenia was similar to that seen in paediatric transplants in cancer, the high numbers of viral infections suggested functional defects. No correlation was found between the numbers of T cells reinfused and the clinical outcome.

In a separate publication, functional ability and exercise tolerance were studied in greater detail in 10 children [17]. Although all children completed exercise testing without complication or the need of supplementary oxygen, cardiorespiratory capacity as measured by VO₂ peak was significantly impaired in this group, although to variable extents among different patients. Evidence was obtained which pointed to underlying defects in mitochondrial oxygen-extraction capacity in skeletal muscles, possibly related to systemic inflammation, steroid myopathy and deconditioning.

Based on the observed favourable long-term outcome in the majority of these patients, the authors concluded that HSCT may still be considered an effective treatment option in drug-resistant JIA, and that an exercise programme should be incorporated as part of post-transplant rehabilitation.

In the UK study, details of seven cases from two tertiary referral centres were reported out of 14 potentially eligible patients with systemic onset, and a polyarticular course ($n = 4$), or a mixed systemic, polyarticular course ($n = 10$). Of the seven patients not transplanted, two in fact died pre-BMT from catheter-related bacterial infections, two received conventional treatment following the advice of independent assessors while the families of three children opted out. The transplant regimens differed slightly between patients, but all included cyclophosphamide (120–200 mg kg⁻¹) and rabbit ATG (10–40 mg kg⁻¹), while one patient also received TBI (400 cGy) and three patients fludarabine (150 mg kg⁻¹). TBI was excluded from the conditioning regimen after an interim data review showed no benefit. Grafts were obtained from bone marrow (in five), or peripheral blood (in two) and T-cell depleted by means of positive CD34 cell selection. One patient died from multi-organ failure due to terminal aspergillus infection and disseminated adenovirus infection resistant to antiviral therapy and T-cell add back, two patients were successfully treated with antiviral and immunomodulatory treatment for virus (EBV and CMV) reactivation-driven haemophagocytic syndrome. Four patients are in complete drug-free remission with 5–8 years follow-up, resulting in significant improvement of functional ability, catch-up growth and good quality of life. Two patients who had undergone the least intensive conditioning experienced relapse presenting with severe polyarticular and systemic disease and MAS, requiring biological and chemotherapeutic therapy. Two of these developed clinical shingles, and one autoimmune thyroiditis whilst in complete remission.

Mechanistic studies on HSCT in RA and JIA

Considerable effort has been spent on trying to understand the mechanism of action of HSCT in RA and JIA, focussing on autoantibodies as surrogate measures of autoreactive B- and T-cell responses, cytokine profiles in blood and phenotypic characterisation of peripheral blood and synovial tissue-infiltrating mononuclear cells. In JIA, improvement of disease activity in JIA patients

was paralleled by the emergence of CD4⁺-CD25⁺-Foxp3⁺ T-regulatory cells [18,19]. In RA, drops in titres and changes of affinity of rheumatoid factor and anti-CCP titres were found, although complete seroconversion was uncommon [20]. Synovial tissue-infiltrate analysis in JIA and RA showed a paucity of lymphocytes immediately post transplant, although memory T cells remained detectable despite their absence in peripheral blood [6,21,22]. At the time of relapse, T cells had re-infiltrated the synovium again [6]. The expression of the RB-isoform of CD45, a marker of recent activation or maturation, on early post-transplant samples and of the RO-isoform on late post-transplant samples was interpreted as a 'smoking gun', indicating T cells might have played a role in the relapse. Taken together, the *ex vivo* studies raised the intriguing possibility that, in RA lesions, T cells had not been sufficiently eradicated, and that relapses arose from homeostatic proliferation of lesional T cells in a lymphopaenic environment with consequent rise of low-affinity autoantibodies as evidence of a new autoimmune response. Studies in RA also highlighted abnormally slow reconstitution of particularly new naive T cells, which was ascribed to low post-transplant interleukin (IL)-7 production by bone marrow stromal cells [23]. As a peculiar finding, persistence of an atypical CD4⁺ T-cell subset was demonstrated, the functional consequences of which remained unclear [24].

Summary HSCT studies

HSCT has been successfully used to treat severe RA and JIA patients, albeit at the expense of significant toxicity and even transplant-related mortality, particularly in JIA patients. HSCT is less common nowadays because of the availability of new, effective treatment alternatives. Nevertheless, despite the introduction of biologicals and new treatment paradigms, cases of therapy-refractory chronic inflammatory arthritis continue to present. Based on the observations of marked and sustained improvements of disease activity, HSCT remains a treatment option for RA and JIA patients who failed combination-DMARD therapy and a number of biologicals (TNF-blockade, B-cell depletion, IL-6RA, etc.), and have steroid-dependent yet not end-stage disease. For such patients, a HSCT-regimen should be considered consisting of intensive immunosuppression including *in vivo* T-cell depletion, *ex vivo* manipulation of graft, followed by post-transplant maintenance with methotrexate to reduce risk of relapse. In patients with a human leucocyte antigen (HLA)-matched sibling donor, non-myeloablative conditioning and allografting may be an option, but the risks of transplant-related morbidity and mortality are significant. In addition, detailed outcome data of patients who prefer not to be transplanted should be collected. These studies will be small in terms of patient numbers, and require multicentre collaboration to reach sufficient numbers for meaningful analyses.

Mesenchymal stromal cell therapy

MSCs are also referred to as mesenchymal stromal cells, although their true 'stemness' has yet to be demonstrated. MSCs are capable of differentiating *in vitro* and *in vivo* to different MSC lineages, including adipose, bone, cartilage, muscle and myeloid-supportive stroma. MSCs may be isolated from bone marrow, skeletal muscle, adipose tissue, synovial membranes and other connective tissues of human adults as well as cord blood and placental products and are defined by using a combination of phenotypic markers and functional properties [25].

In vitro, MSCs have vast proliferative potential, can clonally regenerate and can give rise to differentiated progeny. They also exhibit antiproliferative and anti-inflammatory properties *in vitro* and *in vivo*, making them candidates for treatment of acute inflammatory autoimmune disease (AD) [26]. Irrespective of whether or not MSCs are true stem cells, clinical benefit from MSC may not require sustained engraftment of large numbers of cells or differentiation into specific tissues. It is possible that a therapeutic benefit can be obtained by local paracrine production of growth factors and a provision of temporary anti-proliferative and immunomodulatory properties [27].

MSCs enjoy a degree of immune privilege in that allogeneic MSCs may be infused into patients without any preconditioning, and seem to survive long enough to exert positive clinical effects without acute toxicity.

Mechanism of immunosuppression and anti-proliferation of MSC

The mechanism(s) underlying the immunosuppressive effect remain to be fully clarified with sometimes conflicting data, probably reflecting the variable definitions and experimental conditions. Certainly they are multiple, involving both cell contact and soluble factors including IL-10, tumour growth factor (TGF)- β , hepatocyte growth factor (HGF), IL-1 receptor antagonist and soluble HLA-G. The effects of MSCs on immune cells are shown in Fig. 2.

Animal models of tissue protection and autoimmunity of MSCs

It may be impossible, in fact meaningless, to separate the anti-inflammatory, immunomodulatory and tissue protective ‘trophic’ effects of MSCs. An immunosuppressive effect of MSC *in vivo* was first suggested in a baboon model, where infusion of *ex vivo* expanded donor or third-party MSCs delayed the time to rejection of histoincompatible skin grafts. Since then, many publications have appeared showing a positive outcome in various models of tissue injury and/or inflammation (Table 1).

A notable exception is the murine collagen-induced arthritis (CIA) in which the first study showed a worsening of disease [28]. This study used the allogeneic MSC cell line C3H10T1/2, whereas another study using primary murine MSCs showed a positive outcome [29], as did MSCs engineered to express IL-10 and administered systemically [30]. Further work from the Montpellier group suggested that bone marrow-derived MSCs, both autologous and allogeneic, could prevent and improve, but not cure, murine CIA. This effect was dose and timing dependent, underlining the importance of protocol design (Bouffi C, personal communication). A proposed mechanism was the induction and activation of CD4⁺-FoxP3⁺ T-regulatory cells in addition to induction of effector T-cell anergy.

In vitro studies of human bone-marrow-derived MSCs from healthy donors [31] and from patients with autoimmune disease [32] have shown dose-dependent immunosuppressive and anti-proliferative effects, as have adipose tissue-derived MSCs, in which the generation of antigen-specific T-regs was demonstrated [33].

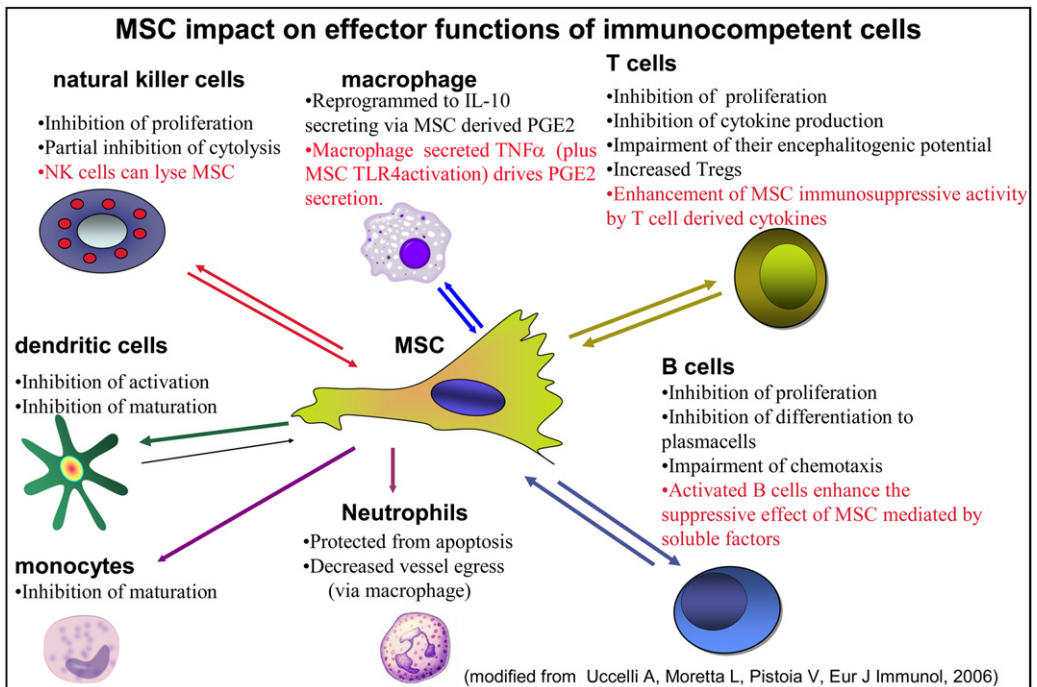


Fig. 2. The impact of MSC on effector functions of immunocompetent cells.

Table 1

Mesenchymal stem cells in clinical protocols.

| Disease indication | Treatment and outcome | Reference |
|-------------------------|--|-----------|
| Acute GvHD | 9 year old boy- full recovery. Required 2 infusions. Allogeneic bone marrow MSC. | 46 |
| Acute GvHD | 30/55 complete recovery | 34 |
| Decompensated cirrhosis | 4 cases Autologous bone marrow MSC | 39 |
| Multiple sclerosis | Improvement in all cases – no toxicity 10 patients – intrathecal autologous bone marrow derived MSC Mixed results. | 36 |
| Multiple sclerosis | Allogeneic adipose vascular stromal fraction (SVF) cells and autologous MSC. IVI and intrathecal in 3 relapsing remitting cases. Improvement of some clinical features, MRI unchanged. | 37 |
| Multiple sclerosis | Single case, allogeneic bone marrow derived. Improved | 38 |
| Renal SLE | 4 cases. Allogeneic bone marrow derived MSC. Improved SLEDAI and proteinuria. 12–18 month follow-up. | 40 |

MSCs and human experience

Ex vivo-expanded allogeneic MSCs have been infused in phase I/II and phase III studies. No adverse events during or after MSC infusion have been observed and no ectopic tissue formation has been noted. After infusion, MSCs remain in the circulation for no more than an hour.

Conditions currently being treated in experimental protocols include acute GvHD after allogeneic HSCT in which 30 out of 55 steroid-resistant patients had a complete response with no immediate toxicity [34]. Other conditions are Crohn's disease [35], multiple sclerosis [36–38], decompensated cirrhosis [39], renal systemic lupus erythematosus (SLE) [40] as well as stroke and myocardial infarction [41] (Table 1).

MSCs from human autoimmune disease

Autologous bone marrow-derived MSCs have been shown to be potently anti-proliferative to stimulated T cells from normal subjects and autoimmune (RA, systemic sclerosis (SSc), Sjogren's syndrome, systemic lupus erythematosus (SLE), etc.) patients, and the in SSc patients these MSCs were normal in respect to proliferation, clonogenicity and differentiation to bone and fat [32,42].

MSCs and inflammatory arthritis in humans

It was proposed some years ago that MSCs migrating from the bone marrow directly into the joints of the CIA mouse model of arthritis somehow 'prepared' the joint in an innate, antigen-independent fashion for the later inflammation [43]. There are direct channels from the subchondral bone marrow into the joint, the channels of Ochi [44], which suggest a more dynamic synovial/bone marrow niche than previously suspected. More recently, the Leeds group has shown that synovial membrane-derived MSCs from the active RA patients are defective in terms of clonogenicity and chondrogenic differentiation potential [45]. The MSCs were compared with those derived from osteoarthritis and suggests that if autologous MSCs are to be applied to inflammatory arthritis clinically, then the lowest level of inflammation should be achieved first if tissue regeneration is the aim.

Summary MSC studies

With respect to the scope of MSC treatment, around 90 clinical trials are now registered online (<http://www.clinicaltrials.gov>), involving various human disorders ranging from tissue engineering, critical ischaemia to inflammatory autoimmune diseases. None involves inflammatory arthritis. Reports of a failed phase III trials with MSCs in acute GvHD have appeared as news items in the financial and scientific press (*Fox Business News*, Tuesday 8 September 2009 and *Nature*, 9 September

2009 doi:10.1038/news.2009.894), although so far no peer-reviewed publications are available. Of importance is the setting of clear therapeutic targets and harmonisation of cell products, especially MSC source and type (autologous or allogeneic), cell-expansion conditions and trial protocols. In addition, long-term safety-data collection across disciplines is required and an international interdisciplinary registry of MSC-treated patients has been launched.

Concluding remarks

The concept of HSCT as treatment of severe rheumatological conditions has been successfully adopted from the transplant community, but gradually been abandoned because of effective and presumed safer treatment options for chronic inflammatory arthritis, including biologicals. Allogeneic HSCT is the only therapy with curative potential, but the risks are significant and, hence, it is recommended that patients are only treated in the context of well-designed multicentre clinical trials in specialised transplant centres. MSCs capture our imagination due to their ability to contribute to tissue regeneration and modulate the immune system, which could be exploited to treat specific inflammatory or vascular conditions.

Practice points

- The introduction of biologicals and more effective use of DMARDs in early chronic inflammatory arthritis has reduced the need for HSCT as a salvage treatment.
- HSCT has resulted in significant improvements of disease activity, functional ability and arrest of joint destruction, but many transplanted RA and JIA patients have relapsed during long-term follow-up. In cases where HSCT is considered a medium-intensity conditioning regimen, it is probably preferred followed by maintenance immunosuppression.
- Most RA patients treated with HSCT received relatively low-intensity regimens, which may explain the high number of relapses. JIA patients have been treated with more lymphoablative therapy, which might explain the more robust responses when compared to RA patients.
- MSCs can be safely procured from autologous and allogeneic sources and their *in vivo* anti-proliferative and tissue regenerative properties are currently being investigated in clinical trials in a range of diseases, including GvHD and SLE. No trials are yet planned in inflammatory arthritis.

Research agenda

- There is a paucity of long-term outcome data on patients with inflammatory arthritis, refractory to combination DMARD therapy and biologicals, who could serve as control cases for future HSCT patients.
- Harmonisation of MSC expansion protocols, phenotypic and functional characterisation of MSC preparations and clinical protocols is required.
- Core data on safety and efficacy of MSC treatment should be centrally collected in a registry.

Conflict of interest statement

The authors declare they have no conflicts of interest that could inappropriately influence (bias) the content of this article.

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